

## The role of multiple pheromones in food recruitment by ants

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### SUMMARY

**In this paper we investigate the foraging activity of an invasive ant species, the big headed ant *Pheidole megacephala*. We establish that the ants' behavior is consistent with the use of two different pheromone signals, both of which recruit nestmates. Our experiments suggest that during exploration the ants deposit a long-lasting pheromone that elicits a weak recruitment of nestmates, while when exploiting food the ants deposit a shorter lasting pheromone eliciting a much stronger recruitment. We further investigate experimentally the role of these pheromones under both static and dynamic conditions and develop a mathematical model based on the hypothesis that exploration locally enhances exploitation, while exploitation locally suppresses exploration. The model and the experiments indicate that exploratory pheromone allows the colony to more quickly mobilize foragers when food is discovered. Furthermore, the combination of two pheromones allows colonies to track changing foraging conditions more effectively than would a single pheromone. In addition to the already known causes for the ecological success of invasive ant species, our study suggests that their opportunistic strategy of rapid food discovery and ability to react to changes in the environment may have strongly contributed to their dominance over native species.**

Key words: ants, pheromone, recruitment, collective decision, dynamic environment, exploration.

### INTRODUCTION

Many group-living animals communicate about the location of food sources. Such communication is especially beneficial when food sources are ephemeral or hard to find (Sherman and Visscher, 2002; Dornhaus, 2002), or when they are too large to be exploited by a single individual (Detrain and Deneubourg, 2002). Recruitment towards food sources also provides a species with the opportunity to quickly monopolize the food source (Visscher and Seeley, 1982; Traniello, 1989; de Biseau et al., 1997; Beekman and Lew, 2008; Nieh, 1999; Nieh, 2004). The best-known examples of food recruitment are found in the social insects: ants, termites and some species of bees and wasps, which have evolved a wide range of signaling mechanisms (Beekman and Dussutour, 2009). For example, ants, termites and stingless bees mark the route between their nest and discovered food sources with a chemical (pheromone), thus indirectly leading nestmates to the food. Thus, the emitter and the receiver do not need to be present simultaneously to exchange information (Hölldobler and Wilson, 1990; Nieh, 2004; Reinhard and Kaib, 2001). Recruitment pheromones are not, however, restricted to the social insects and are found in a variety of taxa (Chapman, 1998; Wyatt, 2003) including caterpillars (Fitzgerald and Costa, 1986; Fitzgerald, 1995), social spiders (Lubin and Robinson, 1982; Vollrath, 1982; Saffre et al., 1999) and mammals (Galef and Buckley, 1996; Judd and Sherman, 1996).

Pheromone trails can enable a rapid mass recruitment to food discoveries, but they also impose constraints on the overall foraging efficiency of a species (Beekman et al., 2001). The characteristics of trail pheromones used, particularly their decay rate, play an important role in determining foraging efficiency and flexibility. Short-lived, volatile trails can rapidly modulate recruitment to ephemeral food sources, whereas long-lived trails will be more suited

to persistent, or recurrent, food sources. Thus, trail longevity must be matched to the foraging ecology of a particular species. Indeed, trail longevity varies from minutes in *Aphaenogaster albisetosus* (Hölldobler et al., 1995) to days in the leaf-cutting ant *Atta cephalotes* (Billen et al., 1992), which exploits permanent food sources. Even when foraging in their natural environments, species with a fixed pheromone decay rate experience a tradeoff between efficient recruitment and a flexible response to changes in the environment. For example, Goss and colleagues (Goss et al., 1989) provided Argentine ants (*Linepithema humile*) first with a long path between nest and food. When a shorter path was added after the ants had established a trail, in most trials the majority of ants continued to forage on the longer path. Similar results have been reported with *Lasius niger* (Beckers et al., 1992b). Mathematical models predict that the ants will remain on an established trail for periods longer than the evaporation rate of the pheromone because ants continue to reinforce the trail on the long path (Goss et al., 1989; Nicolis and Deneubourg, 1999; Sumpter and Pratt, 2003). Thus even short-lasting pheromone trails can, through positive feedback, result in ants becoming 'trapped' in suboptimal situations.

Theoretical predictions about ant foraging usually consider just a single trail pheromone (e.g. Pasteels et al., 1987; Nicolis and Deneubourg, 1999). In practice, ants use a variety of pheromones to mark the path to food discoveries (Wyatt, 2003). For example, *Myrmica sabuleti* uses pheromones from different glands depending upon the type of food it locates (Cammaerts and Cammaerts, 1980).

A combination of long-lasting and short-lived pheromones could allow ants to 'remember' routes to sites that were previously rewarding and may become rewarding again in the near future. An example of pheromone trails that contain components that have

different life times is given by the Pharaoh's ants (*Monomorium pharaonis*). Jackson and colleagues (Jackson et al., 2007) showed that these ants leave a pheromone trail that can be detected up to 2 days after it is laid. This pheromone is deposited even in the absence of food (Fourcassié and Deneubourg, 1994). By offering Pharaoh's ants a choice between a branch on which food had been recently located and a branch which ants had explored in the absence of food, Jeanson and colleagues (Jeanson et al., 2003) showed that the pheromone linked to the presence of food decays within 25 min. Jackson and Chaline (Jackson and Chaline, 2007) report that the intensity of trail laying, in terms of the degree of continuity of the markings made (when pheromone is deposited), changes only slightly between ants returning from a rewarding food source and those exploring. These ants exhibit both rapid exploitation of newly discovered food (Beekman et al., 2001; Sumpter and Beekman, 2003) and rapid abandonment of trails which no longer lead to food (Jeanson et al., 2003). A deposition of distinct chemicals during 'exploration' and 'foraging' is thus the most plausible explanation of the experimental observations (although Jackson and Chaline themselves are cautious about drawing this conclusion). 'Exploration' and 'foraging' pheromones are possibly complemented by a volatile negative pheromone that serves as a 'no entry' signal when food is not found at the extremity of a path (Robinson et al., 2005). Such a combined system seems most beneficial to opportunist species that forage at a wide range of resources.

*Pheidole megacephala* Fabricius, the focus of our current study, is, like Pharaoh's ants, an opportunistic species. Originally native to tropical Africa, it has become one of the most successful invasive ant species (Holway et al., 2002; Hoffman, 1998; Hoffman et al., 1999; Wilson, 2003), reaching a pan-tropical distribution and regarded as a major ecological pest threatening native invertebrate populations (Wilson and Taylor, 1967; Hoffman et al., 1999). We hypothesized that key to its success is its ability to rapidly adjust its foraging focus *via* the use of multiple pheromones. We investigated, first, the possibility that there are two different pheromones with different decay rates, second, how these pheromones affect recruitment in a static environment and, third, the extent to which these pheromones affect the ants' ability to adapt to changing foraging conditions.

## MATERIALS AND METHODS

### Species studied and rearing conditions

We studied the big headed ant *P. megacephala*, a dimorphic species that uses mass recruitment through pheromone trails to exploit abundant food sources. This species favors shaded and moist environments, but can exist in open areas and wherever there is anthropogenic disturbance (Hoffman et al., 1999). We collected 15 colonies which contained 2000–3000 workers and 4–6 queens in Sydney (Australia). Ants were installed in eight test tube nests (10 cm in length, 1.5 cm in diameter) covered with black paper. These tubes were placed in a rearing box (30 cm×20 cm×15 cm) with walls coated with Fluon® to prevent ants from escaping. Colonies were kept at room temperature (25±1°C) with a 12 h:12 h L:D photoperiod. We supplied each colony with water and a mixed diet of vitamin-enriched food (Bhatkar and Whitcomb, 1970) supplemented with mealworms.

We conducted a series of four experiments. The first two experiments were designed to establish the existence of two pheromones and to determine their longevity (i.e. how long the ants responded to them). The third experiment tested the role of pheromones when the ants were offered the choice between two

identical food sources placed at the end of the branches of a Y-shaped bridge. The fourth experiment tested the ants' response to a dynamic environment, where the food source changed position.

