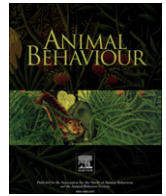




Contents lists available at ScienceDirect

## Animal Behaviour

journal homepage: [www.elsevier.com/locate/anbehav](http://www.elsevier.com/locate/anbehav)

## Orientation in corpse-carrying ants: memory or chemical cues?

Lise Diez\*, Jean-Louis Deneubourg, Lucie Hoebeke, Claire Detrain

Unit of Social Ecology, Université Libre de Bruxelles

## ARTICLE INFO

## Article history:

Received 19 November 2010  
 Initial acceptance 4 January 2011  
 Final acceptance 17 February 2011  
 Available online xxx  
 MS. number: 10-00811

## Keywords:

ant  
 memory  
*Myrmica rubra*  
 necrophoresis  
 trail

In social insects, rejection of dead nestmates is a common hygienic behaviour that allows colonies to reduce pathogen transmission within the nest. We investigated which orientation processes, chemical cues or individual memory, are used by the common red ant, *Myrmica rubra*, when it removes dead nestmates far from the nest. *Myrmica rubra* colonies varied in how efficiently they removed corpses, owing to differences in the number of short-term specialists involved in the transport of several corpses over successive trips. Corpse-carrying ants had to choose between two possible paths leading to areas remote from the nest. Their choices were not influenced by chemical compounds such as a necrophoric trail or any other chemical cues emitted by corpses. On the other hand, corpse-carrying ants that made several successive trips relied on their own spatial memory by choosing the same side as they did before. The strength of this memory was time dependent, an ant being more likely to choose the same side if the time elapsed between two choices was short. This suggests that necrophoresis in field colonies with a low death rate will be likely to lead to dispersion rather than to a piling up of corpses.

© 2011 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Animal groups are regularly faced with the death of conspecifics but few studies have looked into the ways they solve related hygienic problems and behave in the presence of corpses. Specific behaviour towards corpses, such as intraspecific necrophagy, has been reported in several taxa including arthropods, reptiles, amphibians, and mammals (Rudolf & Antonovics 2007). A few vertebrates, such as chimpanzees *Pan troglodytes*, dolphins, *Tursiops truncatus*, and elephants, *Elephas maximus*, may show prolonged interest or touching of dead conspecifics (Douglas-Hamilton et al. 2006; McComb et al. 2006). Such behaviours towards corpses can be hazardous since contact and ingestion are common pathways for acquiring pathogens. Social animals are even more exposed to sanitary problems as pathogen prevalence usually increases with density (Anderson & May 1979; Schmid-Hempel 1998). Concerning social insects, several life traits make them potentially sensitive to the transmission of pathogens: they live at very high densities within societies composed of highly genetically related individuals and overlapping generations. Moreover, they are often settled for several years within the same nest under confined and humid conditions. Insect societies have therefore developed a variety of social defences against the intake and spread of parasites (Cremer et al. 2007). Some defences are prophylactic ones such as the avoidance of infected soil (Drees et al. 1992), the use of antimicrobial material in the nest (e.g. propolis in bees,

Bankova et al. 2000) or the retrieval of tree resin by ant workers (Chapuisat et al. 2007). Frequent grooming coupled to the presence on the cuticle of antibiotic compounds secreted by metapleural glands also contributes to the health maintenance of workers (Hughes et al. 2002). The spatial isolation of infected, moribund or dead individuals is another widely used strategy to reduce the horizontal transmission of parasites and pathogens (Spivak 1996). In some species, diseased or moribund ants leave the nest on their own and die away from their nestmates (Heinze & Walter 2010). When individuals die in the colony, they are removed by nestmates: termites (Crosland 1997) and some ant species (Renucci et al. 2010) bury them in the nest, bees drop dead larvae or adults outside the hive (Wilson-Rich et al. 2009) and most ant species transport corpses away from the nest. Apart from humans, the only cases of active removal of dead conspecifics, called 'necrophoresis' (Wilson et al. 1958), have been reported in bees and ants.

In the limited space of laboratory nests, necrophoresis by ant workers may lead to the formation of 'ant cemeteries'. This piling up results from simple clustering rules: unladen ants pick up corpses with a probability that decreases with cluster size, whereas corpse-carrying ants drop their load with a probability that increases with cluster size (Theraulaz et al. 2003). However, under natural conditions, only a few corpses have to be concurrently removed and dropped within a foraging area that is very large and potentially unlimited. Then, the probability of an ant encountering another corpse is very low, making the formation of piles of corpses through self-amplifying processes unlikely. Even though corpses are not aggregated into 'cemeteries', the removal patterns of dead

\* Correspondence: L. Diez, CP 231, Unit of Social Ecology, Université Libre de Bruxelles, Bd du Triomphe, B-1050 Brussels, Belgium.  
 E-mail address: [lisediez@gmail.com](mailto:lisediez@gmail.com) (L. Diez).

nestmates can nevertheless be structured through time and space. Ideally, corpses have to be removed fast, transported far away from the nest and dumped in areas that are less frequented by nestmates. Such spatial segregation could be facilitated by orientation mechanisms based on trail laying or area marking. Apart from these chemical cues, segregation of waste items could also emerge from the activity of a few ants carrying many corpses successively and showing some memory-based consistency in their orientation towards disposal areas.

