

RESEARCH ARTICLE

Ant foraging on complex trails: route learning and the role of trail pheromones in *Lasius niger*

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SUMMARY

Ants are central place foragers and use multiple information sources to navigate between the nest and feeding sites. Individual ants rapidly learn a route, and often prioritize memory over pheromone trails when tested on a simple trail with a single bifurcation. However, in nature, ants often forage at locations that are reached *via* more complex routes with multiple trail bifurcations. Such routes may be more difficult to learn, and thus ants would benefit from additional information. We hypothesized that trail pheromones play a more significant role in ant foraging on complex routes, either by assisting in navigation or route learning or both. We studied *Lasius niger* workers foraging on a doubly bifurcating trail with four end points. Route learning was slower and errors greater on alternating (e.g. left–right) *versus* repeating routes (e.g. left–left), with error rates of 32 and 3%, respectively. However, errors on alternating routes decreased by 30% when trail pheromone was present. Trail pheromones also aid route learning, leading to reduced errors in subsequent journeys without pheromone. If an experienced forager makes an error when returning to a food source, it reacts by increasing pheromone deposition on the return journey. In addition, high levels of trail pheromone suppress further pheromone deposition. This negative feedback mechanism may act to conserve pheromone or to regulate recruitment. Taken together, these results demonstrate further complexity and sophistication in the foraging system of ant colonies, especially in the role of trail pheromones and their relationship with learning and the use of private information (memory) in a complex environment.

Key words: social information, private information, route memory, navigation, negative feedback.

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INTRODUCTION

Central place foragers find their way back to their nest or roost by a variety of mechanisms, including keeping track of their location (path integration) (Collett and Collett, 2002; Collett et al., 2003), depositing a pheromone trail which can be retraced (Cook, 1971) or navigation (Wallraff, 2010). Many central place foragers are social and also communicate information that assists other individuals in locating the nest or a feeding site. Examples include primates (Dittus, 1984), Lepidoptera larvae (Fitzgerald and Peterson, 1983) and eusocial insects such as bees and ants (Hölldobler and Wilson, 1990; von Frisch, 1967).

For the first few visits to a food source, foraging bees and ants keep track of their location using path integration, allowing them to return directly to the nest from the position in which they find themselves (Collett and Collett, 2002). As foragers gain more experience, they begin to use information gathered during orientation flights (bees and wasps) (Lehrer, 1991; Zeil, 1993) or U-turns (ants) (Judd and Collett, 1998; Nicholson et al., 1999) to guide themselves back to the nest by matching stored images, such as of the view around their nest, with their current view. Eventually, this is superseded by a series of snapshot images acquired en route to the goal, with each image eliciting a specific behaviour that brings the individual to the next image and the beginning of the next segment (Collett and Cartwright, 1983; Judd and Collett, 1998; Graham and Collett, 2006). Such route memories can be very accurate in both bees (Menzel et al., 2011) and ants [% correct choices at a single

bifurcation: >90% in *Formica rufa* (Rosengren and Fortelius, 1986), 95% in *Formica lugubris* (Fourcassie and Beugnon, 1988), 95% in *Lasius niger* (Grüter et al., 2011) and 97% in *Lasius flavus* (Jones et al., in preparation)].

The location of resources is often communicated to nestmates, e.g. by waggle dances in honey bees *Apis mellifera* (von Frisch, 1967) or by trail pheromones in many ants and some bees and termites (Lindauer and Kerr, 1958; Nieh, 2004; Wilson, 1972). One benefit is that the information communicated can guide naive nestmates to a resource such as a feeding site. However, for experienced individuals, communication may result in a conflict between an individual's private information in the form of memory and the social information supplied by nestmates, such as trail pheromone. When a conflict arises, it seems that private information is often prioritized (Grüter et al., 2011; Harrison et al., 1989; Fourcassie and Beugnon, 1988; Grüter et al., 2008) [but see *Linepithema humile* (Beckers et al., 1993)]. Even in naive individuals without private information, social information does not eliminate errors. For example, as few as 32% of honey bees that attend a waggle dance find the advertised feeder (Mautz, 1971) and *L. niger* ants chose the branch at a T-bifurcation marked with trail pheromones only 62 or 70% of the time when it had been marked by one or 20 nestmates, respectively (Grüter et al., 2011). In the pharaoh ant, *Monomorium pharaonis*, only 70% of foragers chose the branch at a bifurcation marked by hundreds of workers (Jeanson et al., 2003) and only 9 to 65% of *Tetramorium impurum* ant foragers

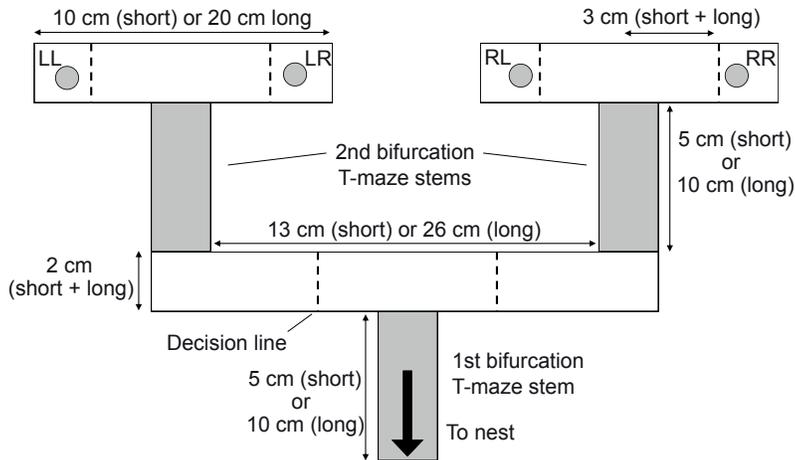


Fig. 1. Double-bifurcation maze. Pheromone depositions were recorded on the T-maze stems (shaded gray). Ants were considered to have chosen left or right at a bifurcation when they crossed the relevant decision line (dashed lines). A syrup feeder (1 mol l^{-1} sucrose) was placed at one of the four end-points of the maze. Each section of the maze was covered in paper, which could be replaced to remove any pheromone on the maze surface. In the long maze (Experiment 1), the heads and stems of the T-maze were doubled in length, but the distance of the decision line from the center of the T-maze stem, and the width of the maze, remained constant. LL: left-left, ants reaching this feeder had to turn left at both bifurcations; LR left-right; etc.

succeeded in following a 10-cm-long pheromone trail (Verhaeghe, 1982).

Apart from providing naive worker insects with information on the location of a food source, what other roles does social information have in foraging? The waggle dance can reactivate foraging in experienced honey bee foragers (von Frisch, 1967), resulting in revisiting of a location that had ceased being rewarding (Grüter and Ratnieks, 2011). Trail pheromones allow experienced ant foragers to reach a food source more rapidly because foragers are able to walk faster and straighter (Czaczkes et al., 2011). It has also been suggested that pheromone trails may assist ants in acquiring route memories (Collett and Collett, 2002). Similarly, pheromone trails might be of importance to experienced ants if the route to the food source is hard to learn. Whilst route learning in ants has often been reported to be both rapid and accurate (Fourcassie and Beugnon, 1988; Grüter et al., 2011; Rosengren and Fortelius, 1986), most studies were conducted on simple trails with a single bifurcation. Trails with multiple choice points may provide a greater challenge, as more information must be stored, and route learning at one bifurcation may interfere with route learning at another.