### Experiments 1 and 2: foraging trail life time

#### Experimental set-up and data collection

Our hypothesis was that ants lay a pheromone as they explore their environment (exploration pheromone) and use a different pheromone to recruit their congeners after the discovery of a food source (foraging pheromone). We also hypothesized that the exploration pheromone (E) is a long-lived signal that acts as an 'external long-term memory' of the environment allowing the colony to rapidly establish a new trail. In contrast, we expect the foraging pheromone (F) to evaporate quickly allowing the colony to abandon a depleted food source. In order to test our hypothesis we designed two different experiments. The first one quantified the decay rate of a trail comprising both exploration and foraging pheromone *vs* no pheromone at all (E+F *vs* N) while the second experiment quantified the decay rate of a trail containing exploration and foraging pheromone *vs* a trail containing only exploration pheromone (E+F *vs* E). We used eight colonies and each of these colonies was subdivided into two subcolonies each containing 1000–1500 workers with brood and queens, yielding a total of 16 colonies. Colonies were food deprived 5 days before an experiment. We decided to create subcolonies so that we could swap pheromone trails between two subcolonies while avoiding colony-specific effects on the ants' behavior (Hölldobler and Wilson, 1990).

#### Experiment 1

In the first experiment (E+F *vs* N), a colony was connected to a Y-shaped bridge which had two branches (1 and 2) of equal length (60 mm, angle between the two branches 60 deg.; Fig. 1). The whole experimental set-up was isolated from any sources of disturbance by surrounding it with white paper walls. There were two main phases during this experiment: a foraging phase and a test phase.

During the foraging phase the colony was first allowed to explore branch 1 and was then given access to the food for 1 h (3 ml of 1 mol l<sup>-1</sup> sucrose solution) placed on a platform (70 mm×70 mm) at the end of branch 1. A piece of masking plastic coated with Fluon blocked access to branch 2 to prevent the ants from depositing any pheromone on this branch during the foraging phase. Traffic flow was assessed by measuring the number of ants on the bridge in 1 min intervals for 1 h. Counting began as soon as the first ant reached the food (Fig. 1).

Before the test phase, the masking plastic and the food source were removed and the bridge was turned 90 deg. to eliminate any information from visual or other cues that might affect branch choice. This took approximately 1 min. During the test phase, which lasted 2 h, ants walking towards each branch were gently removed with a paintbrush as they crossed the decision line to prevent reinforcement of either branch. The number of ants crossing the decision line to either the marked or the unmarked branch was counted at 5 min intervals for 2 h. During this test phase ants had a binary choice between a branch with decaying trail pheromone (marked branch: E+F) and an unmarked branch. The whole experiment was repeated 16 times using the eight pairs of colonies.

#### Experiment 2

During the foraging phase of the second experiment (E+F *vs* E) we gave a colony access to a bridge that had one branch of 60 mm

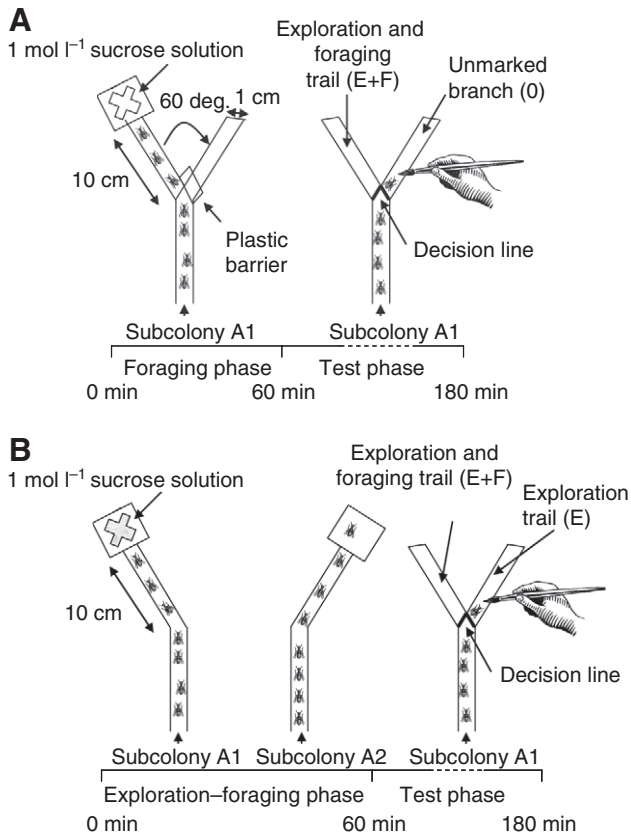


Fig. 1. (A) First experiment: top view of the bridge for the two different phases of a trial: foraging and test phase. Between the two phases the platform was rotated by 90 deg. (B) Second experiment: top view of the bridge for the two different phases of a trial: foraging and test phase. Between the two phases the platform was rotated by 90 deg.

length. The colony was starved for 5 days prior to the experiment. The first colony of a pair (see above; e.g. subcolony A1) was given access to branch 1 in the absence of food (exploration phase) and was then given access to the food for 1 h (3 ml of  $1 \text{ mol l}^{-1}$  sucrose solution) placed on a platform ( $70 \text{ mm} \times 70 \text{ mm}$ ) at the end of the branch. The second colony of the pair (e.g. subcolony A2) had access to a branch and a platform ( $70 \text{ mm} \times 70 \text{ mm}$ ) only (without food), also for 1 h. Subcolony A2 was thus only allowed to explore the bridge and the platform (E) whereas subcolony A1 explored and recruited towards a food source (E+F).

During the foraging phase we again measured the traffic on the bridge in 1 min intervals for 1 h. Counting began as soon as the first ant reached the food source. Traffic was assessed only for the colony which had access to the food source (subcolony A1).

Prior to the start of the test phase, we added the branch explored by subcolony A2 to the bridge used by subcolony A1 in order to obtain a Y-shaped bridge. The bridge was then reconnected to subcolony A1 and turned 90 deg. Thus during the test phase subcolony A1 had the choice between a branch marked during the foraging phase (E+F) and a branch marked during the exploration phase (E). Once an ant had chosen a branch and crossed the decision line it was gently removed using a paintbrush. The number of ants crossing the decision line to either branch was counted in 5 min intervals for 2 h. The whole experiment was repeated using the eight pairs of subcolonies twice yielding a total of 16 replicates.

### Statistical analyses

We used a two-way ANOVA with repeated measures (time) to test for the effects of type of pheromone and time interval on the flow of workers during the foraging and test phase.

For the test phase, we tested whether ants preferred one branch over the other (asymmetric distribution), or whether they showed no preference (symmetric distribution) using a binomial test on the number of ants choosing each branch in each replicate for each 5 min interval. The null hypothesis was that ants chose the two branches with equal probability (Siegel and Castellan, 1988). We assumed that a branch was selected when the binomial test showed a significantly higher number of foragers on one branch.

We then compared the pheromone decay rate in the two experiments. The decay rate was estimated by the slope of the regression line describing the relationship between the proportion of ants choosing the marked branch and time. Specifically, to evaluate evaporation rates of the exploration and foraging pheromone, we adapted the method of Jeanson and colleagues (Jeanson et al., 2003). We assumed that the rates of pheromone decay at time  $t$  are proportional to the pheromone quantity  $C_i$  at this time and to a constant  $\lambda$ , specific for each pheromone so that:

$$C_i(t) = C_i(0)e^{-\lambda t}. \quad (1)$$

In addition we supposed that the probability  $P_{C_i}$  that an individual chooses branch  $i$  is:

$$P_{C_i} = \frac{f(C_i)}{f(C_i) + f(C_j)} \quad (i=1,2), \quad (2)$$

where  $f(C_i)$  and  $f(C_j)$  are functions of the pheromone  $C$  dropped respectively in branch  $i$  and  $j$  (Beckers et al., 1992; Deneubourg et al., 1990). Rewriting Eqn 2 we get:

$$\frac{1}{P_{C_i}} - 1 = \frac{f(C_j)}{f(C_i)}. \quad (3)$$

Because in experiment one there is pheromone on one of the branches only, we can assume that  $C_2=0$ . Thus combining Eqns 1 and 3 we get:

$$\frac{1}{P_{C_i}(t)} - 1 = \frac{f(0)}{f(C_i(0)e^{-\lambda t}}}, \quad (4)$$

where  $P_{C_i}(t)$  is now specifically a function of time since the trial began. Solving for  $t$  gives:

$$\lambda_c t = \ln \left( f^{-1} \left( \frac{f(0)P_{C_i}(0)}{1 - P_{C_i}(0)} \right) \right) - \ln \left( f^{-1} \left( \frac{f(0)P_{C_i}(t)}{1 - P_{C_i}(t)} \right) \right). \quad (5)$$

Thus by transforming the observed probability of taking the marked branch first by taking the inverse of  $f$  and then by a log transform we can use linear regression to estimate  $\lambda_c$ . Determining the correct form of  $f$  for a particular pheromone is by no means straightforward, but is equivalent to specifying what transformation we apply to the data in order to get a linear relationship between a set of observations. We used a multiple regression analysis to test for significant effects of type of pheromone (exploration or foraging) and time on the decay rate.

