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# Pheromone trails in the Brazilian ant *Pheidole oxyops*: extreme properties and dual recruitment action

**Tomer J. Czaczkes · Francis L. W. Ratnieks**

**Abstract** Communication of feeding locations is widespread in social animals. Many ants use pheromone trails to guide nestmates to food sources, but trail properties and how they are used vary. The ant *Pheidole oxyops* retrieves prey cooperatively using multiple workers. The recruited workers are guided to the prey by a pheromone trail laid by the initial discoverer. In comparison to other ants, this trail has extreme properties. Despite being laid by just one ant, freshly laid trails are followed very accurately (84.4 % correct choices at a bifurcation), but decay in only 5–7 min. This extreme accuracy and short duration probably reflect adaptations to underlying differences in feeding ecology. In particular, *P. oxyops* needs to rapidly recruit nestmates to a precise location in a competitive environment. Rapid decay combined with a natural walking speed of 1.4 m/min should set an upper limit of 4 m (an 8-m round trip) on recruitment range. However, experimentally placed food items up to 8 m from the nest entrance were cooperatively retrieved. This greater range is due to the trail having a dual recruitment role. It not only recruits from the nest but also intercepts ants already outside the nest, causing them to join the trail. Seventy-five per cent of ants joining the trail then followed it towards the food item. Even when direct recruitment from

the nest was prevented, this secondary recruitment action resulted in seven times as many ants locating a food source than by chance discovery and in items being moved 46 % sooner.

## Introduction

Animals often forage in a competitive environment. Those possessing adequate weaponry or size may be able to dominate a food source (Hölldobler et al. 1978; Holldobler 1982). Less aggressive animals may use another niche in time or space (Cerdá et al. 1998) or employ other strategies such as moving food to a safe location (Smith and Reichman 1984). Many central place foragers, including social insects, must also retrieve food to the nest to feed developing young. Some ants increase the maximum size of the food items they can retrieve by cooperative transport, in which two or more workers collectively move an item (Sudd 1965; Fowler 1984; Moffett 1988; Czaczkes et al. 2010), thereby making the food safe from more competitors (Hölldobler et al. 1978; Traniello 1983, 1987). Ants employ a variety of strategies and techniques to cooperatively transport food items (Sudd 1960a; Moffett 1988; Czaczkes et al. 2010; Czaczkes and Ratnieks 2011). However, a basic requirement is that sufficient foragers must be recruited to the item to move it. Ideally, recruitment should be accurate and rapid: accuracy to ensure that recruits reach the precise location and rapidity to outpace competitors.

Food gathering locations range along a continuum from point sources to broad areas. In the latter case, the recruitment of nestmates can still be effective even if there is some error in the ability to communicate the location, such as in

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some harvester ants (Greene and Gordon 2007) or honeybees (Weidenmuller and Seeley 1999, p. 272). In some ants, trail-following accuracy is low. For example, in *Lasius niger*, which often exploits honeydew from aphid colonies (Pontin 1963), as few as 62 % of the ants chose the correct branch at a trail bifurcation with only one ant depositing a trail, and only 70 % of ants chose the correct branch with 20 depositing ants (Grüter et al. 2010). Pharaoh's ants, *Monomorium pharaonis*, achieve a comparable accuracy of 70 % on a paper substrate (Jeanson et al. 2003). However, when recruitment is to a single point source, such as to a nest site or a single food item, accuracy is more important (Weidenmuller and Seeley 1999). This study investigates trail following in *Pheidole oxyops*, a neotropical ground-nesting species that retrieves 78 % of its food using cooperative transport of large items, mostly dead insects (Czaczkes et al. 2010). This species provides an excellent opportunity to study adaptations for recruitment where maximum accuracy is expected because recruits are directed via a pheromone trail laid by one or a few food-discovering ants to a precise location (Fowler 1984; Czaczkes et al. 2010; see Electronic supplementary material (ESM) 1). This contrasts to the situation in *L. niger* or *M. pharaonis* where many ants contribute to a single pheromone trail. Indeed, Beekman et al. (2001) claim that in *M. pharaonis*, recruitment to a food source is not possible unless many ants lay the trail pheromone (but see Sudd 1960b).