This study investigated foraging by *L. niger* ants in a doubly bifurcating maze leading from the nest to four end-points, one of which had a food source. We predicted that this more difficult and realistic challenge would lead to greater errors than a route with a single bifurcation. In addition, this design allowed us to investigate the possibility that routes requiring alternating choices (e.g. left at the first bifurcation and right at the second) would be more difficult to learn than routes requiring repeating choices (e.g. left at both bifurcations), as well as whether the presence of trail pheromones affected learning and errors. We find that ants do indeed make more errors on alternating routes, but that trail pheromones can assist experienced forager ants to relocate a feeder, both by decreasing errors at bifurcations and by facilitating route memory formation.

MATERIALS AND METHODS

Study species

We studied eight *Lasius niger* (Linnaeus 1758) colonies collected on the University of Sussex campus. Colonies were housed in plastic foraging boxes ($40 \times 30 \times 20 \text{ cm}$). The bottom of each box was covered with a layer of Plaster of Paris. Each foraging box contained a circular plaster nest box (14 cm diameter, 2 cm high). The colonies were queenless with 500–1500 workers and small amounts of brood. Queenless colonies forage, make pheromone trails and care for brood, and are frequently used in foraging experiments (Devigne

and Detrain, 2002; Evison et al., 2008). Colonies were fed three times per week with Bhaktar diet, a mixture of egg, agar, honey and vitamins (Bhatkar and Whitcomb, 1970), supplemented once per week with dead mealworms (*Tenebrio molitor*). Colonies were deprived of food for 4 days prior to a trial in order to achieve uniform and high motivation for foraging. Water was provided *ad libitum*.

Experiment 1 – comparing repeating routes versus alternating routes, and effect of route length

We investigated route learning and pheromone deposition behaviour using an experimental maze (Fig. 1) that bifurcated twice on the way from the nest to give four end-points. To start an experimental trial, the maze was connected to the colony's foraging box using a paper bridge. Once four ants had found the feeder, the remaining ants were removed from the maze and further ants were prevented from entering. Each feeding ant was individually marked with an acrylic paint dot on her abdomen, and allowed to make seven more trips to and from the feeder. For each ant, we recorded the decision it took on every outward trip at each bifurcation, and the number of pheromone-depositing behaviours it made on each stem section of the maze (see Fig. 1). Decision lines 3 cm to the left and right of each bifurcation were used to define the choices, left or right, made by each individual foraging ant when walking away from the nest. Choice accuracy was scored independently for both bifurcations: if an ant should have taken a left-right route to reach the feeder, but instead took a right-right route, it would be scored as having made an incorrect decision at the first bifurcation and a correct decision at the second bifurcation. The maze was covered by pieces of white printer paper. A drop of 1 mol l^{-1} sucrose solution was placed at one end-point. To reach this feeder, an ant had to make a choice at each trail bifurcation, either left-left or right-right for a repeating route or left-right or right-left for an alternating route.

Pheromone deposition is a characteristic and easily observed behaviour in *L. niger* in which the ant makes a brief, $\sim 0.2 \text{ s}$ pause to touch the tip of the abdomen to the substrate. Every time an ant deposited pheromone, the paper overlay covering that maze section was replaced by a fresh piece, thereby removing pheromone information. Thus, ants had to rely solely on their own route memory. Each ant was tested at only one feeder location, but different individuals from each test colony were tested at all four locations. Tested ants were removed from their colony after being studied in order to prevent the same ant being studied twice.

The entire experiment was repeated using a maze of the same overall shape but with all length dimensions doubled to explore the effect of route length on route learning and pheromone deposition

behavior. The decision lines remained at the same distance (3 cm) from the center of each bifurcation. Longer routes may provide foragers with more opportunity to learn the route, as the image of their surroundings is stable for longer between turns. Similarly, longer routes would give foragers more time between the first and second bifurcation to notice any errors they have made on the first bifurcation, and to correct that error before reaching the second bifurcation. Furthermore, on a larger maze the visual difference between different end-points of the maze will be greater, and consequently errors should be easier for the ants to detect. In addition, on longer mazes errors would be more costly in terms of time and energy wasted so that ants may thus invest more in error checking.

Experiment 2 – effects of trail pheromone

This experiment was carried out to test the effect of trail pheromone on route learning and pheromone deposition. It used the same short-length double T-maze as in Experiment 1. Ants were tested only on alternating routes (right–left or left–right) because this combination proved the most difficult to learn, thus giving a better opportunity to detect any effect of trail pheromones on reducing errors. In half the trials the trail pheromone was removed as above, and in the other half the pheromone was allowed to accumulate. To control for any disruption due to changing the paper overlays in the pheromone removal treatment, we sham removed (removed and replaced) overlays in the trials in which pheromone was allowed to build up. At the end of a trial, an additional 12 naive ants were allowed onto the maze and their decisions were recorded to determine the effectiveness of pheromone without route memory in locating the food source. Each colony was tested twice with pheromone removal (feeder positions right–left and left–right), and twice with pheromone accumulation. When comparing naive and experienced ants, only the last visit of each experienced ant was used, to ensure that both groups experienced a similar high level of pheromone.

Experiment 3 – trail pheromone as an aid to route learning

We investigated whether pheromone trails improve the formation of a route memory on a difficult-to-learn alternating route. The experimental protocol had one change from Experiment 2: trail pheromone was removed after each visit, allowed to accumulate (as in Experiment 2) or allowed to accumulate for the first six visits but removed on the final return to the feeder. Thus, ants in the final treatment had the benefit of an accumulating pheromone trail on visits 1–6, but no pheromone on visit 7, their final visit. The behaviour of these ants ('visit 7 memory test') could be compared with: (1) their own behaviour on visit 6, in which pheromone was present ('visit 6 memory test'), (2) the behaviour of naive ants on a pheromone trail ('naive'), (3) the behaviour of experienced ants on their seventh visit, where pheromone trails were allowed to build up over all visits ('always pheromone visit 7'), or (4) the behaviour of ants on the seventh visit that had never had a pheromone trail, and thus had to rely on memory alone ('never pheromone visit 7'). The final outward journey from the start of the maze to an end-point was timed.

Statistical analysis

Data were analyzed using generalized linear mixed-effect models (GLMMs) (Bolker et al., 2009) using R version 2.9.2 (R Development Core Team, 2009). Models were fitted using the lmer function (Bates et al., 2007). Model selection followed Zuur et al. (Zuur et al., 2009): a model with all pertinent variables and all

interactions was constructed. Random effect structure was explored by comparing different structures using Akaike's information criterion. Random effects included colony, trial and individual ant. By including these as appropriate we controlled for non-independence of data points. Non-significant terms were then removed from the model, beginning with the least significant, until an 'ideal model' – containing only significant terms – was reached. Decision data were analyzed using a binomial distribution, and data on pheromone deposition behaviour were analyzed using a Poisson distribution, after visual verification of the distribution structure. Interactions were explored by subsetting. For example, if we found an interaction between treatment and visit number, the data would be split into the various treatments, and the effect of visit number would be analyzed separately in each subset. All *P*-values presented are corrected for multiple testing using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995).