### Experiment 3: role of exploration pheromone during foraging

In the third experiment, a colony again starved for 5 days was given access to a Y-shaped bridge with branches of equal length (60 mm). At the end of each branch was a platform ( $70 \text{ mm} \times 70 \text{ mm}$ ) on which food sources could be placed. This experiment consisted of three treatments. In the first treatment ('foraging': F), ants were allowed



to forage at two food sources of equal quality (3 ml of  $1 \text{ mol l}^{-1}$  sucrose solution) for 1 h. In the second treatment ('exploration': E), ants had access only to the two platforms and were allowed to explore the bridge for 1 h. In the last treatment ('exploration then foraging': E+F), ants were allowed to explore the bridge for 1 h after which a food source was placed on each platform (both containing 3 ml of  $1 \text{ mol l}^{-1}$  sucrose solution) and ants were allowed to forage for 1 h. Ants were removed from the bridge prior to placement of the food sources. We replicated each treatment 15 times using 15 different colonies of 2000 individuals. To investigate whether the presence of an exploration pheromone enables the colony to more rapidly recruit towards a food source, we measured the flow of outbound ants on each branch every minute for 1 h for the three different treatments. Counting began as soon as the first ant climbed onto the bridge.

#### Statistical analyses

We used a two-way ANOVA with repeated measures on time to test for the effects of treatment and time on the flow of workers. We then tested whether ants preferred one branch over the other or whether they showed no preference using a binomial test.

#### Experiment 4: dynamic environment

In the fourth experiment, a colony starved for 5 days was given access to a Y-shaped bridge which had two branches A and B of equal length (60 mm). The ants had access to one food source placed at the extremity of branch A for 45 min (first phase). After 45 min, the food source was removed and another food source was placed at the extremity of branch B for 45 min (second phase). After 2 h the food source was removed from the extremity of branch B and another food source was placed at the extremity of branch A for 45 min (third phase). Each food source consisted of 3 ml of a  $1 \text{ mol l}^{-1}$  sucrose solution placed in a small cavity carved in a block of paraffin wax placed on a platform (70 mm  $\times$  70 mm). We replicated the experiment 15 times using the 15 colonies. All experiments were filmed by a video camera placed over the bridge. The whole experimental set-up was isolated from any sources of disturbance by surrounding it with white paper walls. To assess traffic flow we measured the traffic on the bridge every minute for the entire experiment. We measured the flow of outbound ants at a point 1 cm from each choice point on each branch. Counting began as soon as the first ant climbed onto the bridge. We repeated this procedure for the 15 replicates.

#### Statistical analyses

To test whether ants preferred one branch over the other or whether they showed no preference for each phase we used a binomial test.

All statistical tests for experiments 1–4 were conducted with SPSS for Windows (v.10, SPSS, Chicago, IL, USA). All means in the text are given  $\pm$ s.e.m. The probabilities given in the text are always two-tailed.

## RESULTS

### Foraging trail life time

In experiments 1 and 2 the flow of ants to the food source prior to the test phase was similar (two-way ANOVA with repeated measures on time interval: experiment effect,  $F_{1,30}=0.04$ ,  $P=0.844$ , interaction experiment  $\times$  time effect,  $F_{59,30}=0.72$ ,  $P=0.692$ ). The flow increased and reached a maximum after 20 min and then stayed constant (time effect:  $F_{59,30}=57.94$ ,  $P<0.001$ ). This is typical of a trail-recruitment process (Pasteels et al., 1987). During the test phase, when ants had a choice between a branch marked during the foraging phase (E+F) and an unmarked branch, ants no longer showed a

preference for the marked branch after 85 min (binomial test:  $P<0.05$  in the 16 replicates; Fig. 2). This decreased to 30 min when the choice was between a branch marked during the foraging phase (E+F) and a branch marked during the exploration phase (E; binomial test:  $P<0.05$  in the 16 replicates; Fig. 2).

The regression analysis of the effect of experiment (1st and 2nd) and time on the proportion of ants choosing the (E+F) marked branch was significant (ANOVA for the whole model:  $F_{3,380}=266.26$ ,  $P<0.001$ ) and accounted for 67.8% of the variance. This analysis shows that time and the proportion of ants choosing the marked branch were negatively correlated in the two experiments ( $t_{380}=24.19$ ,  $P<0.001$ ), i.e. the number of ants choosing the (E+F) marked branch decreased with time. Most importantly, irrespective of time, experiment had an effect on the proportion of ants choosing the (E+F) marked branch ( $t=-19.21$ ,  $P<0.001$ ), i.e. the proportion of ants choosing the marked branch was always higher when the second branch was unmarked than when it was marked during exploration (E). Finally, the interaction term between time and experiment was significant ( $t=13.98$ ,  $P<0.001$ ), i.e. the proportion of ants choosing the (E+F) marked branch decreased quicker during the second experiment than during the first experiment.

#### Evaluation of evaporation rates

For the E+F vs N experiment we assume the following form of  $f(F_i)$  for the choice between two branches (see Eqn 2):

$$f(E_i) = 1 + E_i. \quad (6)$$

In the E+F vs E experiments we assume:

$$f(F_i) = 1 + F_i^2. \quad (7)$$

Both choices of  $f$  are made on empirical grounds. Firstly, they both give a log-linear relationship between the probability of choosing the marked branch and the time since marking stopped. Secondly, they are borne out in experiment three on the role of exploration pheromone (see below). In particular, in these experiments there is no clear preference for a particular branch in tests where no food

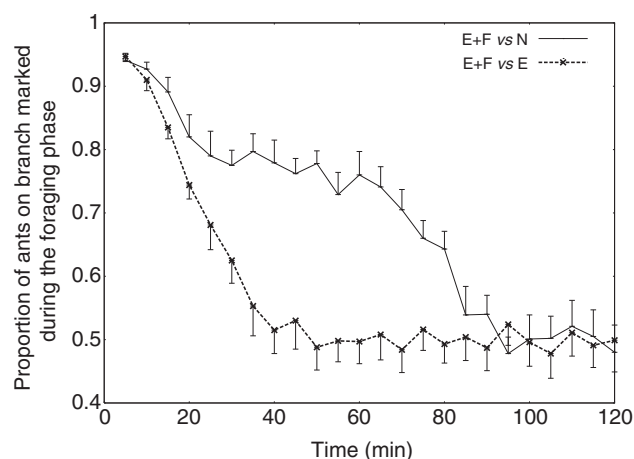


Fig. 2. Mean proportion of ants ( $\pm 95\%$  confidence interval, CI) choosing the branch marked during the foraging phase ( $N=16$  for each experiment) as a function of the time elapsed from the start of the test phase (trail no longer being reinforced). The first random choice occurred at 85 min (binomial test:  $P<0.05$  in the 16 replicates) when ants had a choice between a branch marked during the foraging phase (E+F) and an unmarked branch (N), and at 30 min when the choice was between a branch marked during the foraging phase (E+F) and a branch marked during the exploration phase (E; binomial test:  $P<0.05$  in the 16 replicates).

sources were present (E only treatment in the third experiment), while there is in tests in the presence of food (E+F and E then E+F treatments in the third experiment).

Combining Eqns 5 and 6, and 5 and 7 gives:

$$\lambda_F t = \ln \left( \sqrt{\frac{P_F(0)}{1 - P_F(0)}} - 1 \right) - \ln \left( \sqrt{\frac{P_F(t)}{1 - P_F(t)}} - 1 \right)$$

$$\lambda_E t = \ln \left( \frac{P_E(0)}{1 - P_E(0)} - 1 \right) - \ln \left( \frac{P_E(t)}{1 - P_E(t)} - 1 \right) \quad (8)$$

where  $P_F$  and  $P_E$  are respectively the proportions of individuals on one branch in experiments E+F vs E and E+F vs N.