We investigated orientation of the common red ant, *M. rubra*, while carrying dead nestmates. We first explored whether some individual ants contribute to corpse transport more than average and if they improve their efficiency in corpse removal over time. Second, we investigated whether chemical cues act upon the ants' orientation while they carry corpses. Then we studied the influence of individual memory during necrophoresis. We also modelled the forgetting rate of spatial memory among corpse-carrying ants. Finally, we discuss the consequences of ants' orientation during necrophoresis on the global pattern of removed corpses.

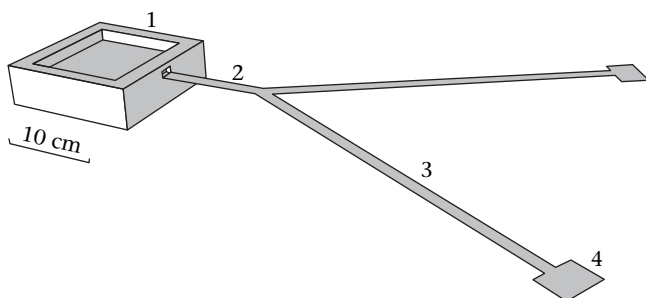
## METHODS

### Collection and Maintenance of Ants

The red ant is a common species inhabiting forests or open areas of Northern Europe. Three *M. rubra* colonies were excavated from earth banks of a semi-open area in the campus of Gembloux Agro-Bio Tech, Wallonia, Belgium. In the laboratory, ants were kept in plaster nests (Janet type, 120 × 120 mm and 2 mm high) connected to a foraging arena (47 × 33 cm) the borders of which were coated with fluon (polytetrafluoroethylene) to prevent ants from escaping. Each nest contained 400–500 workers, brood covering 10–15% of the nest area and three gynes, which is a colony composition commonly found in the field (Elmes 1973). Laboratory conditions were kept at 23 ± 2 °C and 50 ± 5% relative humidity, with a constant light period of 12 h per day. Each nest was provided with a sucrose solution (ad libitum, 0.3 M) and one mealworm, *Tenebrio molitor*, per day as a protein and lipid supply.

### Experimental Design and Protocol

Before each experiment, the tested ant colony was connected to a Y-shaped aluminium bridge placed at the nest entrance (Fig. 1). An ant leaving the nest had to walk 10 cm before facing a 60° fork where it had to choose one branch (40 cm long) until reaching a 4 × 3 cm platform. The bridge was 1.5 cm wide and was covered with paper that was replaced between successive trials to remove any colonial odours or trails possibly laid by walking ants. To prevent ants from falling off the bridge, we placed plastic walls, 1.5 cm high, covered with fluon on each side of the bridge. To avoid



**Figure 1.** Experimental set-up consisting of an ant nest (1), a bridge with a 10 cm common part (2), two 40 cm right and left branches (3) and two platform areas (4).

orientation biases caused by an asymmetry of visual cues, light sources (four neon lamps OSRAM L 18 W) were placed symmetrically with respect to the centre of the set-up, which was surrounded by white walls. Before the introduction of corpses, we observed choices of ants at the bridge's fork for 20 min. The distribution of ants between the two branches did not differ from a binomial distribution (Kolmogorov–Smirnov test:  $D = 0.5$ ,  $P = 0.164$ ), confirming that there was no experimental bias in their orientation. Then three corpses were placed at the nest entrance. All corpses were nestmates that were killed by freezing at –24 °C for 30 min and left at ambient temperature for 7 days before being tested. The trial started as soon as the first corpse was transported from the nest entrance beyond the bridge's fork. During each trial, we kept constant the number of three corpses at the nest entrance by adding a new corpse as soon as one was carried away. Each transported corpse was removed after having been dumped for at least 30 s either on the bridge or on one of the two platforms. The activity at the bridge's fork was video recorded (Panasonic WV-BP330 camera with a WV LZ62/8 lens). Each trial was divided into two phases of 90 min each. Between the two phases, we swapped the pieces of paper covering the left and right branches of the bridge. This was done to test for the possible presence of a necrophoric trail or other chemical cues which would act upon the orientation of corpse-carrying ants and which would redirect their exiting flows after the pieces of paper were swapped. To study the influence of the ant's memory, we observed each corpse-carrying ant during its successive transports of dead nestmates until it returned inside the nest. The low frequency of transports allowed two observers to follow each corpse-carrying individual visually without any physical marking. We quantified on each branch (1) the number of noncarrying ants, (2) the number of corpse-carrying ants and (3) the number of transports made by each corpse-carrying individual. We also recorded the time at which corpse-carrying ants made a choice on the bridge.