RESULTS

Trail choice accuracy

Experiment 1

We provided a feeder at the end of a repeating (left–left or right–right) or alternating route (left–right or right–left). The maze was either short or long (doubled lengths). The model included the terms 'maze length' (short or long), 'bifurcation' (first or second), 'route type' (alternating or repeating routes) and 'visit number' (1–7) as explanatory variables. We found an interaction between bifurcation and route type (GLMM, $Z=-2.802$, $P=0.00817$; Fig. 2): on alternating route, the error probability was greater at the first bifurcation ($Z=10.658$, $P<0.00001$), whilst on repeating routes there was no difference in the error probability between the two bifurcations ($Z=0.981$, $P=0.327$). There was also a significant interaction between route type and visit number ($Z=5.542$, $P<0.00001$). On repeating routes, ants made fewer errors in later visits ($Z=6.233$, $P<0.00001$). On alternating routes, ants also made fewer errors on later visits ($Z=4.595$, $P<0.00001$), but the effect was much weaker than on repeating routes (see Fig. 2). Ants also made more errors on short mazes *versus* long mazes ($Z=-2.808$, $P=0.00817$). Over the whole data set, ants made 97% correct choices per bifurcation on repeating routes *versus* 68% on alternating routes, 76% correct choices at the first bifurcation *versus* 89% at the second, and 81% correct choices on the short maze *versus* 84% on the long maze.

Experiment 2

Trail pheromone was either removed after deposition, as in Experiment 1, or allowed to remain on the trail. Only the short maze was used, and the feeders were always at the end of alternating routes (left–right or right–left). The statistical model included the terms 'bifurcation', 'treatment' (pheromone present or removed) and 'visit number' as explanatory variables. Fig. 3A shows that more errors were made when pheromone was removed (56% *versus* 73% correct choices averaged over all visits, $Z=6.756$, $P<0.00001$). We also found an interaction between bifurcation and visit number ($Z=3.044$, $P=0.00291$): whilst errors were reduced in later trips, the reduction was less on the first *versus* second bifurcation (55% correct choices on first bifurcation *versus* 74% correct choices on second bifurcation, averaged over all visits; see Fig. 3A).

When we compared the error rates of ants in both treatments on their last visit with the error rates of naive ants, we found an interaction between treatment and bifurcation ($Z=-2.871$, $P=0.00614$). On the first bifurcation, experienced ants with no pheromone information were less accurate (46% correct choices)

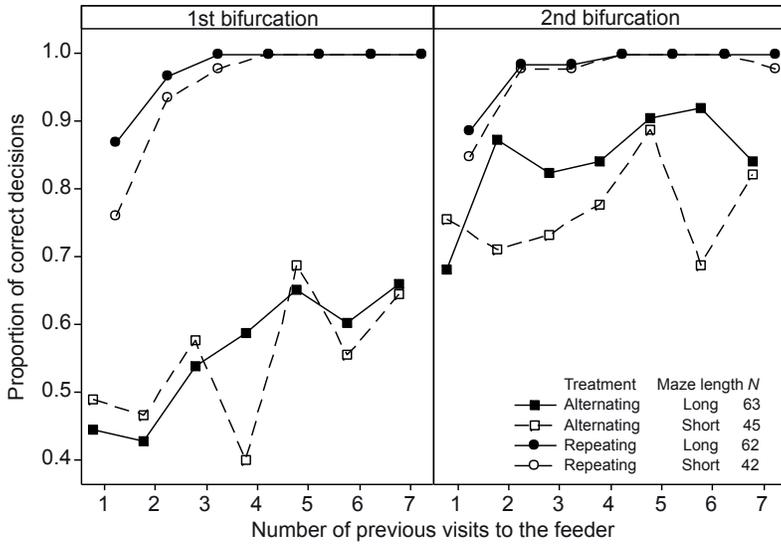


Fig. 2. Experiment 1. Route choice accuracy on a doubly bifurcating maze. Proportions of ants choosing the correct branch at each of the two bifurcations, over seven consecutive re-visits to the food source. The initial, 'naive' visit (visit 0) is not shown. The food source was either at the end of a repeating route (left–left or right–right; circles) or an alternating route (left–right or right–left; squares). The maze may be short (open shapes, dashed line) or long (double length; closed shapes, unbroken line). Ants made more errors on alternating *versus* repeating mazes, at the first *versus* the second bifurcation on alternating but not repeating mazes, and on short *versus* long mazes.

than both experienced ants with pheromone information (84% correct choices, $Z=-2.871$, $P=0.00614$) and naive ants with pheromone information (71% correct choices, $Z=-4.814$, $P<0.00001$; Fig. 3B). Naive ants did not make significantly more errors than experienced ants with trail pheromones ($Z=1.847$, $P=0.0648$; Fig. 3B). On the second bifurcation, however, experienced ants with no pheromone information and naive ants with pheromones were equally accurate (78% *versus* 75% correct choices, $Z=0.315$, $P=0.753$), but ants with experience and pheromone information made fewer errors than the other two groups (97% correct choices, *versus* experienced ants with no pheromone information, $Z=-2.780$, $P=0.00543$; *versus* naive ants with pheromones, $Z=-3.004$, $P=0.00399$; Fig. 3B). Thus, on the first bifurcation pheromone seems more helpful than route memory, and on the second bifurcation trail pheromones and route memory have a synergistic effect on trail choice accuracy.

Experiment 3

To ascertain whether the presence of pheromone assists in route learning, we allowed pheromones to accumulate on the trail and then removed them (1) after each visit, (2) only on the ant's final visit or (3) never. The model included the terms 'bifurcation' and 'treatment' as explanatory variables. Treatment has five levels: experience + pheromone (visit 6), experience, no pheromone (visit 7), only pheromone (naive), pheromone always removed, and experience + pheromone (visit 7). We found significant interactions between some of the treatment comparisons and bifurcation, and in other treatment comparisons we found no effect of bifurcation. A summary of all treatment comparisons at both the first and second bifurcations is given in the Appendix (see Tables A1, A2), and summarized in Fig. 4. Our key findings are, first, that ants that have had access to trail pheromone for the first six visits and then had the pheromone removed on the seventh visit made fewer errors on

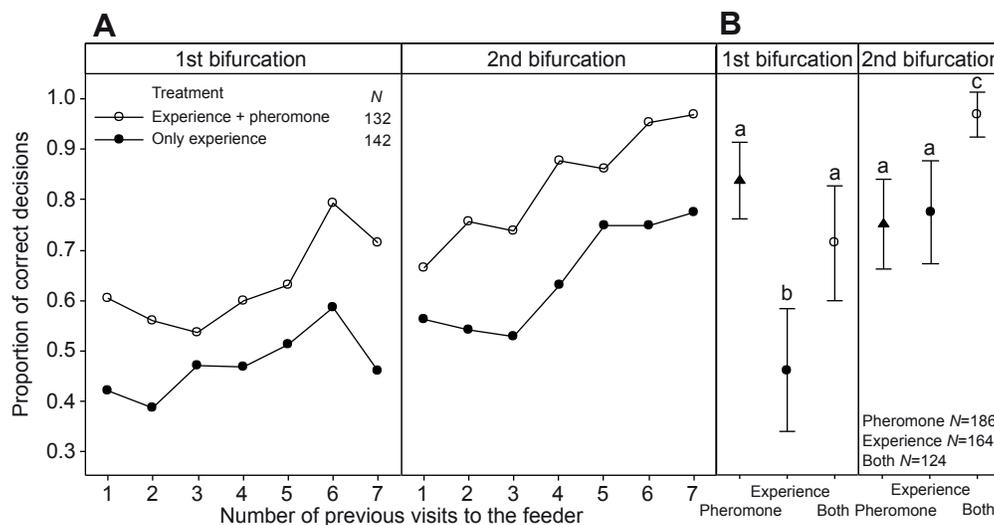


Fig. 3. Experiment 2. Route choice accuracy with or without trail pheromone. (A) Proportion of ants choosing the correct branch at each of two bifurcations, over seven re-visits to the food source and (B) on the final visit. (A) Pheromone is either removed from the trail after each visit (filled circles) or allowed to build up (open circles). (B) Triangles represent naive ants with trail pheromone information. Data in B are means \pm 95% confidence intervals (CI); different letters are significantly different at 95%. At the second bifurcation, naive ants and experienced ants without access to trail pheromone information are equally accurate, whilst experienced ants with access to trail pheromone information are more accurate. Only the alternating direction treatment was used in this experiment.