Fig. 3 shows a fitting of Eqn 8 where the experimental proportions were computed over time until the proportions reached 0.5 (60 min for F and 90 min for E). The slopes of the two regression lines represented in Fig.3 give the decay rate of the two pheromones:  $\lambda_F=0.07$  ( $R^2=0.79$ ,  $F_{1,273}=509.20$ ,  $P<0.001$ ) and  $\lambda_E=0.04$  ( $R^2=0.65$ ,  $F_{1,121}=468.47$ ,  $P<0.001$ ). The decay was significantly higher for the foraging pheromone than for the exploration pheromone (multiple regression analysis: pheromone effect  $t_{394}=4.11$ ,  $P<0.001$ ; interaction between time and pheromone  $t_{394}=-4.55$ ,  $P<0.001$ ). Now we are able to calculate the half-life of each pheromone with the formula:

$$T_{1/2} = \frac{1}{\nu} = \frac{\ln 2}{\text{decay rate}} \quad (9)$$

where  $\nu$  is the disappearance rate of the pheromone, which results in 10.06 min and 17.46 min for the foraging and exploration pheromone, respectively.

**Role of exploration pheromone during foraging**

In experiment 3, ant flow was significantly higher during foraging than during exploration [Fig.4; two-way ANOVA with repeated measures on time interval: treatment effect,  $F_{2,42}=17.88$ ,  $P<0.001$ , mean flow per min  $34.90 \pm 2.47$ ,  $19.18 \pm 2.47$  and  $38.97 \pm 2.47$  for the first (F), the second (E) and the third treatment (E+F), respectively]. We found no significant difference in the total ant flow throughout the experiment when the ants were allowed to explore before foraging (E+F) compared with when they were not (F) (*post-hoc* test,

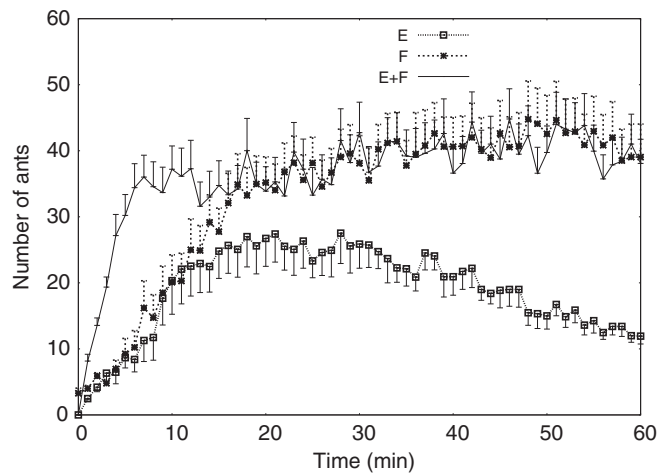


Fig. 4. Average number ( $\pm$ s.e.m.) of outbound ants crossing the two branches of the bridge every minute.  $N=15$  replicates for each treatment.

Bonferroni  $P=0.756$ ). Ant flow increased in all treatments at the beginning of the trial (ANOVA, time effect:  $F_{59,42}=37.45$ ,  $P<0.001$ ). However, when ants were allowed to explore only (E), the flow decreased after 40 min while it remained stable when ants were allowed to forage (F, E+F) (ANOVA, interaction of treatment  $\times$  time:  $F_{59,42}=8.91$ ,  $P<0.001$ ). When ants were allowed to explore prior to foraging, traffic flow increased faster during the foraging phase compared with when the ants were not allowed to explore (ANOVA, interaction of treatment  $\times$  time:  $F_{59,42}=8.91$ ,  $P<0.001$ ). This suggests that exploration accelerated the recruitment process.

Fig. 5A–C shows the proportion of ants taking the right branch during the final 10 min of each trial for the E, F and E+F treatments, respectively. In all treatments there was evidence of an asymmetrical preference for one of the branches (for E only, binomial test:  $P<0.05$  in 11 out of 15 replicates; for F, binomial test:  $P<0.05$  in 11 replicates; and for E+F, binomial test:  $P<0.05$  for all 15 replicates). The asymmetry was weak in the E treatment, with at most 60–80% of ants taking the preferred branch (Fig. 5A). In the absence of exploring prior to the foraging phase (the F treatment), in 11 replicates the majority of ants used one branch (either the right or the left branch) whereas in the other four replicates the two branches were used approximately equally (Fig. 5B). When the ants were allowed to explore before the foraging phase (E+F), most ants traveled on only one branch of the bridge (Fig. 5C). By plotting the flow per minute on each branch vs the total flow per minute we can estimate how the asymmetry changes with total flow (Fig. 5D–F). In all treatments the asymmetry increases with the total flow, with the strongest asymmetry occurring in the E+F experiments.

**Mathematical model**

To capture the trail-laying dynamics underlying the above experiments we propose a two pheromone extension of the classic Deneubourg ‘mean-field’ model of single pheromone recruitment (Goss et al., 1989; Beckers et al., 1992a; Beckers et al., 1992b; Beckers et al., 1993; Nicolis and Deneubourg, 1999; Nicolis et al., 2003). The new element is the presence of an exploration pheromone, which coexists and interacts with the foraging pheromone. We make two assumptions about the interaction between the pheromones. Firstly, the exploration pheromone has a positive feedback on the deposition of foraging pheromone. This assumption is justified by the more rapid recruitment to the food

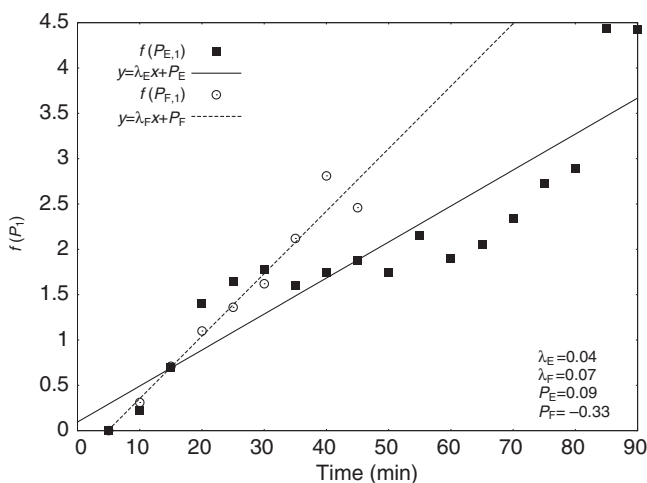


Fig. 3. Solution of Eqn 8 where the experimental proportions  $P_E, P_F$  are computed over time until the proportions reach 0.5 (45 min for F and 90 min for E).