### Data Analysis

We carried out five trials on each of the three colonies. We used all the trials ( $N = 15$ ) to assess the efficiency of necrophoresis as well as memory effects on choices of individuals. To study collective choice of one branch by corpse-carrying ants, we took into account only the trials with a minimum of 10 transports of corpses in each of the two phases. Only 12 trials fulfilled these conditions and could be used for further analysis. The numbers of exploring and corpse-carrying ants were normally distributed; therefore we performed an ANOVA for mean comparisons. For data that were not normally distributed, we used nonparametric tests. Correlations between variables were tested with a Pearson product–moment correlation test. We compared the distributions between branches of carrying and noncarrying ants with expected theoretical distributions by using Kolmogorov–Smirnov tests. To assess changes in the orientation of corpse-carrying ants, we used a Wilcoxon signed-ranks test to compare the percentages of corpse-carrying ants on the winning branch, that is, the branch chosen by more than 50% of corpse-carrying ants during the first phase, before and after we swapped the paper on the branches of the bridge. Survival curves were drawn to assess the persistence of choice between two successive transports as a function of time elapsed. These survival curves were compared by applying the log-rank test. Deviation from binomial distribution was assessed by using an exact binomial test to compare expected and observed probabilities of a corpse-carrying ant choosing the same path several times. We used software R 2.9.2 (<http://www.r-project.org>) for all statistical analyses and modelling. All tests were two tailed with alpha set at 0.05.

## RESULTS

*Dynamics of Corpse Removal*

Throughout the trials (3 h including first and second phases), corpses were always available at the nest entrance and  $56.5 \pm 23.4$  dead nestmates were transported by ants with an average rate of one transport every 3 min. For each trial, cumulated flows of corpse-carrying ants increased linearly as a function of time (linear regression:  $R^2 \geq 0.949$ ). This indicates that there was no amplification process such as the recruitment of nestmates that would speed up the corpse-carrying activity. Noncarrying ants were observed exploring the bridge at a rate of one worker every minute ( $175.8 \pm 32$  passages in 3 h). Even though colonies differed in the number of noncarrying ants (ANOVA:  $F_{2,12} = 10.97$ ,  $P = 0.002$ ), they did not differ in the number of corpses carried (ANOVA:  $F_{2,12} = 1.14$ ,  $P = 0.350$ ). Because of this high variability in the exploration level (Table 1), the percentage of ants whose activity was related to necrophoric behaviour varied between colonies ( $24 \pm 18\%$ ).

A majority of corpse-carrying ants (86.3%) transported only one corpse, but a few individuals kept on going back to the nest entrance (up to 26 successive transporting trips). Consequently, a minority of ants (3.2%) that transported more than three corpses contributed to a large part of all the transport of corpses (20%).

The dynamics of necrophoresis was highly variable between trials and ranged from 15 to 94 corpses removed in 3 h. The total number of corpses removed during trials was positively correlated with the number of corpse-carrying ants ( $r = 0.91$ ; Pearson product–moment correlation test:  $t_{13} = 7.75$ ,  $P < 0.001$ ), but not with the mean number of corpses transported per carrying ant ( $r = 0.37$ ; Pearson product–moment correlation test:  $t_{13} = 1.41$ ,  $P = 0.179$ ). This suggests that an increase in the necrophoric efficiency was due to an increased number of ants participating in corpse carrying rather than to a higher carrying performance per ant.

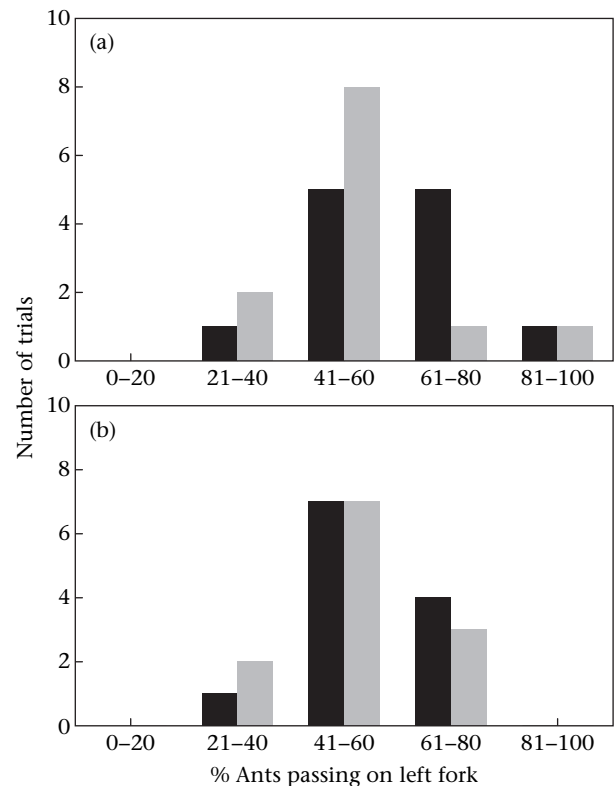
*Searching for a Necrophoric Chemical Cue*

During the first phase of the trials, the distributions between the two branches of noncarrying ants or of corpse-carrying ants did not differ significantly from a binomial distribution (Kolmogorov–Smirnov test: corpse-carrying ants:  $D = 0.4$ ,  $N = 12$ ,  $P = 0.401$ ; noncarrying ants:  $D = 0.5$ ,  $N = 12$ ,  $P = 0.164$ ; Fig. 2). Moreover, no significant difference was observed between the distributions of corpse-carrying ants and noncarrying ants (Kolmogorov–Smirnov test:  $D = 0.2$ ,  $N = 12$ ,  $P = 0.988$ ). Likewise, during the second phase of the trials, after we swapped the pieces of paper, distributions of carrying or noncarrying ants did not differ significantly from a binomial distribution (Kolmogorov–Smirnov test: carrying ants:  $D = 0.5$ ,  $N = 12$ ,  $P = 0.164$ ; noncarrying ants:  $D = 0.5$ ,  $N = 12$ ,  $P = 0.164$ ; Fig. 2b). Thus, throughout the trials, ants were as likely to choose the right or the left branch, which confirms the lack of external bias influencing workers' orientation. Moreover, since there was no clear asymmetry in the activity level between branches this indicates that very weak amplification mechanisms