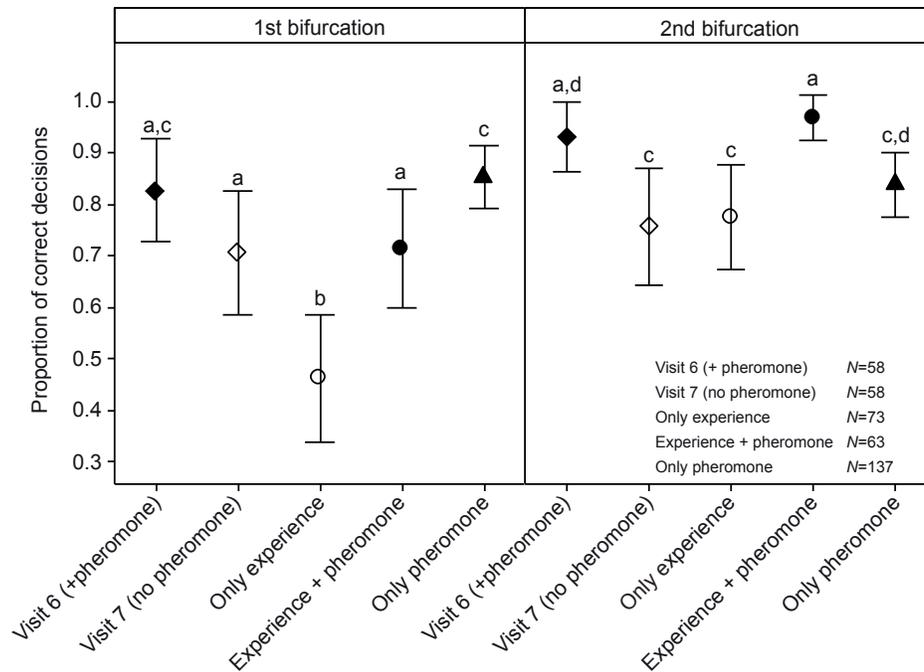


Fig. 4. Does the presence of trail pheromone assist in route learning? Proportions of correct choices at the first and second bifurcations for: (1) ants on their sixth visit, trail pheromone present (closed diamond), (2) the same ants on the seventh visit, trail pheromone absent (open diamond), (3) ants on their seventh visit, trail pheromone absent throughout all visits (open circles), (4) ants on their seventh visit where trail pheromones were not removed (closed circles) and (5) naive ants that are walking on a maze where trail pheromone was not removed (closed triangles). Data are means \pm 95% CI. At the first bifurcation, where most errors occur, fewer errors are made by ants in category 2 than ants in category 3, indicating that trail pheromone can assist in route learning, and that the effects remain even once pheromone has been removed.

both bifurcations than ants on the seventh visit that never had access to trail pheromone information (73% *versus* 62% correct choices $Z=2.322$, $P=0.0326$). Second, ants that have had access to trail pheromone for the first six visits and then had the pheromone removed on their last visit were less accurate on their last than on their penultimate visit on the second bifurcation (93% *versus* 75% correct choices, $Z=2.476$, $P=0.0275$), but not on the first bifurcation (83% *versus* 71% correct choices, $Z=1.563$, $P=0.148$). These ants also made more errors than ants that always had access to trail pheromones on the second bifurcation (76% *versus* 97% correct choices, $Z=2.937$, $P=0.00674$), but error rates were equal on the first bifurcation (71% *versus* 71% correct choices, $Z=0.052$, $P=0.9586$; interaction term $Z=2.584$, $P=0.01956$). Thus, trail pheromone aids both the learning of a complex route and also the use of a complex route by an experienced ant revisiting a location for the seventh time.

We also found that experience and trail pheromone act synergistically, allowing ants to reach the feeder faster. The time taken for experienced ants on a trail without pheromone and naive ants on a trail with pheromone to reach the end of the maze was not significantly different (mean \pm s.d. = 18.3 ± 2.2 s *versus* 17.8 ± 1 s, $Z=0.211$, $P=0.833$), but experienced ants with trail pheromone are ~34% faster (13.7 ± 1.5 s) than both experienced ants without trail pheromone ($Z=5.804$, $P<0.00001$) and naive ants with trail pheromone ($Z=2.047$, $P=0.0407$).

Trail pheromone laying behaviour

Experiment 1

The model included the terms 'bifurcation', 'route type' (alternating or repeating), 'travel direction', 'correct last visit?' and 'maze length' as explanatory variables. Travel direction is a very important determinant of pheromone-deposition behaviour, and it interacts with many other factors. We found an interaction between travel direction and treatment ($Z=4.198$, $P=0.000153$): ants deposited more pheromone on an alternating *versus* repeating route when returning to the nest ($Z=5.311$, $P<0.00001$), but there was no difference between route types when going to the feeder ($Z=2.109$, $P=0.134$; Fig. 5A). Thus, ants deposit more pheromone on their homeward

journey when faced with a route that is difficult to learn (2.1 ± 0.04 *versus* 1.5 ± 0.03 depositions per 5 cm for alternating and repeating routes, respectively, averaged over all visits, maze lengths and travel directions).

There was also an interaction between travel direction and maze length ($Z=8.442$, $P<0.00001$): ants deposited more pheromone on shorter mazes when returning to the nest ($Z=6.982$, $P<0.00001$), but there was no difference when going to the feeder ($Z=0.137$, $P=0.9726$).

There was a three-way interaction between visit number, bifurcation and direction ($Z=3.91$, $P=0.000305$). When returning to the nest, ants deposited less pheromone on the second bifurcation in later visits ($Z=4.836$, $P<0.00001$), but there was no change over visit number on the first bifurcation ($Z=1.125$, $P=0.266$). When going towards the feeder, ants deposited more pheromone in later visits on the second bifurcation ($Z=2.618$, $P=0.0401$), but there was no change over visit number on the first bifurcation ($Z=0.814$, $P=0.519$).

Lastly, there was a three-way interaction between bifurcation, direction and whether the ants made an error on their previous visit to the feeder ($Z=3.133$, $P=0.004170$). When returning to the nest, ants that made an error on the outwards journey of their current visit deposited more pheromone on both the first and second bifurcation, although the effect was strongest on the first bifurcation, where more errors were made (first bifurcation, $Z=12.388$, $P<0.00001$; second bifurcation, $Z=9.432$, $P<0.00001$; 2.2 ± 0.04 *versus* 3.0 ± 0.1 depositions per 5 cm, averaged over both bifurcations; see Fig. 5B). However, when going towards the feeder, ants that made an error on their previous visit did not change their pheromone-deposition behaviour on the first bifurcation ($Z=1.719$, $P=0.1713$), and deposited slightly more pheromone on the second bifurcation ($Z=2.188$, $P=0.0344$; 1.5 ± 0.08 *versus* 1.5 ± 0.03 depositions per 5 cm, averaged over both bifurcations). Thus, it seems that ants monitor how successfully they navigate a route, and if they make an error they lay more pheromone (i.e. provide more information for nestmates or their own subsequent journey) on their return journey (Fig. 5B).