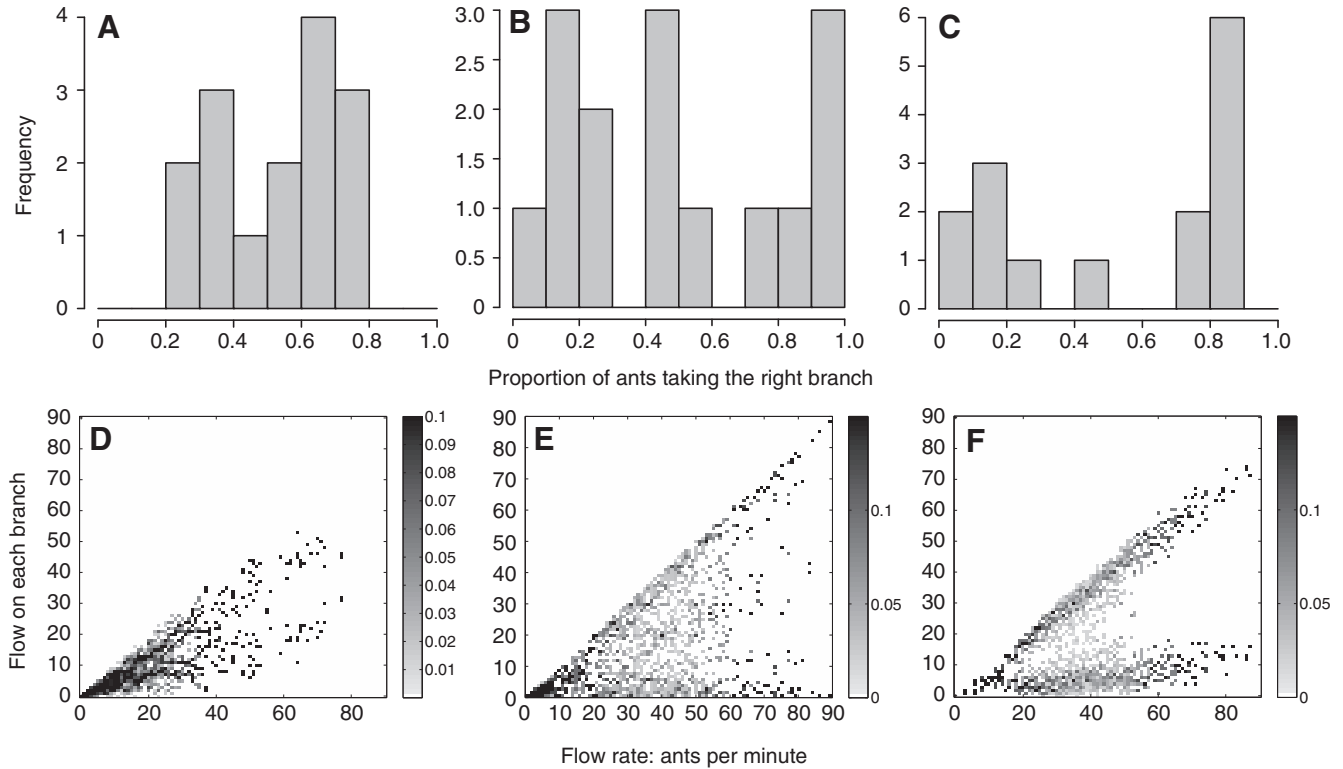


Fig. 5. Proportion of replicates with between 0 and 100% ant flow on the right branch of the bridge for each treatment 1 h after the beginning of the experiment ( $N=15$  for each treatment). (A–C) Data from the final 10 min of each trial for the E, F and E+F treatments, respectively. (D–F) The number of ants per minute for 1 min intervals on the right-hand branch as a function of the total number of ants per minute on both branches for the E, F and E+F treatments, respectively.

source in the E+F treatment in experiment 3 in comparison with the F only treatment (Fig. 4). The second assumption is that the foraging pheromone has a negative feedback on the deposition of exploration pheromone. This assumption arises simply from the supposition that the more ants there are leaving recruiting pheromone the less there are available to leave exploration pheromone.

From these assumptions we propose a model in the form of four coupled non-linear differential equations:

$$\begin{aligned} \frac{dF_1}{dt} &= \phi_F E_1 \frac{k^n + F_1^n}{2k^n + F_1^n + F_2^n} - v_F F_1 \\ \frac{dF_2}{dt} &= \phi_F E_2 \frac{k^n + F_2^n}{2k^n + F_1^n + F_2^n} - v_F F_2 \\ \frac{dE_1}{dt} &= \phi_E (1 - \gamma F_1) - v_E E_1 \\ \frac{dE_2}{dt} &= \phi_E (1 - \gamma F_2) - v_E E_2 . \end{aligned} \quad (10)$$

Here  $E_1$  and  $E_2$  are the concentrations of exploration pheromone, and  $F_1$  and  $F_2$  are the concentrations of foraging pheromone in trails 1 and 2.  $\phi_F E_i$  and  $\phi_E$  are respectively the fluxes of exploring and recruiting ants,  $v_F$  and  $v_E$  are respectively the disappearance rates of the foraging and exploration pheromones, and  $\lambda$  is a parameter measuring the strength of the negative feedback of  $F$  on  $E$ . Parameter  $k$  represents a concentration threshold from which the foraging pheromone becomes active and parameter  $n$  the strength of the choice process during recruitment. In what follows  $n$  is fixed to  $n=2$ .

It is convenient to non-dimensionalize Eqn 10. One obtains the following form (to simplify notation we use the same symbols to design the original variables and the scaled ones):

$$\begin{aligned} \frac{dF_1}{dt} &= E_1 \frac{1 + F_1^2}{2 + F_1^2 + F_2^2} - F_1 \\ \frac{dF_2}{dt} &= E_2 \frac{1 + F_2^2}{2 + F_1^2 + F_2^2} - F_2 \end{aligned} \quad (11)$$

$$\begin{aligned} \frac{dE_1}{dt} &= a(1 - cF_1) - bE_1 \\ \frac{dE_2}{dt} &= a(1 - cF_2) - bE_2 , \end{aligned}$$

where

$$\begin{aligned} a &= \frac{\phi_E \phi_F}{v_F^2 k} \\ b &= \frac{v_E}{v_F} \\ c &= k\gamma . \end{aligned} \quad \text{Definitions 1}$$

Note also that the time and the variables have been scaled in the following way:

$$\begin{aligned} t_{\text{new}} &= v_F t_{\text{old}} \\ F_{\text{new}} &= \frac{F_{\text{old}}}{k} \\ E_{\text{new}} &= \frac{\phi_F}{v_F} E_{\text{old}} . \end{aligned} \quad \text{Definitions 2}$$

The model is thus characterized by three key parameters:  $a$ , which is proportional to the total flow of ants from the nest divided by the disappearance rate of the foraging pheromone;  $b$ , the ratio of the disappearance rates of the foraging and exploration pheromones; and  $c$ , the strength of the negative feedback of foraging pheromone on the production of exploration pheromone.

Resolving Eqn 11 and testing the stability of the stationary solutions led to the bifurcation diagrams of Fig. 6A,B where the different solutions are shown as a function of the parameter  $a/b$ , for two different values of  $b$ . For  $b=1$  (the two pheromones disappear at the same rate) we find that both sources are exploited equally (stable homogeneous state) up to a critical value of  $a/b$  after which only one branch is exploited (unstable homogeneous state and two stable non-homogeneous states; Fig. 6A). The unstable homogeneous state becomes stable again at a second critical value of  $a/b$ , where two unstable non-homogeneous branches are also born and subsequently collide and annihilate with the aforementioned stable non-homogeneous solutions. We thus see that there exists a parameter region in which there is coexistence between the homogeneous and inhomogeneous states, implying that the colony selects with a certain probability homogeneous or non-homogeneous modes of exploitation. For higher values of  $a/b$  there are no more non-homogeneous solutions, and only the homogeneous stable branch remains.

We know from our experiments that the decay rate of the two pheromones is not identical but is instead equal to  $b=10.06/17.46=0.576$ . When we again plot the solutions against  $a/b$

using a more realistic value of  $b$  (Fig. 6B), we see that the homogeneous state remains unstable, i.e. even for higher values of  $a/b$  no stable solution appears that corresponds to the simultaneous exploitation of the two branches. The majority of the colony exploits one of the two sources for the parameter region where the non-homogeneous stable branches exist. This corresponds to the asymmetry we see in Fig. 5C. Fig. 6C shows the evolution in time of the full system of Eqn 11 for  $a/b=24$ . We see that when the concentration of the foraging pheromone on one branch ( $F_1$ ) is high in comparison to  $F_2$ , the exploration pheromone ( $E_1$ ) is low (and thus  $E_2$  is high). For larger values of  $a/b$  (Fig. 6D,  $a/b=40$ ) no stable solutions exist and an intriguing phenomenon appears, i.e. self-sustained oscillations where the concentration of foraging pheromone builds up first on one bridge and then switches to the other bridge. The onset of such oscillations may be discernable in some of the trials for the F treatment (e.g. Fig. 7). However, the experiments were too short to properly establish oscillations between food sources.

Although the decay rate of the exploration pheromone is only 17 min, its disappearance time is approximately 80 min. As the duration of the foraging experiments is 60 min the bifurcation diagrams (which provide predictions about steady-state pheromone concentrations) cannot fully capture the experimental outcome. We thus implemented a Monte Carlo simulation based on Eqn 11 where all the processes involved are now probabilistic (see Appendix). To compare with the experimental time scale we ran the simulation for only 1 h (60,000 time steps for a time step equal to 0.001 min) and