**Table 1**  
Number (mean  $\pm$  SD) of corpse-carrying and exploring ants for each colony

Colony	Corpse-carrying ants	Noncarrying ants
1	54.6 $\pm$ 28.0	71.6 $\pm$ 16.4
2	60.2 $\pm$ 22.3	91.2 $\pm$ 56.0
3	37.8 $\pm$ 22.2	326.6 $\pm$ 163.5

$N =$  five replicates per colony.

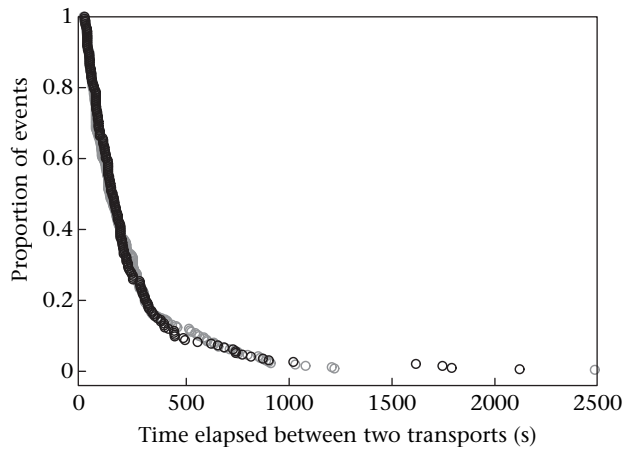


**Figure 2.** Distribution of trials ( $N = 12$ ) depending on the percentage of ants choosing the left branch of the bridge. Grey bars: corpse-carrying ants; black bars: noncarrying ants. (a) First phase and (b) second phase of the trials.

or even none at all were involved in the collective process of corpse carrying.

We investigated whether any attractive chemicals such as a necrophoric trail was laid by corpse-carrying ants that attracted and acted upon the orientation of further ants carrying corpses. Therefore, after identifying the winning branch that was chosen by a majority of transporting ants in the first phase, we compared its relative attractiveness to corpse-carrying ants (i.e. distribution of carrying ants between the two branches) in the two phases. Swapping the pieces of paper slightly, but not significantly, influenced the distribution of corpse-carrying ants on the winning branch between the two phases (Wilcoxon signed-ranks test:  $V = 63$ ,  $N = 12$ ,  $P = 0.064$ ). In addition, during the first phase, we observed no significant correlation between the percentage of ants choosing the winning branch and the number of transports ( $r = 0.13$ ; Pearson product–moment correlation test:  $t_{13} = 0.46$ ,  $P = 0.656$ ). This suggests that the number of passages, and potential marking, previously made by corpse-carrying ants did not influence the orientation of nestmates.

We also checked whether any volatile chemicals could be emitted and/or perceived by corpse-carrying ants that would enhance necrophoresis over a short timescale. Therefore, we tested whether the choice of one branch by a carrying ant favoured the choice of the same branch by the next carrying ants. In this case, the probability of choosing the same branch as the preceding corpse-carrying ants should decrease with the time elapsed. In our experiment, the mean time elapsed between two transporting ants was similar whether ants made the same choice ( $4.4 \pm 5.7$  min) or a different choice ( $4.5 \pm 5.2$  min). The survival curves of the time elapsed between two successive transports (Fig. 3) did not differ whether ants made the same choice or not (log-rank test:  $\chi^2_1 = 0$ ,  $P = 0.96$ ).



**Figure 3.** Survival curves of sets of two successive transports made by two different ants as a function of the time elapsed between them (e.g. 50% of all pairs of transports were separated in time by less than 200 s). Black circles: two successive ants made different choices; grey circles: two successive ants made identical choices.

### Memory and Short-term Specialization

We investigated whether ants that transported more than one corpse showed some persistence in their choice of a branch for depositing dead nestmates. If the choice of one branch occurs at random, the expected probability of an ant choosing the same branch while successively transporting several corpses obeys a binomial law with a probability of choosing one branch equal to 0.5 (Table 2). For two to six corpses removed by the same ant, probabilities of choosing the same branch during successive transports were significantly higher than expected from a binomial distribution (exact binomial test: all  $P$  values  $<0.05$ ). Corpse-carrying ants were thus very likely to choose the same path during successive corpse transports: they showed a spatial specialization most probably based on individual memory.