Pheromone trails should be easier to follow if more of the chemical is present in the headspace around the trail. This will require the chemical to be more volatile, especially if the trail is laid by only one ant. A more volatile trail pheromone will, however, be shorter lived. As *P. oxyops* forages mainly on non-renewable food sources, a long-lasting trail pheromone is not necessary and would in fact be harmful as recruitment would continue long after the item has either been retrieved or lost to competitors. However, there is a time delay equivalent to one round trip between food discovery and recruits from the nest reaching the item. In the first part of this study, we found that the foraging range of a *P. oxyops* colony was double the maximum distance from the nest at which recruitment from the nest should be able to function. This led to the hypothesis that the trail of *P. oxyops* also intercepted nestmates already searching for food near the food item, thereby resulting in the longer than expected colony foraging range.

## Methods

### Site and study organism

Data were collected in February 2011 at Fazenda Aretuzina, a farm located near São Simão, in São Paulo State, Brazil. The study species, *P. oxyops*, nests in the ground and naturally scavenges and hunts for dead and living arthropods, which are often carried back by groups of minor ants (2–30). *P. oxyops* display cooperative transport and recruitment behaviour: when an ant attempts to move a food item and fails, it returns to the nest, laying a pheromone trail that recruits nestmates to the item (Fowler 1984; Detrain and Deneubourg 1997; Czaczkes et al. 2010). A surge of recruits can be triggered by providing an immovable food item. In this study, we used a piece of mozzarella cheese fixed to a large piece of modelling clay to cause such surges. Eleven field colonies were studied in total, labelled A–H and X–Z. A subset was used for each individual experiment.

### Experiment 1: Determining trail pheromone decay rate and trail-following accuracy

Our first experiment determined the properties of the pheromone trails of *P. oxyops* by examining the trail-following accuracy of recruits and the decay rate of a trail laid by a single minor worker ant that had discovered an immovable food item. A T-maze was formed by placing a plastic platform, 20×220 mm, which acted as the arms of the T, 30 cm from a nest entrance (see Fig. 1). Each arm was 100 mm long, with a 20-mm central section between them. The platform was raised on stilts surrounded by a water moat to prevent ants gaining access except via a cardboard ramp, which formed the stem of the T. The ramp was 100 mm long, tapering from 50 mm in width at the base to 20 mm where it connected to the central section of the platform. The platform was overlaid with clean printer paper, with vertical decision lines marked 50 mm from the centre of the overlay. A bait item was placed at the end of one arm. Within a few minutes, this would be found by a minor worker ant from the study colony. The discoverer ant would unsuccessfully attempt to move the bait and then return to the nest via the ramp laying trail pheromone. We can be certain that the ant is laying a pheromone trail as in other experiments ants could be seen to accurately follow the path of recruiting ants (see ESM 1, and the results of this experiment and experiment 4). On reaching the nest, the discoverer caused a surge of recruits which ran up the ramp and onto the platform. Ants passing a decision line (see Fig. 1) were considered as having chosen either left or right. These ants were then removed from the platform by allowing them to walk onto a piece of paper and were then shaken off at arm's length from the apparatus. Although the ants could in theory return to the apparatus, resulting in pseudo-replication, it seems likely that this disturbance would prevent most ants from reaching the apparatus or following the trail up onto the platform. We continued monitoring the decisions of ants for up to 7 min as new ants left the nest. As the surge of recruits triggered by the first ants can be short-lived, lasting sometimes <2 min, new surges of recruits must be triggered.
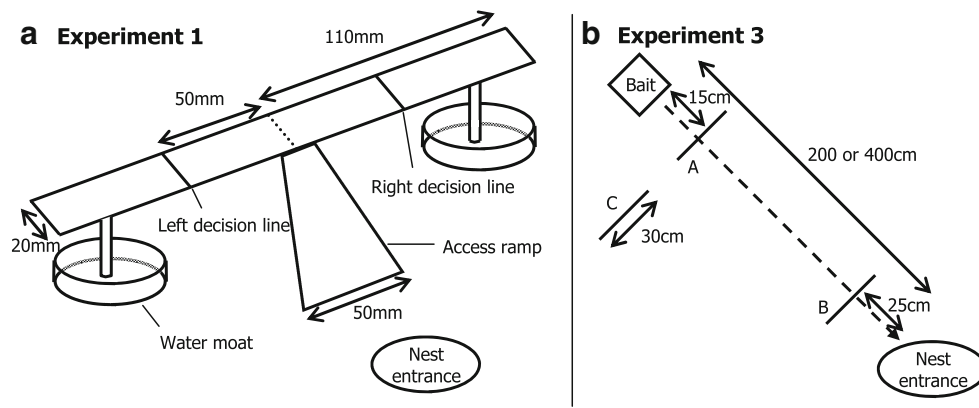
Fig. 1 **a** T-maze apparatus used in experiment 1 (not to scale). An unmovable food item was placed at the end of one arm the T-maze. A worker ant would find this and return to the nest, depositing a pheromone trail. Recruits emerge from the nest entrance almost immediately and enter the apparatus via the access ramp. Ants crossing a particular decision line were considered to have chosen either left or right. **b** Experiment 3 setup. An ant eventually found the bait and returned to the nest, depositing a pheromone trail. This discoverer ant was either allowed to return to the nest and cause a recruitment surge (treatment 1) or was removed 5 cm from the nest entrance (treatment 2). The numbers of ants crossing lines A and B for 6 min after the scout left the food item were recorded. Prior to the trial, the number of ants crossing lines A and C had been recorded for 2 min