Experiment 2

In this experiment, pheromone deposited on the maze was either removed or allowed to build up. The short maze and alternating

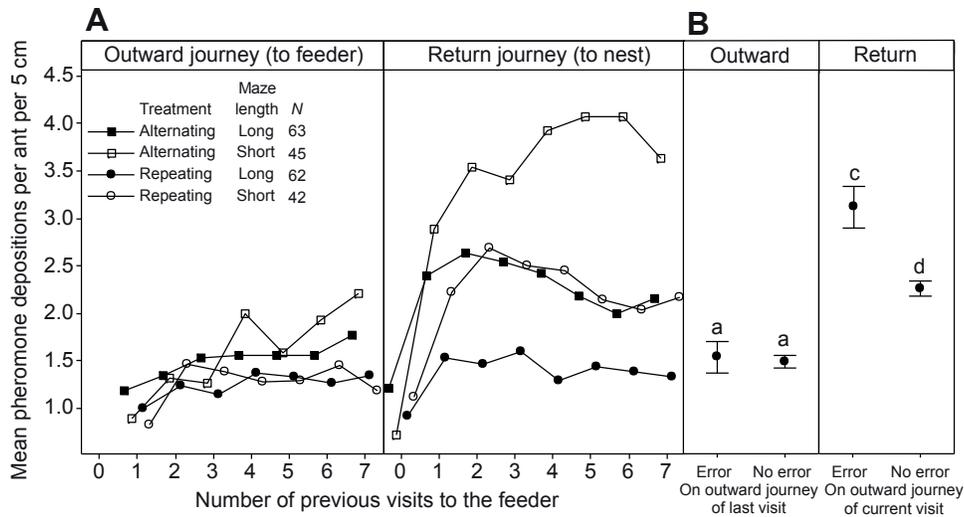


Fig. 5. Effects of alternating *versus* repeating direction, maze length and ant visit number on pheromone deposition. (A) Significantly more pheromone is deposited on alternating routes, in shorter mazes and on the return journey. (B) Ants that made a mistake in their outward journey to the feeder deposit more pheromone when returning from the feeder once they eventually find it. Data are means \pm 95% CI. Different letters signify a statistically significant difference at 95%. Pheromone laying behaviour rates were divided by two on the long maze to allow comparison with the data from the short maze. Error bars for the means in A have been omitted for clarity.

direction treatment were used. The model included the terms ‘bifurcation’, ‘treatment’ (pheromone present or removed), ‘travel direction’ and ‘visit number’ as explanatory variables. We found a significant three-way interaction between travel direction, visit number and treatment ($Z=-4.067$, $P=0.0001$): when pheromone was removed from the maze, ants returning to the nest deposited more pheromone in later visits on both bifurcations ($Z=2.346$, $P=0.0190$; Fig. 6B), with a non-significant trend for more pheromone to be deposited on the second bifurcation ($Z=1.863$, $P=0.0625$). When pheromone was allowed to build up, ants increased pheromone deposition on the first visit (when pheromone had not yet been deposited) and decreased pheromone deposition in later visits ($Z=-5.539$, $P<0.00001$; Fig. 6B). This pattern held true for both bifurcations, although pheromone deposition was higher on the second bifurcation ($Z=2.955$, $P=0.00469$). Ants walking towards the food source did not change their deposition rates in later visits (pheromone allowed, $Z=-0.365$, $P=0.955$; pheromone removed, $Z=0.649$, $P=0.774$; Fig. 6A) on either bifurcation (pheromone allowed, $Z=0.056$, $P=0.955$; pheromone removed, $Z=-0.1$, $P=0.92$).

DISCUSSION

Route choice

Our results show clearly that a more complex trail with two sets of bifurcations is more difficult for ants to learn than the single bifurcation trail that has been most studied by researchers. *Lasius niger* foragers make over 95% correct choices after three visits to a feeder *via* a single bifurcation (Grüter et al., 2011), compared with foragers after three visits to a feeder reached *via* an alternating route (left–right or right–left), which make 79% correct decisions at the second bifurcation and only 56% correct decisions at the first. Foragers made more errors on alternating routes than on repeating routes (left–left or right–right), but error rates on alternating trails could be reduced by 30% by the provision of trail pheromones. Trail pheromones not only helped guide ants, but also facilitated the formation of route memories.

Ants making return journeys to the feeder were most likely navigating by attempting to match learned landmarks or their visual

panorama to their current position (Collett, 2009). During the first visits, the ants may have had a memory restricted to the view from around the food source, and as route memory developed, intermediate views along the path to the food source were likely learned. As *L. niger* cannot learn routes in the dark (Jones et al., in preparation), we are confident that ideothetic cues were not being learned in this case, although other ants can learn such cues (Macquart et al., 2008).

It is unclear why more errors were made on alternating routes. One possibility is that the memory of the right direction to turn at one bifurcation interferes with the (different) decision at the other bifurcation. The experiment was conducted in heterogeneous laboratory space with many large objects that could serve as landmarks for the ants. The view to the left on the maze was very different to the view to the right, providing ample information for landmark learning. To return to a goal using view-based navigation, an ant attempts to match its current image of the landscape with images acquired close to the goal (Collett and Cartwright, 1983; Graham and Cheng, 2009; Judd and Collett, 1998; Wehner and Rüber, 1979; Wehner et al., 1996). On repeating routes, attempting to match the visual panorama acquired at the goal with their current view will lead ants in the correct direction, as on both bifurcations the ants must match their view with the view seen when arriving at the feeder to reach the feeder. However, on alternating routes, ants attempting to match their view with the view seen when approaching the feeder will take the wrong turn at the first bifurcation, as to make a correct decision at the first bifurcation they must head away from the feeder location. Short-term memories from one learning event can be very unstable and prone to interference from contradictory information arriving too soon after the original learning event (Menzel, 1979). Scouting ants also tend to take repeating turns so as to perform an outline-tracing search pattern (Jander, 1990), in which ants follow the edge of an area so as to systematically explore the entire circumference of the area. This may also apply to ants making foraging trips to a known food source. The reason for higher accuracy on the second bifurcation is also unclear. It may be linked to the panorama-matching

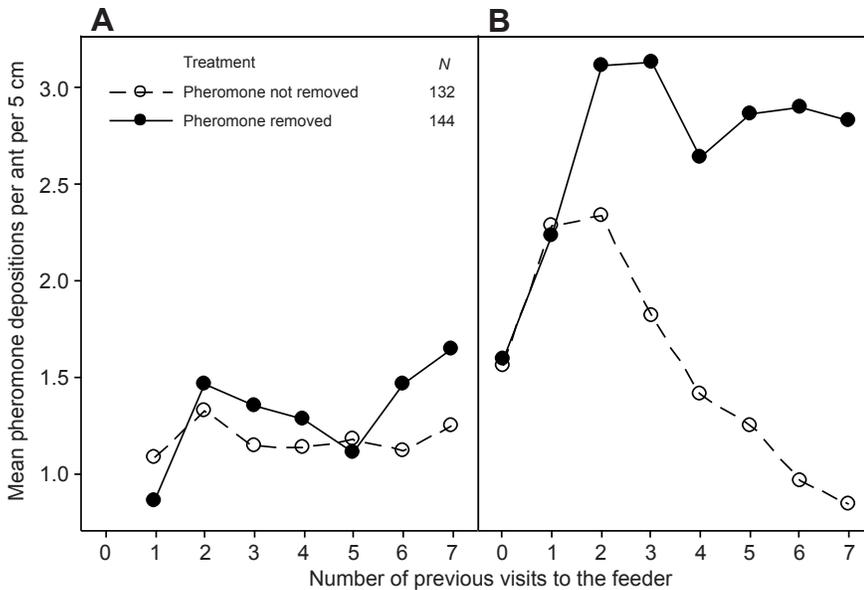


Fig. 6. Effect of trail pheromone presence on trail pheromone deposition. (A) Outward journey from nest to feeder. (B) Return journey from feeder to nest. The presence of pheromone on the trail greatly reduces pheromone deposition rates on the return journey but has little effect on the outward journey. Error bars for the means have been omitted for clarity.