One may assess the persistence of this spatial memory by measuring how the probability of an ant choosing the same path depends on the time elapsed between two successive transports. To estimate how this probability decreases with time, we formulated a model about the dynamics of forgetting a path as a function of the time elapsed between two choices. In this model, an event was defined as two successive transports made by the same ant. First, we described the survival curve of the proportion of events  $N$  where the same ant carried two corpses, whichever path was followed, for a given time elapsed between the two transports ( $t$ ). The experimental data were well fitted by the exponential

$$N = e^{-\mu t} \quad (1)$$

where  $\mu$  is the probability of carrying a new corpse per time unit. Using nonlinear least-square estimation, we obtained the best

**Table 2**

Observed and expected probabilities of choosing the same path each time during two to six successive transports

Number of successive transports	Observed proportion of success	Expected proportion of success	Exact binomial test $P$
2	0.58 (198)	0.5	0.027
3	0.40 (124)	0.25	0.0002
4	0.27 (95)	0.125	0.0001
5	0.21 (78)	0.0625	$<0.0001$
6	0.17 (64)	0.03125	$<0.0001$

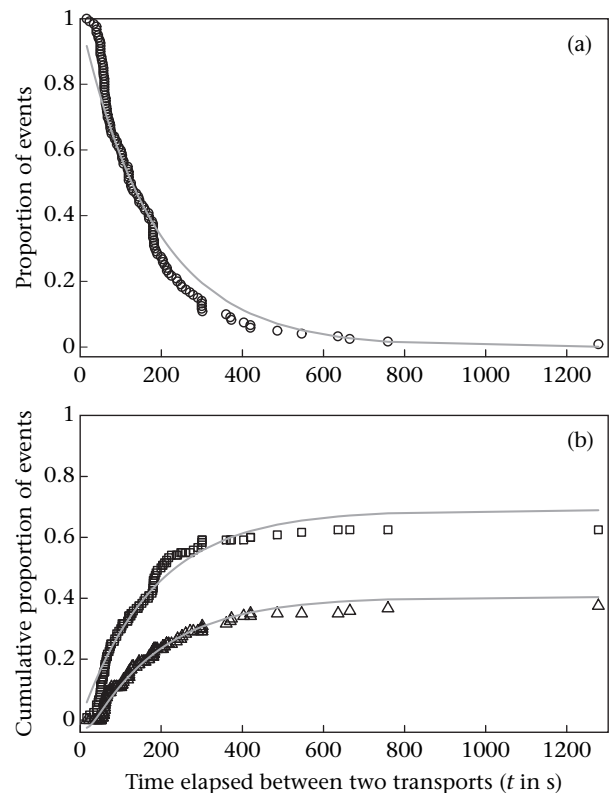
Sample sizes are given in parentheses.

fitting for  $\mu = 0.0054 \pm 0.00012$  (Fig. 4a). The inverse of  $\mu$  provides an estimate of the mean time elapsed between two transports carried out by the same ant. This estimated time (183.8 s) is close to the experimental one ( $171.6 \pm 168.6$  s,  $N = 120$ ). Next, we described the decay of spatial memory with time. Indeed, the probability of an ant choosing the same path twice is a decreasing function of the time elapsed between the two transports. As for previous forgetting models validated in honeybees, *Apis mellifera* (Zhang et al. 2005) and humans (Raaijmakers & Mensink 1988), the decay of spatial memory observed during our experiment can be approximated by an exponential function. Then, the probabilities of choosing the same branch twice ( $p_i$ ) or different branches ( $p_d$ ) are:

$$p_i = (0.5 + ae^{-\alpha t}) \quad (2a)$$

$$p_d = (0.5 - ae^{-\alpha t}) \quad (2b)$$

where  $\alpha$  is the rate of forgetting. The parameter  $a$  gives an estimate of the maximal impact of individual memory on the choice of path by corpse-carrying ants. Indeed, when the time between two successive transports is null ( $t = 0$ ), the probability of choosing the same path ( $p_i$ ) is equal to  $0.5 + a$ . On the other hand, when the time between two transports is very large ( $t \rightarrow \infty$ ), corpse-carrying ants are no longer able to use their spatial memory and will choose a path at random ( $p_i = p_d = 0.5$ ). Based on these equations, we can



**Figure 4.** (a) Survival curve of two successive transports made by the same ant as a function of the time elapsed between them (e.g. 50% of all pairs of transports by the same ant were made in less than 180 s). Circles: experimental data values for corpse-carrying ants; grey line: curve fitted using function (1) with parameter set as  $\mu = 0.0054$ . (b) Cumulative proportion of events in which two successive transports are made by the same ant as a function of the time elapsed between them. Circles: two identical choices ( $N_i$ ); triangles: two different choices ( $N_d$ ); grey lines: curve fitted with the functions (3b) and (3c), respectively, for identical and different choices with parameters set as  $\mu = 0.0054$ ,  $a = 0.24$  and  $\alpha = 0.0036$ .

specify the cumulative proportion of events in which the ants choose the same path twice ( $N_i$ ) or follow different paths ( $N_d$ ). By coupling equations (1) and (2a), one can calculate how the proportion of transports made on the same path ( $N_i$ ) varies with the time  $t$  elapsed between two transports:

$$\frac{dN_i}{dt} = \mu p_i N = \mu p_i e^{-\mu t} = \mu \left( 0.5 e^{-\mu t} + a e^{-(\alpha+\mu)t} \right) \quad (3a)$$