To trigger a new recruitment surge, the bait was replaced at the centre of the T-maze when the flow of recruits ceased. This elicited a new surge of recruits without reinforcing the trail pheromone on the branch of the T-maze where the food item had been previously located. Thus, we could observe trail-following accuracy up to 7 min after the initial recruitment occurred. During some trials, no ants crossed a decision line in some of the minutes, hence the varying number of trials per minute in Fig. 2. The number of ants tested per minute and the number of trials from which they stem are presented in Fig. 2. Trials were videoed using a Sony HD XR520 camcorder. From the videos, the number of ants making left or right decisions per minute was determined. After every trial, the platform was cleaned with ethanol and the paper overlay replaced. All trials were conducted in the shade. Six colonies, B–G, were tested in this experiment. Each colony was tested two or three times.

## Experiment 2: Effect of distance to nest entrance on discovery of food items

The main aim of this experiment was to determine the maximum distance at which a *P. oxyops* colony can locate and retrieve food items. Individually numbered 5×5×1-mm cheese pieces were laid out evenly in concentric circles around colony X. The nest entrance was then monitored for 4 h, and we noted any food items being retrieved. All retrieved food items were removed before they entered the nest, preventing satiation of the colony. Three trials were conducted. In the first trial, four items were placed 0.5 m from the entrance, 8 at 1 m, 16 at 2 m, 24 at 3 m and 32 at 4 m. The second trial used longer distances, with 20 items at 5 m and 40 at 6 m. In the third trial, 92 food items were placed around the nest entrance. Beginning on the outer circle, we laid 30 items at 10 m, 26 at 8 m, 18 at 6 m, 12 at 4 m and 6 at 2 m. A larger number of bait items were placed at the more distant locations to maintain a similar item frequency at the various distances, although food item frequency did differ at different distances and in different trials (see ESM 2). All items returning to the nest were being cooperatively transported. The study colony was located on
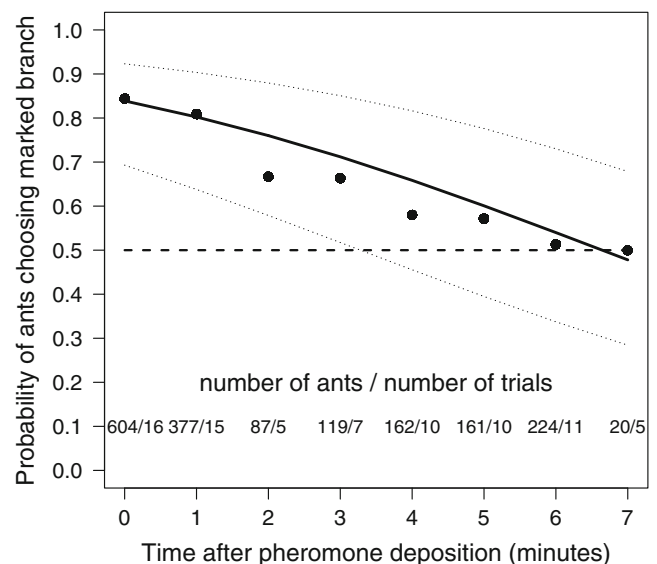


Fig. 2 Experiment 1. Decay rate of trails laid by a single discoverer ant. Trail choice accuracy decays rapidly after pheromone deposition. Ants follow a freshly laid pheromone trail with high accuracy, but within 6–7 min, branch choice is random, with probability 0.5 (*dashed line*). The *thick line* represents the model estimate (GLMM: $z=-9.747$, $P<0.001$). The *dotted lines* represent the 95 % confidence interval for the estimate. *Dots* show the mean for data within 1-min blocks (i.e. 0 min=0–59 s after the pheromone was laid, 1 min=60–119 s, etc.). *Numbers* are the number of ants tested/number of trials

the edge of tree cover, at least 12 m away from the nearest neighbouring *P. oxyops* colony. This colony was chosen as it was active, with many foragers, and isolated, thus suitable for determining the maximum foraging range for the species. We were unable to study other colonies as their foraging ranges overlapped.