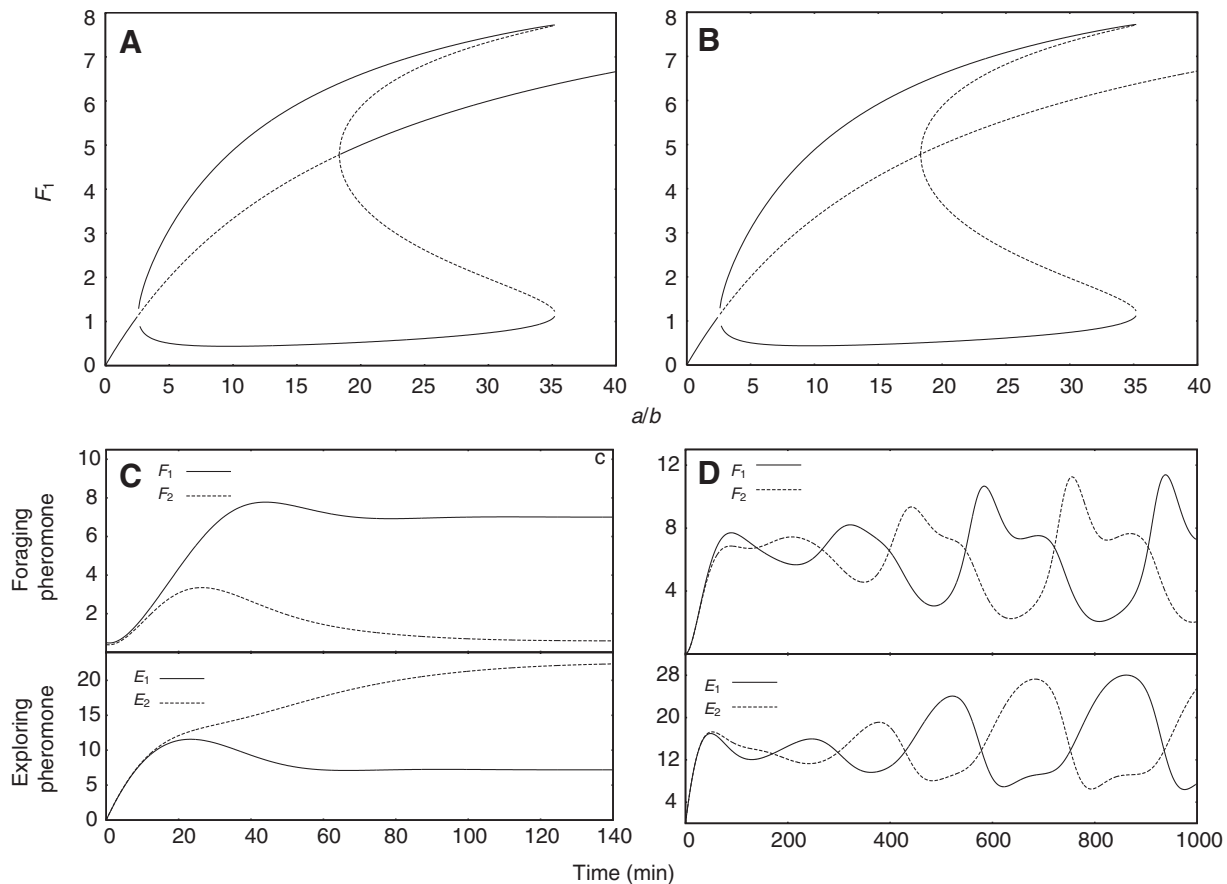


Fig. 6. (A,B) Bifurcation diagrams of the steady-state solutions of Eqn 11 for parameter value of  $b=1$  (A) and  $b=0.576$  (B). Solid lines indicate stable solutions and dashed lines unstable ones. (C,D) Numerical integrations of Eqn 11 for parameter value of  $b=0.576$  and  $a=13.824$  (C) and  $a=26.04$  (D). Parameter  $c$  is set to 0.1.  $E_1$  and  $E_2$  are the concentrations of exploration pheromone and  $F_1$  and  $F_2$  the concentrations of foraging pheromone in trails 1 and 2, respectively.

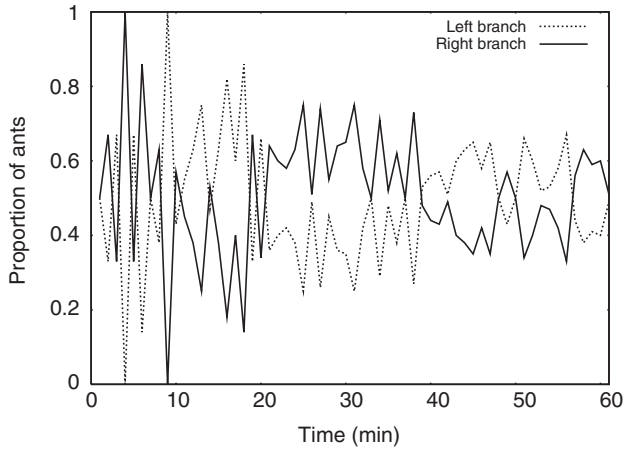


Fig. 7. Example of one experiment showing oscillating behavior.

used uniform random initial conditions between 0 and 1 for  $F_1$  and  $F_2$ . We are mainly interested in comparing simulations with initial conditions of  $E_1$  and  $E_2$  equal to zero (Fig. 8A corresponding to ‘F only’) with initial conditions equal to the stationary state of  $E_i$  when  $F_i=0$ ; that is to say,  $a/b=24$  (Fig. 8B, corresponding to ‘E then F’). In these two cases we see a good match with the experiments reflected by a quasi-uniform distribution for ‘F only’ and by a ‘U-shaped’ distribution for the case ‘E then F’. Comparison of Fig. 8A with Fig. 5B and Fig. 8B with Fig. 5C shows a qualitative similarity in the predictions of the model in each case and the outcome of the experiments. In both model and experiments, the existence of the exploration pheromone facilitated a stronger collective choice.

**Dynamic environment**

A key question is whether and to what degree two different pheromones better allow the ants to respond to changes in a dynamic environment. To investigate this first theoretically we come back to the mean-field model (Eqn 11). The idea is to test whether the presence of two pheromones, and in particular in the presence of negative feedback of foraging pheromone on the production of exploration pheromone, results in more efficient switching between paths when the position of the food is changed. We assume that at the start of the experiment there is food on branch 1, then at time  $T_1$  the food is switched to branch 2, at time  $T_2$  the food is switched back to branch 1 and, finally, at time  $T_3$  the experiment is terminated. Assuming that foraging pheromone is produced only when the source is available, the equations for the foraging pheromone become:

$$\begin{aligned}
 0 < t < T_1 & \begin{cases} \frac{dF_1}{dt} = E_1 \frac{1 + F_1^2}{2 + F_1^2 + F_2^2} - F_1 \\ \frac{dF_2}{dt} = -F_2 \end{cases} \\
 T_1 < t < T_2 & \begin{cases} \frac{dF_1}{dt} = -F_1 \\ \frac{dF_2}{dt} = E_2 \frac{1 + F_2^2}{2 + F_1^2 + F_2^2} - F_2 \end{cases} \\
 T_2 < t < T_3 & \begin{cases} \frac{dF_1}{dt} = E_1 \frac{1 + F_1^2}{2 + F_1^2 + F_2^2} - F_1 \\ \frac{dF_2}{dt} = -F_2 \end{cases}
 \end{aligned} \tag{12}$$

We start by simulating the above equations in the presence of an exploration pheromone as in Eqn 11:

$$0 < t < T_3 \quad \begin{cases} \frac{dE_1}{dt} = a(1 - cF_1) - bE_1 \\ \frac{dE_2}{dt} = a(1 - cF_2) - bE_2 \end{cases} \tag{13}$$

Fig. 9A shows the integration of Eqns 12 and 13 for  $T_1=45$  min and  $T_2=90$  min (we allow the system to reach the steady state), with  $T_3=180$  min. We see that after the second switch of the source, the branch with the food source has a greater concentration of foraging pheromone than the other branch after only 11 min. These results can be compared with a situation where there is no negative feedback on the production of exploration pheromone as a result of foraging. In this case, exploration pheromone evolves independently of the foraging pheromone:

$$0 < t < T_3 \quad \begin{cases} \frac{dE_1}{dt} = a - bE_1 \\ \frac{dE_2}{dt} = a - bE_2 \end{cases} \tag{14}$$

In this case, Fig. 9B shows that after the second switch of the source, the time until the branch with the food source has a higher concentration of foraging pheromone is 22 min. Thus the negative feedback of foraging pheromone on the production of exploration pheromone allows a more rapid response to changing foraging conditions.

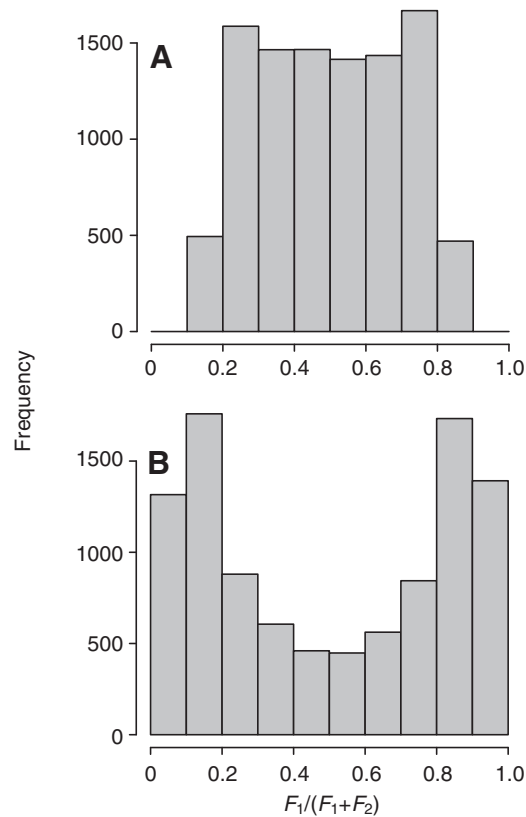


Fig. 8. Probability histograms after 60 min (corresponding to 60,000 time steps) in the case of  $b=0.576$  and  $a/b=24$ . Initial conditions of  $F_1$  and  $F_2$  are random between 0 and 1, and  $E_1$  and  $E_2$  are equal to 0 (A) and 24 (B). Other parameter values as in Fig. 6B; number of realizations is 5000.