The integration of (3a) gives the cumulative proportion of events  $N_i$  for a given time  $t$ :

$$N_i(t) = \left( 0.5 \left( 1 - e^{-\mu t} \right) + \frac{\mu a}{(\alpha + \mu)} \left( 1 - e^{-(\alpha+\mu)t} \right) \right) \quad (3b)$$

Similarly, the cumulative proportion of events in which ants choose two different paths ( $N_d$ ) for a given time  $t$  obeys the following equation

$$N_d(t) = \left( 0.5 \left( 1 - e^{-\mu t} \right) - \frac{\mu a}{(\alpha + \mu)} \left( 1 - e^{-(\alpha+\mu)t} \right) \right) \quad (3c)$$

Using equations (3b) and (3c) we fitted parameters on the observed data (Fig. 4b) using nonlinear least squares:  $a = 0.24 \pm 0.0065$  and  $\alpha = 0.0036 \pm 0.00037$ . Parameter  $a$  indicates that when the time between two transports is very short, corpse-carrying ants will choose the same path with a probability of 0.74, which represents the maximal impact of individual memory on path choice. The inverse of  $\alpha$  (278 s) provides an estimate of the mean time during which memory is effective and may influence path choice by corpse-carrying ants. For a very large time elapsed between two transports ( $t \rightarrow \infty$ ), the spatial memory no longer influences path choice by corpse-carrying ants and expected values  $N_i$  and  $N_d$  reach a plateau:

$$N_i \Big|_{t \rightarrow \infty} = \left( 0.5 + \frac{\mu a}{(\alpha + \mu)} \right) = 0.64 \quad (4a)$$

$$N_d \Big|_{t \rightarrow \infty} = \left( 0.5 - \frac{\mu a}{(\alpha + \mu)} \right) = 0.36 \quad (4b)$$

Experimental values (Fig. 4b) show that this plateau is reached for a time elapsed between two transports larger than 400 s. The  $N_i$  plateau value is an estimate of the mean effect of an ant's memory on its path choice. Then, on average, corpse-carrying ants that make two successive transports have an individual memory that increases by 14% on average the probability of corpse-carrying ants choosing the same path twice.

## DISCUSSION

*Mymica rubra* showed a high variability between colonies in their dynamics of corpse removal which was due to variability in the number of ants involved as well as to the presence of short-term specialists carrying several corpses over successive trips. Likewise, there is a high variability between honeybee hives in how efficiently they maintain colony healthiness. Hygienic and non-hygienic bee colonies can be differentiated by their ability to remove varroa-infested pupae (Spivak 1996) and by their behavioural thresholds for corpse removal (Arathi & Spivak 2001; Oxley et al. 2010). Future studies should investigate whether performance of ant colonies in preventing the spread of diseases is also determined by individual response thresholds to cues triggering corpse carrying such as oleic acid, a chemical compound released during corpse decomposition (Wilson et al. 1958; Gordon 1983; Akino & Yamaoka 1996).

Chemical communication is well known to play a key role in the organization of the ant colony. In our study, the spatial distribution of *M. rubra* corpse-carrying ants was slightly influenced by a shift in the location of the most frequented, and potentially more marked, path. This effect, however, was not significant and was probably caused by footprint marks passively left by walking workers (Devigne & Detrain 2002; Lenoir et al. 2009). In addition, *M. rubra* ants may emit trail pheromones that recruit nestmates and focus their foraging activity (Cammaerts-Tricot 1974; Cammaerts et al. 1981). However, in the case of necrophoresis, neither trail recruitment nor collective choice of a path was observed. The spatial distribution of workers between paths did not differ between corpse-carrying ants and exploring ants. This suggests that neither the laying of a necrophoric trail nor the passive emission of volatiles by dead corpses rules the orientation of *M. rubra* corpse-carrying ants.

Our results show that necrophoresis in ants is rather an individual-based process in which the differences in carrying efficiency of workers, as well as the spatial memory of individuals, plays a key role. Individualities matter in the removal of dead nestmates: a minority of *M. rubra* carrying individuals was responsible for a large part of the removal of corpses and behaved as short-term specialists for the corpse-carrying tasks. In leaf-cutting ants, subgroups of workers are also highly specialized in the management of fungus wastes and corpses (Julian & Cahan 1999; Ballari et al. 2007). Likewise, a few honeybee workers are responsible for the rejection of dead nestmates (Visscher 1983) and infected larvae (Arathi et al. 2000). At the colony level, specialization and individual experience are known to improve collective performance (Langridge et al. 2007). Moreover, in the case of necrophoresis, specialization limits the number of workers that are exposed to pathogen transmission by contact during removal of corpses (Schmid-Hempel 1998).

While carrying several corpses, *M. rubra* workers used their spatial memory and were likely to choose the same path several times. Spatial learning is well known in several ant species including *M. sabuleti*, a species close to *M. rubra*, which uses olfactory and visual cues for orientation while foraging for food (Collett 1992; Cammaerts 2004; Cammaerts & Rachidi 2009). Moreover, ants can use sensorimotor cues and navigate in the absence of landmarks (Macquart et al. 2008). Here, we have shown that the spatial orientation of corpse-carrying ants relies on short-term memory. When at least 7 min elapsed between two transports, the orientation of the ant was no longer influenced by its previous path choice. This duration of memory is within the range reported for other social insects such as honeybees (Hammer & Menzel 1995).