Experiment 3: Ability of the pheromone trail to intercept and direct foragers already outside the nest to the food item

The discoverer ant causes a surge of recruits from the nest along the trail that it has laid. But is the trail also effective at directing foraging ants who are already outside the nest scouting for food to the food source? We tested this by placing a bait item either 2 or 4 m from a nest entrance. A single discoverer ant was allowed to find the item and return to the nest, laying a pheromone trail. When the ant left the bait, the bait item was removed and video recording of the area surrounding the bait location began. The scout was allowed either to enter the nest, causing a normal recruitment surge (treatment 1: full trail with recruitment from the nest), or was removed 5 cm from the nest entrance, resulting in an equivalent pheromone trail across the ground but without causing a recruitment surge (treatment 2: full trail without recruitment from the nest). The number of ants approaching the location of the food item from the direction of the nest was determined from the videos for 6 min after the scout had left the bait. A 30-cm line perpendicular to the line from the bait to the nest, 150 mm from the location of the bait (line A in Fig. 1b), was used as the criterion for an ant approaching the bait. We also recorded the number of ants crossing a similar line on the pheromone trail 25 cm from the nest (line B in Fig. 1b). As a control, before the trial began, line A and a similar line 20 cm away (line C) were monitored for 2 min and the number of ants crossing the lines heading away from the nest counted. This provided a measure of how many ants arrive at the location of the food by chance. For the 2-m distance, five colonies (A, G, X, Y and Z) were studied, with two repeats of the two treatments. At 4 m, two repeats of each treatment were carried out on three colonies (G, X and Y). Only a partial dataset was obtainable for colony A, and colony Z could not be tested at 4 m as it was too close to the other colonies.

Experiment 4: Effect of recruitment and the pheromone trail on the movement of food items

This experiment was designed to test the effect of a trail pheromone, with or without recruitment from the nest, on the ability of a colony to assemble sufficient ants to move a food item. A smooth ceramic tile, 15×15 cm, was sunk with its surface level to the soil surface 2 m from the nest entrance, providing a surface with regular friction. A 15× 1-mm square of cheese was placed in the centre. The nest entrance was either left open (treatment 1: full trail with recruitment from the nest) or temporarily blocked using a 90-cm diameter Petri dish, thus preventing recruitment (treatment 2: full trail without recruitment from the nest). We waited until an ant found the food item, attempted to move it, failed and returned to the nest, laying a pheromone trail. We then measured the time from the moment the scout left the food item until the item was first moved by recruiting ants and also until it had been moved 5 cm from its original position to represent the successful initiation of the collective retrieval of a large food item. The numbers of ants grasping the food item when it was first moved, and when it had been displaced by 5 cm, were also recorded. To get a measure of the rate at which ants find a food item without any form of recruitment, we also ran controls in the absence of a pheromone trail by removing ants as they found the food item by chance. Using data from this last sub-experiment, we constructed a model describing the buildup of ants at a food source without recruitment using the same methodology as described for constructing generalized linear mixed-effect models (GLMM; see "Statistical analysis" below and ESM 3 for more detail on the modelling of the buildup of ants by chance alone). We then compared the model's results with the time needed to build up sufficient ants to move the item when a pheromone trail (treatment 2), or pheromone trail and recruitment (treatment 1), was present (see "Results" for further details).