hypothesis mentioned above, or to possible pheromones emitted by feeding nestmates, or to visual orientation to feeding nestmates. Also unclear is why accuracy is greater on long routes. Longer routes may provide more time and opportunities to learn intermediate route images, facilitating learning. Visual differences between maze end-points will be greater on longer routes, also facilitating learning and error-checking. Lastly, errors are more costly on longer routes, so ants may invest more in error-checking on these routes. These possibilities are not mutually exclusive, and these questions remain open for future studies.

As predicted, the results show that pheromone trails are of value even to experienced foragers. This is in contrast to the results obtained using a trail with a single bifurcation, which by definition is not alternating, where pheromones did not seem to be used by experienced ants (Grüter et al., 2011). Trail pheromone and route memory information are used additively, not redundantly, with pheromone trails increasing accuracy in experienced foragers beyond that achievable with memory alone (Fig. 3). In Experiment 2 when considering just the last visit, path choice accuracy of ants guided by memory alone is in fact lower than that of naive ants guided by pheromones alone. This is likely due to the memory of the correct turn at the second bifurcation interfering with the decision at the first bifurcation. However, some of this pattern could be attributed to the experienced ants depositing pheromone on their last visit to the feeder, thus making the pheromone trail the naive ants experience slightly stronger. This slight increase in trail strength could also have affected the results of the naive ants in Experiment 3 to some extent. The order in which ants visited the feeder would also affect to some degree the amount of pheromone experienced on the trail, as later ants would have access to the newly deposited pheromone from previous ants.

The results also show that in addition to helping in navigation, the presence of trail pheromone also improves learning. These two effects are somewhat separate, and demonstrated by the results of different experiments. In Experiment 2 we can see that the benefit of pheromone trails in navigation can be immediate: in the presence of trail pheromones, ants on alternating mazes make significantly more accurate choices even on their first return visit to the feeder (Fig. 3A). The effect of trail pheromones on improving route memory formation can be seen in Experiment 3. On the first

bifurcation, where most errors occurred, ants that walked on mazes with trail pheromone for six visits, but then walked on an unmarked route on the seventh, made fewer errors than ants that never had access to trail pheromone information (Fig. 4). The fact that this effect is only present on the first bifurcation suggests that the presence of trail pheromones helps ants learn to reduce interference from memories relevant to later parts of the journey. Collett and Collett (Collett and Collett, 2002) suggested that pheromone trails might assist learning, either by constraining ants onto a narrow route and thus facilitating the formation of intermediate snapshot memories, or by providing a training signal, informing ants that they are on the trail and thus should learn the surrounding landmarks. The role of trail pheromones as a training signal is especially reasonable in the case of a difficult-to-learn route: foragers that are unsure of their location should not invest effort in memorising a route and location. The presence of trail pheromone may thus act to 'reassure' ants that they are on the correct path, and thus that the location and route is worth learning. As ants in this experiment were constrained by the maze, our data support the second suggested role of trail pheromones as a training signal. However, the two proposed roles of trail pheromones in improving route learning are not mutually exclusive. It is possible that trail pheromones not only promote route learning, but also promote learning in general, for example associative learning of odours with rewards. This possibility is very amenable to experimental investigation.

Pheromone-deposition behaviour

The deposition of trail pheromones by ants mirrors their success at navigating a route. Ants deposited more pheromone on routes in which they made more mistakes: the alternating and short routes (Fig. 5A). Moreover, ants walking on a straight route with no bifurcations deposit even less pheromone [mean \pm s.d. = 0.45 ± 0.93 depositions per 5 cm, data taken from Czaczkes et al. (Czaczkes et al., 2011)] than ants walking on repeating routes. Thus, ants deposit more pheromone on more complex or more difficult-to-learn routes. This is due, at least in part, to ants that made a mistake on their outward journey upregulating pheromone deposition on the subsequent return journey (Fig. 5B). Similarly, when honey bees experience a delay in finding their goal they recommence performing learning flights (Wei et al., 2002) to assist memory formation. In

addition, honey bee workers that have difficulty in finding the nest entrance release an attractant pheromone to assist nestmates in finding the entrance (Butler et al., 1970). By increasing pheromone deposition after experiencing difficulties in finding the food source, an ant assists both her nestmates and herself on a subsequent visit.

One reason that an ant increases her pheromone deposition rate on the return journey may be that it is only once she has reached her goal that she can evaluate whether she made an error in getting there. If an ant were to increase pheromone deposition on the outward journey and make a mistake, this could result in more ants being lead into error. However, this reasoning assumes that ants make more errors on their outward than return journey. Although we did not collect data on how accurately ants returned to the nest, there are several reasons why this is likely. Firstly, by definition, ants on a return journey have travelled a route once more than when they were making their previous outward journey. Secondly, on return journeys, ants can rely on path integration (Collett et al., 2003) to guide them to the nest, without having to rely on possibly confusing and conflicting landmark information. Thirdly, ants may spend less time at the feeder than in the nest, and so their memory of the route on the return journey may be more recent than on their outward journey. Lastly, ants may simply avoid recruiting heavily during their outward journeys, as they cannot be sure that the food source is still productive.

Negative feedback also occurs in the *L. niger* recruitment system. Our results (Fig. 6) show that the presence of high levels of trail pheromone suppresses further pheromone deposition. This may have important implications for the organization of colony-level foraging. Ants that successfully find a food source return to the nest depositing trail pheromone (Beckers et al., 1992). This elicits more ants to exit the nest (Wilson, 1962). Some of these ants will follow the trail successfully, feed and return, also depositing trail pheromone. This positive feedback quickly results in a strong trail being established. By depositing more pheromone for higher quality food sources, such positive feedback loops can allow ant colonies to concentrate their foraging on one or a few best feeding locations from multiple possible feeders (Aron et al., 1993; Beckers et al., 1990). However, as recruitment in many mass-recruiting ant species is non-linear (Detrain and Deneubourg, 2008; Sumpter and Beekman, 2003),

foraging trails can rapidly become very strong. If a new feeding location is located once foraging at the first feeding location is well underway, a colony may not be able to switch feeding locations, even if the newly discovered feeder is of a higher quality (Beckers et al., 1990; Sumpter and Beekman, 2003). This is because the pheromone trail to the first feeder is too strong. The negative feedback system described here – trail pheromone presence suppressing further pheromone deposition – may counteract the positive feedback system responsible for fixing forager allocation. This may act to protect colonies from becoming too firmly entrenched in the exploitation of any one feeding location, without sacrificing the speed at which a consensus ‘decision’ is made, as the initial rapid build-up of pheromone would not be hindered, only the later increase to extremely high levels. Furthermore, once feeding is well underway and the pheromone trail is sufficiently strong to guide ants accurately, this reduction of pheromone deposition in response to pheromone presence will result in less metabolically expensive pheromone being used unnecessarily.