While removing waste, ant species show a variety of strategies by either piling it up or by scattering it in the environment. Short-term spatial memory is a mechanism that can explain these different patterns depending on the amount of waste that has to be removed. Occasionally, an ant colony can be exposed to massive death of nestmates because of fights with competitors, mortality following winter lethargy or generalized infection by a pathogen. Corpse-carrying ants then have to remove a huge number of corpses and may become short-term specialists. Together with their increased probability of dropping items when faced with a pile (Theraulaz et al. 2003), the corpse-carrying ants develop a spatial memory. This may speed up the growth of heaps at locations where the density of waste was previously high. Such heaps of corpses or fungus waste has been observed for army ants (Hölldobler & Wilson 1990) and some leaf-cutting ants, respectively (Hart & Ratnieks 2001; Ballari et al. 2007). In ant species with a large colony size, the aggregation of corpses and waste in limited areas may reduce the risk of encounters and pathogen transmission by contact. Most frequently, only a few dead ants are concurrently present within the nest and no piling up of corpses is observed. In

this latter case, the corpse-carrying ants also show a short-term spatial memory that leads each individual to explore its own zone, as reported for foragers in ponerine (Fresneau 1985) and desert ants (Wehner 1970). However, this spatial memory results in the scattering of corpses around the nest (Howard & Tschinkel 1976) since the number of corpses is not high enough to lead to an amplification of depots. Sanitary risks related to scattering are most probably negligible owing to the low density of dead nestmates. In the future, it would be interesting to investigate how the organization of waste removal, as well as the specialization of corpse-carrying ants, differs depending on colony size, how frequently nesting sites are changed or the diet of ant species.

## Acknowledgments

We thank Dr Pablo Servigne, Madeleine and Lynn Bochner for their constructive remarks on the manuscript. We also thank the anonymous referees for their comments. This study was funded by a Ph.D. grant from FRIA (Fonds pour la Recherche dans l'Industrie et dans l'Agriculture) and by a FRFC grant 2.4600.09. C.D. and J-L.D. are senior research associates from the Belgian National Fund for Scientific Research (F.N.R.S.).