Experiment 5: Joining and following a pheromone trail by ants already outside of the nest

To determine the probability that ants already outside the nest will follow a trail that they discover and the direction they take, a runway (40×100 cm) of smooth ceramic tiles was placed flush with the ground in front of colony X, leading directly to the nest entrance. The tiles were covered with white printer paper. An immovable food bait was placed at the far end of the runway and was soon found by an ant which would attempt to move the item, fail and return in a relatively straight line to the nest along the paper whilst laying a pheromone trail. The food item was then immediately removed and the entire runway video recorded for 7 min. Eight repeat trials were performed. When analysing the videos using Virtualdub software (Lee 1998), the path of the returning scout was marked on screen with a line. Other ants were scouting for food in this area, and every ant to cross this line was scored as to whether it followed the pheromone path or not and, if it did, whether it followed the path towards the nest or towards the food item. An ant was considered to follow the pheromone trail when it followed the line for 10 cm or more if the ant had initially been walking in a different direction to the pheromone trail (>10°

different). If the ant had been walking in a similar direction (<10° different), we required the ant to follow the trail for at least 20 cm before scoring it as following the pheromone trail. This was to reduce the chance of including ants that walked in the trail direction by chance alone.

Statistical analysis

We analysed the data using a GLMM (Bolker et al. 2009) and general linear models using R2.9 (R Development Core Team 2009). Models were fitted using the lmer function (Bates et al. 2007). Where appropriate, we included 'colony' as a random effect to control for the non-independence of data points from these sources (Bolker et al. 2009; Zuur et al. 2009). Saturated models (containing all measured variables and random effects) were produced and non-significant terms sequentially removed until we arrived at a model containing only significant terms (Zuur et al. 2009). Heterogeneity of variance was controlled for when discovered by adding the appropriate term to models (Zuur et al. 2009). Data were square-root-transformed where necessary to achieve normality of error. Sign tests, one-sample $Z$ tests and one-sample $t$ tests were carried out in Minitab 14.

## Results

Experiment 1: Determining trail pheromone decay rate and trail-following accuracy

Every ant that discovered the food item tried to move it, failed, returned to the nest and immediately caused a surge of ants which walked in the direction from which the discovering ant had come. Thus, we are confident that every discoverer ant deposited a pheromone trail. The statistical model contained only one explanatory variable—time after recruitment—and one random effect—colony (with intercept free to vary, but a fixed slope). A binomial error structure was used. Recruit ants initially chose the branch of the T-maze marked with a trail pheromone with high accuracy (84.4 % correct, Fig. 2). The age of the pheromone trail was strongly correlated with the proportion of ants taking the correct branch (GLMM: $z=-9.747$, $P<0.001$; see Fig. 2). Mistakes increased rapidly, and 7 min after trail laying, there was no difference between the branches (mean 50 % correct, Fig. 2). The difference between the branches was non-significant after only 5 min (one-sample $t$ test: $N=161$, mean$=0.57.1$, $T=1.83$, $P=0.07$). Although trail pheromones are normally assumed to decay exponentially (Jeanson et al. 2003; Dussutour et al. 2009), we found that our data were best explained by a linear distribution (sequential ANOVA: linear $F=158.54$, $P<0.0001$; exponential $F=0.97$, $P=0.324$). Although a function with an

asymptote of 0.5 would be a more realistic biological description, over the timescale in this experiment, a linear function is a reasonable, and simpler, approximation.

Experiment 2: Effect of distance to nest entrance on discovery of food items

Colony X was effective at retrieving nearby food items, collecting 100 % within 1 m of the nest (Fig. 3). The proportion dropped with distance, decreasing to 45 % at 5 m and to 12 % at 8 m. At 10 m, no items were retrieved within 4 h. However, it must be noted that our results may have differed if each distance was tested separately. The maximum range at which food items were collected may have been exaggerated by the appearance of a large amount of food, resulting in a higher than normal amount of workers leaving the nest. Alternatively, the unusually large number of concurrent recruitment events may have acted to reduce the maximum foraging distance by intercepting scouts close to the nest (see results from experiments 3 and 5). Furthermore, in the first trial, some items laid down at 3 m were already being returned when observation of the nest entrance started. Thus, the measurement for 3 m is a slight underestimate.

A search of the area surrounding colony X was performed after each trial. Only one food item was discovered. This food item was dominated by an unidentified ant species. As the food items were very light in colour, and the bare earth comprising most of the area surrounding colony X was a dark red, it seems likely that the food items not retrieved by colony X were removed by either conspecific or allospecific competitors.
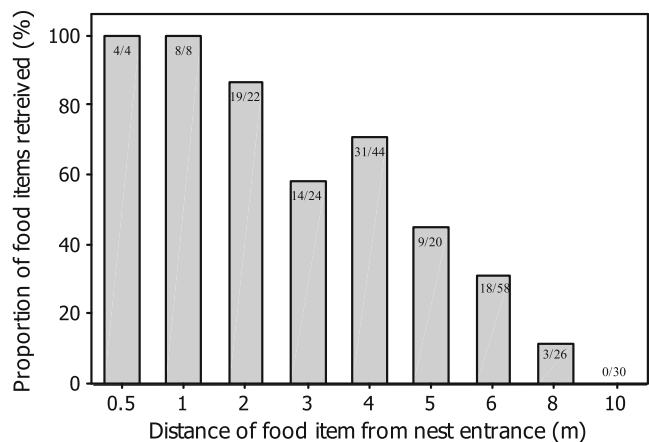