Foraging in ants is a complex process involving the use of route memories and pheromone trails, and is both a collective and an individual behaviour. The simplified situations tested in laboratory studies often mask much of this complexity. By introducing a slightly more complex and realistic situation, we detected features of ant foraging and recruitment that would otherwise have remained hidden. For example, *L. niger* appears to have an innate bias towards learning repeating routes, and this may have ecological implications. We predict that ants would disproportionately exploit food sources at the end of repeating routes. However, when the bias in route memory formation hinders exploitation of food sources, an increase in trail pheromone deposition can compensate, by helping to guide ants to food sources that require an alternating route, and enhancing route learning. These results are a compelling example of the sophistication of ant foraging, and the interplay and complementarity of different information sources in collective organization.

Appendix

In Experiment 3, trail pheromone was allowed to accumulate on the trail, and was then removed (1) after each visit, (2) only on the ants’ final visit or (3) never, in order to ascertain whether the

Table A1. First bifurcation

	Full pheromone visit 7	Memory test visit 7	Memory test visit 6	No pheromone visit 7	Naive + pheromone
Full pheromone visit 7	N/A	Not different Z=0.052 P=0.9586	Not different Z=-1.536 P=0.156	More accurate Z=2.972 P=0.00493	Less accurate Z=-2.411 P=0.0199
Memory test visit 7	Not different Z=-0.052 P=0.9586	N/A	Not different Z=-1.563 P=0.1475	More accurate Z=2.862 P=0.00526	Less accurate Z=-2.415 P=0.0199
Memory test visit 6	Not different Z=1.536 P=0.1556	Not different Z=1.563 P=0.1475	N/A	More accurate Z=4.199 P<0.00001	Not different Z=-0.493 P=0.6220
No pheromone visit 7	Less accurate Z=-2.972 P=0.0105	Less accurate Z=-2.862 P=0.0158	Less accurate Z=-4.199 P<0.0001	N/A	Less accurate Z=-5.737 P<0.00001
Naive + pheromone	More accurate Z=2.411 P=0.0265	More accurate Z=2.414 P=0.0263	Not different Z=0.493 P=0.622	More accurate Z=5.737 P<0.00001	N/A

Data indicate whether the ants in the groups in each row were more accurate, less accurate or no different than the ants in the groups in each column in choosing the correct path at the first bifurcation in Experiment 3.

Full pheromone visit 7, pheromone never removed from path.

Memory test visit 7, pheromone removed from path only on the seventh visit.

Memory test visit 6, the same ants as Memory test visit 7, but on the visit before, where pheromone remains on the path.

No pheromone visit 7, pheromone always removed from path.

Naive + pheromone, ants that have never visited the food source before, on a path with trail pheromone.

Table A2. Second bifurcation

	Full pheromone visit 7	Memory test visit 7	Memory test visit 6	No pheromone visit 7	Naive + pheromone
Full pheromone visit 7	N/A	More accurate Z=2.937 P=0.00828	Not different Z=0.906 P=0.3650	More accurate Z=2.875 P=0.01010	Borderline more accurate Z=2.292 P=0.0548
Memory test visit 7	Less accurate Z=-2.937 P=0.00674	N/A	Less accurate Z=-2.476 P=0.0275	Not different Z=-0.146 P=0.88404	Not different Z=-1.443 P=0.1861
Memory test visit 6	Not different Z=-0.906 P=0.36500	More accurate Z=2.476 P=0.02215	N/A	More accurate Z=2.397 P=0.02754	More accurate Z=1.629 P=0.1723
No pheromone visit 7	Less accurate Z=-2.875 P=0.00674	Not different Z=0.146 P=0.88407	Less accurate Z=-2.397 P=0.0275	N/A	Not different Z=-1.319 P=0.1872
Naive + pheromone	Less accurate Z=-2.292 P=0.02737	Not different Z=1.443 P=0.18626	Not different Z=-1.629 P=0.1292	Not different Z=1.319 P=0.23411	N/A

Data indicate whether the ants in the groups in each row were more accurate, less accurate or no different than the ants in the groups in each column in choosing the correct path at the second bifurcation in Experiment 3. See Table A1 for group descriptions.

presence of pheromone assists in route learning. We found significant interactions between some of the treatment comparisons and bifurcation, and in other treatment comparisons we found no effect of bifurcation. We summarise the significant findings below.

1. Ants that never had access to pheromone are less accurate than ants that always had access to pheromone, at both bifurcations ($Z=-3.030$, $P=0.00815$).

2. On their last visit, ants that never had access to pheromone are less accurate than ants that had access to pheromone in all but the last visit ($Z=-2.922$, $P=0.0326$).

3. Naive ants with trail pheromone are more accurate than ants that had access to pheromone in all but the last visit ($Z=-2.480$, $P=0.02196$).

4. Ants that had access to pheromone in all but the last visit are less accurate on their last visit than on their penultimate visit ($Z=-4.269$, $P=0.000196$).

5. There was a significant interaction between bifurcation and experienced ants with no pheromone on only the seventh visit *versus* ants with pheromone on all visits ($Z=-2.584$, $P=0.01956$). There was no difference at the first bifurcation (Table A1), but at the second bifurcation, ants with experience but no pheromone on the seventh visit were less accurate than ants that always had access to trail pheromones (Table A2).

6. There was a significant interaction between bifurcation and naive ants with pheromone *versus* ants that always had access to trail pheromones ($Z=-3.170$, $P=0.00762$): at the first bifurcation, naive ants with pheromone were more accurate (Table A1); at the second bifurcation, naive ants with pheromone were less accurate (Table A2).

7. There was a significant interaction between bifurcation and experienced ants with no pheromone on only the seventh visit *versus* ants that never had access to trail pheromones ($Z=-2.584$, $P=0.01956$): at the first bifurcation, ants that never had access to trail pheromones were less accurate than experienced ants with no pheromone on only the seventh visit (Table A1); there was no difference in accuracy at the second bifurcation (Table A2).

8. There was a significant interaction between bifurcation and ants that never had access to trail pheromone *versus* naive ants with pheromone ($Z=-3.170$, $P=0.00762$): at the first bifurcation, ants that never had access to trail pheromone were less accurate than naive ants with pheromone (Table A1); there was no difference at the second bifurcation (Table A2).