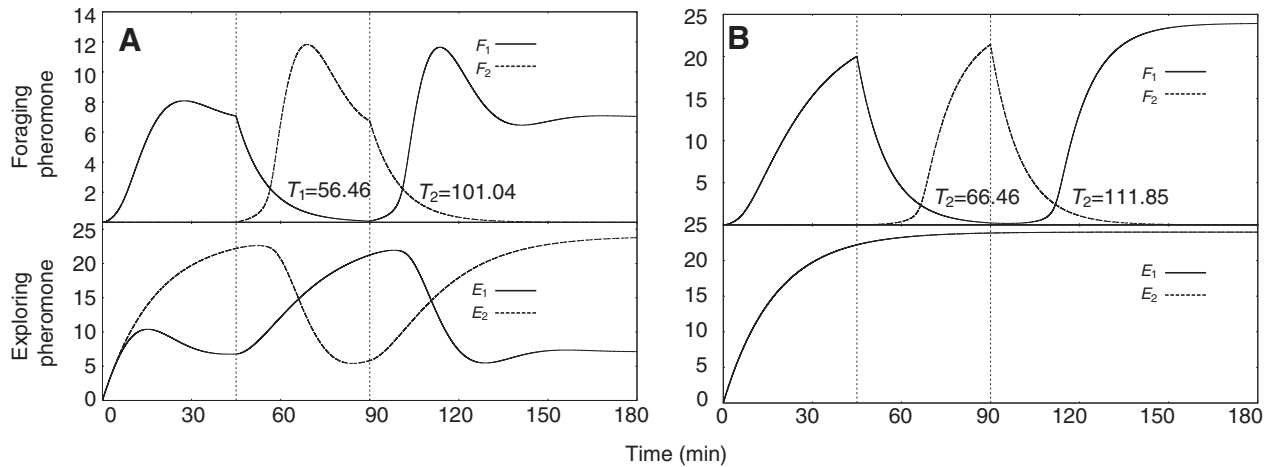


Fig. 9. Integration of Eqns 12 and 13 (A) and Eqns 12 and 14 (B).  $T_1$  and  $T_2$  are respectively equal to 60 and 120 min. Initial conditions of  $F_1$  and  $F_2$  are random between values of 0 and 1, and  $E_1$  and  $E_2$  are equal to 0. Parameter values as in Fig. 6C.

The model predictions can be understood qualitatively as follows. In the absence of coupled negative feedback of exploitation on exploration (Fig. 9B),  $E$  reaches a level close to steady state,  $a/b$  (i.e. the equilibrium solution of Eqn 14). As a result  $F_1$  increases faster during the first stage ( $0 < t < 45$  min), and at  $t = 45$  min it has reached a value higher than in the presence of feedback (Fig. 9A). During the next stage ( $45 < t < 90$  min)  $F_1$  decays in both cases but when pheromone is no longer laid on branch 1, the difference in the concentration of exploration pheromone (i.e.  $E_1 < E_2$  in Fig. 9A at  $t = 45$  min) allows the ants with coupled feedback to switch more rapidly (at  $T_1 = 56.45$ ) than those with uncoupled feedback (at  $T_1 = 66.46$ ). The situation is similar during the third stage ( $90 < t < 135$  min). Although in both the uncoupled and coupled system foraging has dropped to zero on the branch without the food, the difference in the amount of exploration pheromone on the two branches leads to a faster switch.

Experiments confirmed our theoretical predictions. Here we measured ant traffic on the two branches, rather than pheromone concentrations. However, we know that the time at which the traffic switches from one branch to another will correspond approximately to the time at which the branch on which there is a larger amount of foraging pheromone has changed. When food was present on branch A most ants traveled on this branch in all 15 replicates (binomial test:  $P < 0.05$  in all cases; Fig. 10). When the food source was moved to branch B, ants reorganized their traffic onto branch B in all replicates (binomial test:  $P < 0.05$  in these cases; Fig. 10). Finally, when the food source was moved back to branch A, in all 15 replicates ants showed a significant preference for branch A (binomial test:  $P < 0.05$  in these cases; Fig. 10). The latency to switch was longer for the first switch than for the second switch (mean  $\pm$  s.d.:  $20.8 \pm 5.9$  vs  $12.2 \pm 7.7$  min, paired  $t$ -test:  $t_{14} = 3.16$ ,  $P = 0.003$ ).

## DISCUSSION

As in a number of other ant species (for reviews, see Jackson and Ratnieks, 2006; Detrain et al., 1999; Hölldobler and Wilson, 1990) and ants of the same genus [*Pheidole pallidula* (Detrain and Cammaerts, 1991)], *P. megacephala* uses mass-chemical communication both when foraging for food and during exploration. We have provided behavioral evidence that *P. megacephala* uses two different pheromones, a long-lasting pheromone during exploration and a short-lasting foraging pheromone during recruitment to a food source. Without identifying the chemicals

involved we cannot provide categorical evidence of the use of two pheromones, but several aspects of our experiments strongly support this hypothesis. Indeed, we have been able to fit a two-pheromone model to explain all the experiments, while single pheromone models have failed for several reasons. For example, the observation that the initial frequency of ants choosing the E+F branch is the same in the E+F vs N and E+F vs E experiments but these frequencies diverge after 15 min (Fig. 2) is difficult to explain with a single pheromone. Similarly, the different nature of the bifurcation for comparable flow levels (Fig. 5D–F) indicates a difference in reaction to trails left during exploration and foraging.

The use of different pheromones has previously been found in other pest species such as Pharaoh's ants (Fourcassié and Deneubourg, 1994; Jeanson et al., 2003; Jackson et al., 2004; Jackson and Ratnieks, 1991), Argentine ants (Deneubourg et al., 1990) and fire ants (Vander Meer et al., 1981; Vander Meer et al., 1990). The presence of two pheromones in at least four different ant species begs the question why two pheromones are required. The long-lasting exploration pheromone with which ants construct a trail network throughout their foraging territory has been suggested to act as an external long-term memory for the colony (Beugnon

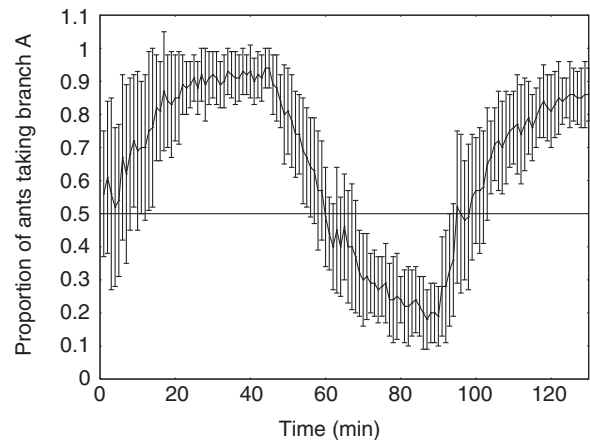


Fig. 10. Experiment in a dynamic environment ( $N = 15$  replicates). Food source on branch A is transferred to branch B and subsequently back to branch A. Means  $\pm$  s.d.

and Dejean, 1992; Traniello, 1989; Jackson et al., 2006; Jackson and Ratnieks, 2006; Billen, 2006; Witte and al., 2007; Couzin, 2009). Rather than signaling to other workers that no more exploration is required in a particular area, exploration pheromone is weakly attracting. Exploration pheromone thus provides an underlying structure for the movement of ants through their territory. This structure ensures that when food is found near to a particular point there is an already existing trail from which other workers can be quickly mobilized to the discovery. It is then the short-lasting foraging pheromone that indicates the discovery of and strongly enhances recruitment to the resource.