**Fig. 3** Experiment 2. Proportions of food items placed on the ground that were returned to the nest entrance within 4 h as a function of distance from the nest entrance in colony X. *Numbers in bars* indicate the number of items retrieved out of the total. The *graph* represents data pooled from three separate trials, in which the frequency of food items per metre differ (see "Methods" and ESM 2 for details). All retrieved items were being transported cooperatively

**Experiment 3: Ability of the pheromone trail to intercept and direct foragers already outside the nest to the food item**

The full statistical model (comprising distance of the food item, time taken for the discoverer ant to reach the nest, treatment, and the interactions between all of these fixed effects and colony as a random effect) was pared down to a final model comprising only treatment as a fixed effect and colony as a random effect. A normal error structure was used. Unsurprisingly, more ants arrived at the location of a discovered food item when recruitment from the nest was allowed than when the trail-laying ant that discovered the food item was removed 5 cm from the nest entrance [mean $n=26.5$ ants from trail+ recruitment (treatment 1) vs. 9.7 from trail alone (treatment 2); GLMM: $DF=34$, $t=3.04$, $P=0.0046$]. However, the trail itself acted as an important recruitment mechanism as only 1.5 ants on average located a food item with no trail leading to it (GLMM: $DF=16$, $t=7.86$, $P<0.0001$; see Fig. 4). Indeed, when the discoverer ant was removed 5 cm from the nest entrance, more ants passed the point at which the food was found (line A in Fig. 1b) than a point on the trail 25 cm from the nest entrance (line B in Fig. 2b), even though this point was closer to the nest and so would be expected to have more ants passing by on their way to and from the nest entrance (mean ants at 25 cm=3.7, mean ants at full distance=9.7; GLMM: $DF=16$, $t=6.15$, $P<0.0001$). The distance of the food bait from the nest (2 or 4 m) had no significant effect on the number of ants reaching the food. Therefore, distance was not included as an explan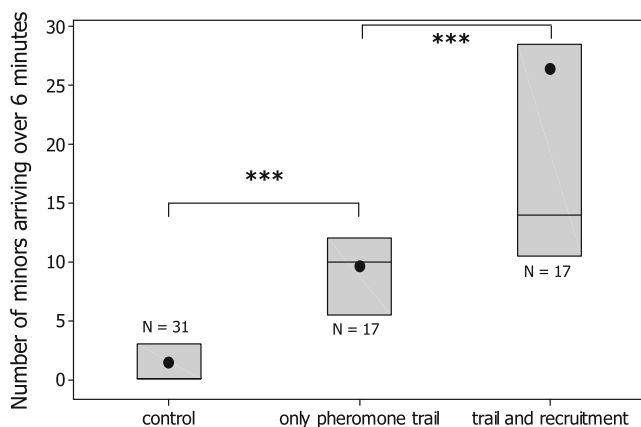atory variable in the final model. Indeed, of all fixed effects in this experiment, treatment was the only significant explanatory variable.

**Experiment 4: Effect of recruitment and pheromone trail on the movement of food items**

On average, 2.2 (SD=1.96) ants were required initially to move a 15-mm$^2$ food item, and an average of 6.55 (SD=2.68) ants were grasping the item when it had been moved 5 cm from its original location. These figures were almost identical when scout ants were allowed to reach the nest and recruit or prevented from doing so [mean number of ants at initial move: only pheromone trail, no recruitment from nest=2.11, SD=0.93(treatment 2); pheromone trail and recruitment from nest=2.27, SD=2.57 (treatment 1); mean ants at 5 cm: treatment 2=6.67, SD=3.32; treatment 1=6.46, SD=2.16]. Using the data on the number of ants that discovered the food item by chance, we modelled the buildup of ants at a food item without recruitment of any form, resulting in a final model of the time (in seconds) required to give $n$ ants at a food item: $n=-5.71+0.0199$ s. We could use this to interpolate the amount of time necessary for a specific number of ants to find the food item. Thus, it would take 345 s for 2.2 ants and 747 s for 6.55 ants to find the food item by chance without the help of a pheromone trail. Using these numbers as the expected values (null hypothesis), we tested whether food items were moved sooner when scouts laid a pheromone trail but were prevented from recruitment at the nest (treatment 2) than by scouts encountering a food item by chance. Items were indeed moved significantly sooner when a pheromone trail was present (one-sample $Z$ test: mean time required for food to be moved=232.1 s, SD=453.7, $N=10$, $Z=-2.12$, $P=0.034$) and were also displaced by 5 cm sooner (one-sample $Z$ test: mean time to move the item 5 cm=468.9 s, SD=110.315, $N=10$. $Z=-5.21$, $P<0.001$).