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REFERENCES

- Aron, S., Beckers, R., Deneubourg, J. and Pasteels, J. M. (1993). Memory and chemical communication the orientation of two mass-recruiting ant species. *Insectes Soc.* **40**, 369-380.
- Bates, D., Maechler, M. and Bolker, B. (2012). lme4: Linear mixed-effects models using Eigen and Eigen. Available at <http://cran.r-project.org/web/packages/lme4/index.html>.
- Beckers, R., Deneubourg, J. L., Goss, S. and Pasteels, J. M. (1990). Collective decision making through food recruitment. *Insectes Soc.* **37**, 258-267.
- Beckers, R., Deneubourg, J. and Goss, S. (1992). Trail laying behaviour during food recruitment in the ant *Lasius niger* (L.). *Insectes Soc.* **39**, 59-72.
- Beckers, R., Deneubourg, J. L. and Goss, S. (1993). Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. *J. Insect Behav.* **6**, 751-759.
- Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289-300.
- Bhatkar, A. and Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *Fla. Entomol.* **53**, 229-232.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J.-S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127-135.
- Butler, C. G., Fletcher, D. J. C. and Watler, D. (1970). Hive entrance finding by honeybee (*Apis mellifera*) foragers. *Anim. Behav.* **18**, 78-91.
- Collett, M. (2009). Spatial memories in insects. *Curr. Biol.* **19**, R1103-R1108.
- Collett, T. and Cartwright, B. (1983). Eidetic images in insects: their role in navigation. *Trends Neurosci.* **6**, 101-105.
- Collett, T. S. and Collett, M. (2002). Memory use in insect visual navigation. *Nat. Rev. Neurosci.* **3**, 542-552.
- Collett, T. S., Graham, P. and Durier, V. (2003). Route learning by insects. *Curr. Opin. Neurobiol.* **13**, 718-725.
- Cook, S. B. (1971). A study of homing behavior in the limpet *Siphonaria alternata*. *Biol. Bull.* **141**, 449-457.
- Czaczkes, T. J., Grüter, C., Jones, S. M. and Ratnieks, F. L. W. (2011). Synergy between social and private information increases foraging efficiency in ants. *Biol. Lett.* **7**, 521-524.
- Detrain, C. and Deneubourg, J.-L. (2008). *Collective Decision-making and Foraging Patterns in Ants and Honeybees*, pp. 123-173. New York, NY: Academic Press.
- Devigne, C. and Detrain, C. (2002). Collective exploration and area marking in the ant *Lasius niger*. *Insectes Soc.* **49**, 357-362.
- Dittus, W. P. J. (1984). Toque macaque food calls: Semantic communication concerning food distribution in the environment. *Anim. Behav.* **32**, 470-477.
- Evison, S. E. F., Petchey, O. L., Beckerman, A. P. and Ratnieks, F. L. W. (2008). Combined use of pheromone trails and visual landmarks by the common garden ant *Lasius niger*. *Behav. Ecol. Sociobiol.* **63**, 261-267.
- Fitzgerald, T. D. and Peterson, S. C. (1983). Elective recruitment by the eastern tent caterpillar (*Malacosoma americanum*). *Anim. Behav.* **31**, 417-423.
- Fourcassie, V. and Beugnon, G. (1988). How do red wood ants orient when foraging in a three dimensional system? I. Laboratory experiments. *Insectes Soc.* **35**, 92-105.
- Graham, P. and Cheng, K. (2009). Ants use the panoramic skyline as a visual cue during navigation. *Curr. Biol.* **19**, R935-R937.

- Graham, P. and Collett, T. S.** (2006). Bi-directional route learning in wood ants. *J. Exp. Biol.* **209**, 3677-3684.
- Grüter, C. and Ratnieks, F. L. W.** (2011). Honeybee foragers increase the use of waggle dance information when private information becomes unrewarding. *Anim. Behav.* **81**, 949-954.
- Grüter, C., Balbuena, M. S. and Farina, W. M.** (2008). Informational conflicts created by the waggle dance. *Proc. R. Soc. Lond. B* **275**, 1321-1327.
- Grüter, C., Czaczkes, T. J. and Ratnieks, F. L. W.** (2011). Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. *Behav. Ecol. Sociobiol.* **65**, 141-148.
- Harrison, J. F., Fewell, J. H., Stiller, T. M. and Breed, M. D.** (1989). Effects of experience on use of orientation cues in the giant tropical ant. *Anim. Behav.* **37**, 869-871.
- Hölldobler, B. and Wilson, E. O.** (1990). *The Ants*. Cambridge, MA: Belknap Press.
- Jander, R.** (1990). Arboreal search in ants: search on branches (Hymenoptera: Formicidae). *J. Insect Behav.* **3**, 515-527.
- Jeanson, R., Ratnieks, F. L. W. and Deneubourg, J. L.** (2003). Pheromone trail decay rates on different substrates in the pharaoh's ant, *Monomorium pharaonis*. *Physiol. Entomol.* **28**, 192-198.
- Judd, S. P. D. and Collett, T. S.** (1998). Multiple stored views and landmark guidance in ants. *Nature* **392**, 710-714.
- Lehrer, M.** (1991). Bees which turn back and look. *Naturwissenschaften* **78**, 274-276.
- Lindauer, M. and Kerr, W. E.** (1958). Die gegenseitige Verständigung bei den stachellosen Bienen. *J. Comp. Physiol. A* **41**, 405-434.
- Macquart, D., Latil, G. and Beugnon, G.** (2008). Sensorimotor sequence learning in the ant *Gigantiops destructor*. *Anim. Behav.* **75**, 1693-1701.
- Mautz, D.** (1971). Der kommunikationseffekt der schwanzeltanze bei *Apis mellifica carnica* (Pollm.). *J. Comp. Physiol. A* **72**, 197-220.
- Menzel, R.** (1979). Behavioural access to short-term memory in bees. *Nature* **281**, 368-369.
- Menzel, R., Kirbach, A., Haass, W.-D., Fischer, B., Fuchs, J., Koblotsky, M., Lehmann, K., Reiter, L., Meyer, H., Nguyen, H. et al.** (2011). A common frame of reference for learned and communicated vectors in honeybee navigation. *Curr. Biol.* **21**, 645-650.
- Nicholson, D. J., Judd, S. P., Cartwright, B. A. and Collett, T. S.** (1999). Learning walks and landmark guidance in wood ants (*Formica rufa*). *J. Exp. Biol.* **202**, 1831-1838.
- Nieh, J.** (2004). Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* **35**, 159-182.
- R Development Core Team** (2009). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rosengren, R. and Fortelius, W.** (1986). Ortstreue in foraging ants of the *Formica rufa* group – hierarchy of orienting cues and long-term memory. *Insectes Soc.* **33**, 306-337.
- Sumpter, D. J. T. and Beekman, M.** (2003). From nonlinearity to optimality: pheromone trail foraging by ants. *Anim. Behav.* **66**, 273-280.
- Verhaeghe, J.** (1982). Food recruitment in *Tetramorium impurum* (Hymenoptera: Formicidae). *Insectes Soc.* **29**, 67-85.
- von Frisch, K.** (1967). *The Dance Language and Orientation of Bees*. Cambridge, MA: Harvard University Press.
- Wallraff, H. G.** (2010). *Avian Navigation: Pigeon Homing as a Paradigm*. New York, NY: Springer-Verlag.
- Wehner, R. and Räber, F.** (1979). Visual spatial memory in desert ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). *Experientia* **35**, 1569-1571.
- Wehner, R., Michel, B. and Antonsen, P.** (1996). Visual navigation in insects: coupling of egocentric and geocentric information. *J. Exp. Biol.* **199**, 129-140.
- Wei, C. A., Rafalko, S. L. and Dyer, F. C.** (2002). Deciding to learn: modulation of learning flights in honeybees, *Apis mellifera*. *J. Comp. Physiol. A* **188**, 725-737.
- Wilson, E. O.** (1962). Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith) 1. The organization of mass-foraging. *Anim. Behav.* **10**, 134-147.
- Wilson, E. O.** (1972). *The Insect Societies*. Cambridge, MA: Harvard University Press.
- Zeil, J.** (1993). Orientation flights of solitary wasps (Cerceris; Sphecidae; Hymenoptera). *J. Comp. Physiol. A* **172**, 207-222.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M.** (2009). *Mixed Effects Models and Extensions in Ecology with R*. New York, NY: Springer-Verlag.