## References

- Akino, T. & Yamaoka, R. 1996. Origin of oleic acid: corpse recognition signal in the ant *Formica japonica* Motschulsky (Hymenoptera: Formicidae). *Japanese Journal of Applied Entomology and Zoology*, **40**, 265–271.
- Anderson, R. M. & May, R. M. 1979. Population biology of infectious diseases: part I. *Nature*, **280**, 361–367.
- Arathi, H. S. & Spivak, M. 2001. Influence of colony genotypic composition on the performance of hygienic behaviour in the honeybee, *Apis mellifera* L. *Animal Behaviour*, **62**, 57–66.
- Arathi, H., Burns, I. & Spivak, M. 2000. Ethology of hygienic behaviour in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): behavioural repertoire of hygienic bees. *Ethology*, **106**, 365–379.
- Ballari, S., Farji-Brener, A. G. & Tadey, M. 2007. Waste management in the leaf-cutting ant *Acromyrmex lobicornis*: division of labour, aggressive behaviour, and location of external refuse dumps. *Journal of Insect Behavior*, **20**, 87–98.
- Bankova, V. S., Castro, S. L. D. & Marcucci, M. C. 2000. Propolis: recent advances in chemistry and plant origin. *Apidologie*, **31**, 14.
- Cammaerts, M. 2004. Operant conditioning in the ant *Myrmica sabuleti*. *Behavioural Processes*, **67**, 417–425.
- Cammaerts, M. & Rachidi, Z. 2009. Olfactive conditioning and use of visual and odorous cues for movement in the ant *Myrmica sabuleti* (Hymenoptera: Formicidae). *Myrmecological News*, **12**, 117–127.
- Cammaerts, M., Evershed, R. & Morgan, E. 1981. Comparative study of the mandibular gland secretion of four species of *Myrmica* ants. *Journal of Insect Physiology*, **27**, 225–231.
- Cammaerts-Tricot, M. 1974. Piste et phéromone attractive chez la fourmi *Myrmica rubra*. *Journal of Comparative Physiology*, **88**, 373–382.
- Chapuisat, M., Oppliger, A., Magliano, P. & Christe, P. 2007. Wood ants use resin to protect themselves against pathogens. *Proceedings of the Royal Society B*, **274**, 2013–2017.
- Collett, T. S. 1992. Landmark learning and guidance in insects. *Philosophical Transactions of the Royal Society B*, **337**, 295–303.
- Cremer, S., Armitage, S. A. & Schmid-Hempel, P. 2007. Social immunity. *Current Biology*, **17**, 693–702.
- Crosland, M. 1997. Division of labour in a lower termite: the majority of tasks are performed by older workers. *Animal Behaviour*, **54**, 999–1012.
- Devigne, C. & Detrain, C. 2002. Collective exploration and area marking in the ant *Lasius niger*. *Insectes Sociaux*, **49**, 357–362.
- Douglas-Hamilton, I., Bhalla, S., Witemyer, G. & Vollrath, F. 2006. Behavioural reactions of elephants towards a dying and deceased matriarch. *Applied Animal Behaviour Science*, **100**, 87–102.
- Drees, B. M., Miller, R. W., Vinson, B. S. & Georgis, R. 1992. Susceptibility and behavioral response of red imported fire ant (Hymenoptera: Formicidae) to selected entomogenous nematodes (Rhabditida: Steinernematidae Heterorhabditidae). *Journal of Economic Entomology*, **85**, 365–370.
- Elmes, G. W. 1973. Observations on the density of queens in natural colonies of *Myrmica rubra* L. (Hymenoptera: Formicidae). *Journal of Animal Ecology*, **42**, 761–771.
- Fresneau, D. 1985. Individual foraging and path fidelity in a ponerine ant. *Insectes Sociaux*, **32**, 109–116.
- Gordon, D. M. 1983. Dependence of necrophoric response to oleic acid on social context in the ant *Pogonomyrmex badius*. *Journal of Chemical Ecology*, **9**, 105–111.
- Hammer, M. & Menzel, R. 1995. Learning and memory in the honeybee. *Journal of Neuroscience*, **15**, 1617–1630.
- Hart, A. G. & Ratnieks, F. L. 2001. Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. *Behavioral Ecology and Sociobiology*, **49**, 387–392.
- Heinze, J. & Walter, B. 2010. Moribund ants leave their nests to die in social isolation. *Current Biology*, **20**, 249–252.
- Hölldobler, B. & Wilson, E. O. 1990. *The Ants*. Cambridge, Massachusetts: Harvard University Press.
- Howard, D. F. & Tschinkel, W. R. 1976. Aspects of necrophoric behavior in the red imported fire ant, *Solenopsis invicta*. *Behaviour*, **56**, 157–180.
- Hughes, W. O. H., Eilenberg, J. & Boomsma, J. J. 2002. Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society B*, **269**, 1811–1819.
- Julian, G. E. & Cahan, S. 1999. Undertaking specialization in the desert leaf-cutter ant *Acromyrmex versicolor*. *Animal Behaviour*, **58**, 437–442.
- Langridge, E. A., Sendova-Franks, A. B. & Franks, N. R. 2007. How experienced individuals contribute to an improvement in collective performance in ants. *Behavioral Ecology and Sociobiology*, **62**, 447–456.
- Lenoir, A., Depickère, S., Devers, S., Christidès, J. & Detrain, C. 2009. Hydrocarbons in the ant *Lasius niger*: from the cuticle to the nest and home range marking. *Journal of Chemical Ecology*, **35**, 913–921.
- McComb, K., Baker, L. & Moss, C. 2006. African elephants show high levels of interest in the skulls and ivory of their own species. *Biology Letters*, **2**, 26–28.
- Macquart, D., Latil, G. & Beugnon, G. 2008. Sensorimotor sequence learning in the ant *Gigantiops destructor*. *Animal Behaviour*, **75**, 1693–1701.
- Oxley, P. R., Spivak, M. & Oldroyd, B. P. 2010. Six quantitative trait loci influence task thresholds for hygienic behaviour in honeybees (*Apis mellifera*). *Molecular Ecology*, **19**, 1452–1461.
- Raaijmakers, J. G. & Mensink, G. J. 1988. A model for interference and forgetting. *Psychological Review*, **95**, 434–455.
- Renucci, M., Tirard, A. & Provost, E. 2010. Complex undertaking behavior in *Temnothorax lichtensteini* ant colonies: from corpse-burying behavior to necrophoric behavior. *Insectes Sociaux*, **58**, 9–16.
- Rudolf, V. H. & Antonovics, J. 2007. Disease transmission by cannibalism: rare event or common occurrence? *Proceedings of the Royal Society B*, **274**, 1205–1210.
- Schmid-Hempel, P. 1998. *Parasites in Social Insects*. Princeton, New Jersey: Princeton University Press.
- Spivak, M. 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*. *Apidologie*, **27**, 16.
- Theraulaz, G., Gautrais, J., Camazine, S. & Deneubourg, J. 2003. The formation of spatial patterns in social insects: from simple behaviours to complex structures. *Philosophical Transactions of the Royal Society A*, **361**, 1263–1282.
- Visscher, P. K. 1983. The honey bee way of death: necrophoric behaviour in *Apis mellifera* colonies. *Animal Behaviour*, **31**, 1070–1076.
- Wehner, R. 1970. Etudes sur la construction des cratères au-dessus des nids de la fourmi *Cataglyphis bicolor*. *Insectes Sociaux*, **17**, 83–94.
- Wilson, E. O., Durlach, N. I. & Roth, L. M. 1958. Chemical releaser of necrophoric behavior in ants. *Psyche: A Journal of Entomology*, **65**, 108–114.
- Wilson-Rich, N., Spivak, M., Fefferman, N. H. & Starks, P. T. 2009. Genetic, individual, and group facilitation of disease resistance in insect societies. *Annual Review of Entomology*, **54**, 405–423.
- Zhang, S., Bock, F., Si, A., Tautz, J. & Srinivasan, M. V. 2005. Visual working memory in decision making by honey bees. *Proceedings of the National Academy of Sciences, U.S.A.*, **102**, 5250–5255.