Our results are the first to show how the use of two pheromones allows the colony to allocate its foragers to food sources. When offered a choice between two identical food sources in the presence of exploration pheromone, the ants focused exclusively on one source in all trials (Fig. 5). In contrast, in the absence of exploration pheromone this was reduced to 11 out of 15 trials (Fig. 5). This again supports the idea of the exploration pheromone creating an underlying structure which increases the efficiency of recruitment when food is found.

The interplay of two pheromones is even more important under dynamically changing foraging conditions. When the location of the food sources changed, the colony was able to redirect its recruitment activity onto the rewarded branch. Our model explains our experimental results if we make two assumptions. Our first assumption is that exploration pheromone has a positive feedback on the deposition of foraging pheromone. This assumption is justified by the more rapid recruitment to the food source in the presence of exploration pheromone in the third experiment (E+F, Fig. 4). A similar effect has been found in other ant species [*Cataglyphis cursor* (Mayade et al., 1993); *P. pallidula* (Cammaerts and Cammaerts, 1998); *Tetramorium* spp. (Cammaerts and Cammaerts, 2000); *L. niger* (Devigne et al., 2004)]. Our second assumption is that foraging pheromone has a negative feedback on the deposition of exploration pheromone. This assumption arose simply from the supposition that when more ants leave foraging pheromone a smaller number of ants are available to deposit exploration pheromone. Alternatively, ants cease depositing exploration pheromone when exploiting a known food source.

Although flexibility could potentially be achieved through the modulation of a single chemical signal, a process based on several signals seems intuitively more reliable. The modulation of a single signal needs to be precisely tuned to allow flexible foraging behavior, something that is difficult when the signal (i.e. the trail pheromone) is deposited on substrates that differ in their adsorption properties (Detrain et al., 2001; Jeanson et al., 2003). Hence, when environmental conditions change, the use of an exploration and a foraging pheromone may minimize the time needed for discovering food sources and may maximize foraging efficiency. The coupling of two pheromones in the recruitment process with competition between recruitment trails appears to be a powerful strategy to optimize foraging success. The model showed that the existence of negative feedback of foraging pheromone on the production of exploration pheromone allows an efficient switch back to the path where the source was located previously, after the intermediate period of unavailability of this source (2nd switch in our experiment). Being flexible and able to easily shift to more rewarding food patches provides obvious benefits to the colony in terms of optimal exploitation of environmental resources.

One key prediction of our model is that in situations where there is more foraging pheromone on one of the two branches, there will be more exploration pheromone on the other. This prediction is

particularly relevant in situations where ants need to know that one path does not lead to food. Robinson and colleagues (Robinson et al., 2005) showed that ants can for example use 'a negative, repellent' pheromone to mark unrewarding branches. They performed two experiments on Pharaoh's ants, a species known to use an exploration pheromone which lasts for a number of days (Fourcassie and Deneubourg, 1992) and a 'foraging' pheromone which lasts for approximately 25 min (Jeanson et al., 2003). Similar to our experimental set-up Robinson and colleagues used a double-branch experiment, offering a colony two branches, only one of which led to food. They then transferred a section of the branch that did not lead to food to the entrance of one of two branches of a similar set-up offered to another colony. Both branches had previously led to food. On the other branch of this second colony they placed a control section with a background level of exploration pheromone as this section had been placed in the arena of the first colony. They found that the majority of ants chose the branch with the control section and avoided the branch that originated from the empty branch in the first colony, implying that ants use a negative pheromone to mark unrewarding branches. Our model suggests that big headed ants might not need to use a negative pheromone to mark an empty branch. It predicts that a high level of exploration pheromone would build up on the branch that does not lead to food in the first colony, as exploration is suppressed by foraging pheromone. When part of this branch is then offered to the second colony the ants could use the fact that the branch has a high concentration of exploration as a signal that the alternative branch is preferable. While such a mechanism also involves a form of negative feedback it does not require ants to leave specific markings on unprofitable branches in their foraging networks, and uses only the two pheromone signals. In our current model, negative feedback for a high level of exploration pheromone was not incorporated and more investigation, both theoretical and experimental, is needed to establish how multiple pheromones can be used in different contexts. However, our main point here is that a combination of two pheromones allows for a wider variety of signals than simply 'here is where has been explored' and 'here is where there is food'.

One fascinating prediction of our model is that of cycles (Fig. 6B). Here, when the ants are offered two identical branches leading to identical food sources, the foraging pheromone first builds up on one of them and the exploration pheromone on the other, then after some time this switches and the ants swap to the other branch. In 3 of the 15 trials when only foraging pheromone was present there was some evidence of weak oscillation between the branches (Fig. 7 gives one such example). Rigorous experimental testing, however, would require long-term observations of trails. Such observations are made difficult because once the ants have collected sufficient sugar the flow of ants from the nest is reduced. However, establishing the existence of cycles could prove a way of testing how pheromones interact to create foraging patterns.

The big headed ant *P. megacephala* is an invasive species that exploits ephemeral food sources (Dejean et al., 2005; Dejean et al., 2007). In addition to the already known causes of its ecological dominance in areas where it has been introduced, including its intrinsic ability to achieve unicoloniality and the absence or rarity of enemies (Holway et al., 2002; Hoffmann et al., 1999; Wilson, 2003), our study suggests that its opportunist strategy of rapid food discovery and ability to react to changes in the environment may have contributed to the dominance of *P. megacephala* when introduced into new areas. Indeed, *P. megacephala* workers are able to quickly respond to the removal of a food source and effectively redistribute their foragers when a new opportunity is detected. These

abilities are likely prerequisites to its capacity to have a high impact on native ants in areas where it has been introduced.

### APPENDIX Monte Carlo simulations

To be able to identify the main effects arising from the fluctuations (probabilistic deposition of foraging and exploration pheromone) we used Monte Carlo simulations (Gillespie, 1992). The advantage of this type of approach is that one can simulate the process of interest directly instead of solving master-type equations (Van Kampen, 1981), modeling it at a probabilistic level. In such a numerical experiment the random aspects of the process are thus automatically incorporated. We can summarize the different steps as follows.

#### Initial conditions

Exploration pheromone concentration on each branch is fixed to zero and foraging pheromone concentration on each branch is set to a random number between 0 and 1. This can be viewed as a point in a 4-dimensional lattice whose characteristic length is equal to the change undergone by the relevant variable at each simulation step.

#### Decision process and evolution over time

At each time step there are eight probabilistic processes in competition that we normalized as follows

(a) Probability of depositing an exploration pheromone:

$$P_{1,i} = \frac{a(1 - cF_i)}{\sum_{j=1}^2 a(1 - cF_j) + \sum_{j=1}^2 bE_j + \sum_{j=1}^2 E_j \frac{1 + F_j^2}{\sum_{k=1}^2 1 + F_k^2} + \sum_{j=1}^2 F_j} \quad i = 1, 2. \quad (\text{A1})$$

(b) Probability that exploration pheromone decays:

$$P_{2,i} = \frac{bE_i}{\sum_{j=1}^2 a(1 - cF_j) + \sum_{j=1}^2 bE_j + \sum_{j=1}^2 E_j \frac{1 + F_j^2}{\sum_{k=1}^2 1 + F_k^2} + \sum_{j=1}^2 F_j} \quad i = 1, 2. \quad (\text{A2})$$

(c) Probability of depositing foraging pheromone:

$$P_{3,i} = \frac{E_i \frac{1 + F_i^2}{\sum_{k=1}^2 1 + F_k^2}}{\sum_{j=1}^2 a(1 - cF_j) + \sum_{j=1}^2 bE_j + \sum_{j=1}^2 E_j \frac{1 + F_j^2}{\sum_{k=1}^2 1 + F_k^2} + \sum_{j=1}^2 F_j} \quad i = 1, 2. \quad (\text{A3})$$

(d) Probability that a foraging pheromone decays:

$$P_{4,i} = \frac{F_i}{\sum_{j=1}^2 a(1 - cF_j) + \sum_{j=1}^2 bE_j + \sum_{j=1}^2 E_j \frac{1 + F_j^2}{\sum_{k=1}^2 1 + F_k^2} + \sum_{j=1}^2 F_j} \quad i = 1, 2. \quad (\text{A4})$$

At each time step each of these probabilities is updated. The process is therefore viewed as a generalized random walk in the aforementioned 4-dimensional lattice. We ran the simulation 10,000 times and are interested in the probability histograms corresponding to the selection of a branch.

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