**Experiment 5: Non-recruited ants joining and following a pheromone trail**

The probability that an ant walking in the environment that encounters a pheromone trail will join the trail is strongly negatively correlated with the age of the pheromone trail. For a freshly laid trail, the probability is almost 80 %, decreasing to 0 % at 7 min (see Fig. 5). Of the ants that did join the trail, 75.5 % walked towards the food item, which is significantly more than expected by chance (sign test: $N=46$, $H_0=0.5$, $P=0.0016$).

**Discussion**

The results support our prediction that the pheromone trail laid by a single *P. oxyops* worker recruiting to a single large



**Fig. 4** Experiment 3. Number of ants arriving at the location of the food item per 6 min before it was presented (control—a measure of discovery rate alone) and after a forager had found it and returned to the nest. Returning foragers were either prevented from reaching the nest (treatment 2—only pheromone trail: discovery+ants intercepted by the pheromone trail) or allowed to enter the nest and cause a surge of recruitment (treatment 1—trail and recruitment: discovery+interception+recruitment from nest). *Circles* represent the means, *central horizontal lines* represent medians (median for control, 0), and *grey boxes* represent the interquartile range. *Three asterisks* represent highly significant differences ($P<0.001$) between neighbouring treatments. The median in the control bar is 0 and so is not represented

food item can be followed with considerable accuracy (85 % correct choices at a T bifurcation; also see ESM 3). In contrast, in *L. niger*, a trail laid by a single forager is followed through a T bifurcation with a probability of only 62 % (Grüter et al. 2010). Even when the trail is laid by 20 ants, the proportion choosing the correct branch increases only to 70 %. Similar results were obtained for the Pharaoh's ant, *M. pharaonis*, in which only 70–80 % chose a branch that had been marked by hundreds of nestmates (Jackson et al. 2006). In both these species, there is unlikely to be as great a need for a precise communication of location as in *P. oxyops* as *L. niger* recruit mainly to clustered, long-lasting food sources (Pontin 1963) and neither *L. niger* nor *M. pharaonis* perform cooperative transport, so both can rely on many ants making return trips to the food patch to strengthen the pheromone trail.

The decay rate of the *P. oxyops* trail pheromone also supported our predictions. The trail no longer provided useful information 5–7 min after it was laid. There was close agreement in the results of our two different bioassays, one on trail choice at a T bifurcation (experiment 1, Fig. 3) and one on trail joining (experiment 5, Fig. 5). In contrast, pheromone trails of *L. niger* are usually laid by many individuals and are still effective up to 20 h later (Evison et al. 2008). This difference matches the different feeding ecology of the two species. Whilst the aphid patches which *L. niger* primarily recruit to replenish over time, and can persist for months (Salo and Rosengren 2001), the single large food items which are the main food source of *P. oxyops* do not replenish and must be exploited immediately.
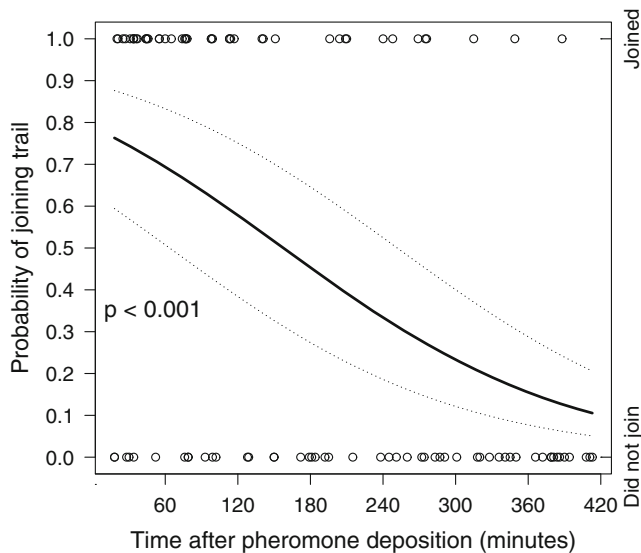


**Fig. 5** Experiment 4. The probability that an ant walking outside the nest that intercepts a pheromone trail will follow the trail as a function of trail age. The *thick black line* represents the modelled probability of joining (*left Y-axis*) based on the data. The *white circles* represent individual ants which either joined or did not join the pheromone trail (*right Y-axis*)

Evaporation should lead to a trade-off between trail-following accuracy and pheromone decay rate. *P. oxyops* makes this trade-off firmly on the side of high accuracy and short duration. Indeed, trail-following accuracy is particularly high when we consider that the trail is laid by a single ant as compared to tens or hundreds in other species. As individual *P. oxyops* foragers rarely have to reinforce pheromone trails multiple times, one might expect them to use a larger proportion of their pheromone store compared to mass-recruiting species. The behaviour of the recruits may also be adjusted to allow for fast and accurate trail following. One might also expect that ants relying on single discoverers will tend to deposit fairly continuous trails, whilst ants recruiting to long-lasting food sources might conserve pheromone by depositing a series of dots, as *L. niger* does. These are open questions for future investigation.

The rapid decay rate of *P. oxyops* pheromone trails sets a maximum recruitment distance. In our study (experiment 3), returning scouts took on average between 33 and 45 s to return from a bait 2 and 4 m away, respectively, walking on natural substrate. This gives a mean walking speed of SD= $1.36\pm0.22$ m/min. Given a 6-min life span of the pheromone trail, the maximum recruitment range of *P. oxyops* should be $6\times1.36/2=4.08$ m. However, we found that a colony can retrieve food items using cooperative transport from as far away as 8 m (Fig. 3). This discrepancy can be explained by the results of experiments 3 and 5 that show that the trail intercepts ants already out of the nest and directs most of these (75.5 %) towards the food item. Even without recruitment from the nest (treatment 2), seven times as many ants reach a food item with a trail pheromone leading to it than would be expected by chance discovery (experiment 3, Fig. 4), thus allowing food retrieval to begin 47 % sooner than it would if ants relied on chance discovery alone (experiment 4).

Local recruitment of nestmates has also been reported in the ant *Aphaenogaster cockerelli* (Hölldobler et al. 1978), where workers finding a food item emitted a pheromone which attracted nearby ants. The recruitment effect of the pheromone trail in *P. oxyops* has a similar effect, but works via a different mechanism. Whilst the local recruitment signal of *A. cockerelli* is from a point source using diffusion and air movement, in *P. oxyops*, it is in the form of an interception line from the food item to the nest. Effectiveness is increased as the interception line is directed towards the nest, an area where nestmates are likely to occur. Seventy-five per cent of nestmates walking in the environment who crossed the pheromone trail walked towards the food source, demonstrating that *P. oxyops* foragers have additional information, presumably in the form of personal information about the direction in which the nest entrance lies (perhaps by learning local landmarks or by using path integration; Collett and Collett 2002), which increases the

effectiveness of interception. Because pheromone trails recruit ants already outside the nest, the foraging range of a *P. oxyops* colony is increased and the response time is decreased compared to a situation in which the trail only serves to direct recruits from the nest.

The pheromone trails of ants have traditionally been seen as way markers from a resource to the nest and particularly as a means of directing foragers at trail bifurcations. However, recent research indicates that ant trail systems are more sophisticated than this, including new roles for ant trail pheromones such as activation signals for old trails (Robinson et al. 2008), allowing the reactivation of long-lasting trail pheromones, or as a reassurance to route memory, allowing higher movement speeds (Czaczkes et al. 2011). Here, we have demonstrated that pheromone trails also have a dual recruitment effect, directing ants from the nest itself to a food item and also intercepting and directing foragers already in the environment to the food item. This second mechanism allows a colony *of P. oxyops* to forage at greater distances and to start moving large food items more quickly than would be possible if the trail only recruited nestmates from the nest itself. We have also demonstrated that a pheromone trail has the potential to be very effective in its traditionally assigned role, with a trail laid by a single ant being capable of guiding 85 % of recruits down the correct branch at a bifurcation.

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