

RESEARCH ARTICLE

Landmarks and ant search strategies after interrupted tandem runs

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ABSTRACT

During a tandem run, a single leading ant recruits a single follower to an important resource such as a new nest. To examine this process, we used a motorized gantry, which has not previously been used in ant studies, to track tandem running ants accurately in a large arena and we compared their performance in the presence of different types of landmark. We interrupted tandem runs by taking away the leader and moved a large distant landmark behind the new nest just at the time of this separation. Our aim was to determine what information followers might have obtained from the incomplete tandem run they had followed, and how they behaved after the tandem run had been interrupted. Our results show that former followers search by using composite random strategies with elements of sub-diffusive and diffusive movements. Furthermore, when we provided more landmarks former followers searched for longer. However, when all landmarks were removed completely from the arena, the ants' search duration lasted up to four times longer. Hence, their search strategy changes in the presence or absence of landmarks. Even after extensive search of this kind, former followers headed back to their old nest but did not return along the path of the tandem run they had followed. The combination of the position to which the large distant landmark behind the new nest was moved and the presence or absence of additional landmarks influenced the orientation of the former followers' paths back to the old nest. We also found that these ants exhibit behavioural lateralization in which they possibly use their right eye more than their left eye to recognize landmarks for navigation. Our results suggest that former follower ants learn landmarks during tandem running and use this information to make strategic decisions.

KEY WORDS: *Temnothorax albipennis*, Navigation, Search behaviour, Behavioural lateralization

INTRODUCTION

Humanity has long been fascinated by the ability of animals to navigate (Santschi, 1911; Waterman, 1989; Mackintosh, 2002; Rodrigo, 2002; Cheung et al., 2012; Gould and Gould, 2012). One reason for this fascination is that many of us know from direct experience how difficult it can be to navigate. Even when we have access to accurate maps, compasses and an ability to track how far we have moved we can struggle to navigate accurately. Difficulties

often arise because of miscalculation and the accumulation of errors (for a review, see Ronacher, 2008). Moreover, after arriving at a goal, to make the journey again, we often rely on learning not only specific landmarks but also the order in which we should encounter them. In addition, we may resort to asking other members of our species for advice on the route to take. Then again, we may prefer to follow knowledgeable individuals as they take a good route to a goal that is important both for them and for us. It is not surprising, therefore, that we so admire the abilities of animals to navigate effectively. Our admiration may even peak when the organism is small, only possesses a tiny brain, and has to make relatively long journeys through complicated terrain. Thus the feats of navigation of individual ants have long fascinated biologists (Santschi, 1911; Wehner et al., 1996; Collett et al., 1998; Giurfa and Capaldi, 1999; Wehner et al., 2002; Collett et al., 2003; Durier et al., 2004; Collett, 2012).

Here we look at an unusual aspect of navigation in ants. The behaviour in question is tandem running in which one ant literally leads another ant to an important resource such as food or a new nest (Möglich, 1978). Once the follower of a tandem run has arrived at that goal it may return completely independently to the old nest site and initiate another tandem run in which it becomes the leader and solicits following from a nestmate (Möglich, 1978; Möglich, 1979). In this way, tandem-running ants may teach others to navigate to a specific goal (Franks and Richardson, 2006).

Tandem running raises a whole series of fascinating issues in ant navigation. Despite many studies that have been conducted to understand tandem running in the ant *Temnothorax albipennis* (Pratt et al., 2005; Franks and Richardson, 2006; Richardson et al., 2007; Franks et al., 2010; Franklin et al., 2011; Franklin and Franks, 2012), there are still many questions that need to be answered. For example, what exactly is the follower of a tandem run learning? Is she learning landmarks (Pratt et al., 2001; McLeman et al., 2002; Merkle and Wehner, 2008)? Such landmarks might include local landmarks, or so-called 'way-posts' along the route (Wehner and Rüber, 1979; Durier et al., 2004) plus large distant landmarks beyond the new goal or close to the old nest that might serve as beacons for the outward or return journeys (Graham et al., 2003; Wystrach et al., 2011). A previous study with no control over landmarks showed that despite not knowing the precise location of a new nest, former follower ants were still able to extrapolate forward towards the new nest after separation from the leader ants (Franks et al., 2010). Hence, we hypothesized that the former follower ants could have learned certain cues during the tandem run that then helped them to extrapolate forward towards the new nest after losing their leader ants. Thus, in the study reported here we provided a large landmark behind the new nest to investigate whether follower ants use such a large landmark as a beacon for their goal. We also carried out experiments with and without additional small local landmarks together with a large distant

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List of abbreviations

C	centre
Ci	confidence interval
E	experiment
FF	former follower
HSL	hue, saturation, luminance
L	left
N	no landmark
PSU	power supply unit
R	right
r.m.s.	root mean square
SP	search phase
TD	tandem direction
TR	tandem run

landmark behind the old nest to test whether former follower ants use them in combination with the large distant landmark behind the new nest for orientation after losing their leader. Finally, given that tandem run followers do not slavishly follow the route of the tandem run on their return journeys (Franks and Richardson, 2006), we hypothesised that they might be learning landmark locations to help them orientate on the way back home.

For the first time in ant studies we used a motorised gantry to track tandem running ants very accurately in a large arena and we compared their performance not only with different types of landmarks but also with displaced landmarks. We interrupted tandem runs by taking away leaders before they reached the goal to determine what information followers had obtained from incomplete tandem runs and to understand how the former followers behave after a tandem run has been interrupted. In particular, we were interested in how former followers of tandem runs search for: (1) their leader; (2) the goal to which they were heading; or (3) the old nest to which they must eventually return. To this end, we performed two experiments. In experiment 1, we placed a large landmark centrally behind the new nest while the tandem run was in progress. In experiment 2, in addition to the large landmark behind the new nest we placed a large landmark centrally behind the old nest and four small landmarks in the arena. In the two experiments we tracked follower ants both during the tandem run and after their leaders have been removed, under four treatment manipulations of the large landmark behind the new nest: (1) control (picked up and replaced in its central position); (2) moved to the right; (3) moved to the left; and (4) removed (no landmark).

RESULTS**Heading directions and trajectories of follower ants during tandem running and after interruption**

As expected, the mean bearings of all followers in all treatments for both experiments during tandem running tended to be orientated towards the new nest because the 95% confidence interval (CI) overlaps 0 deg in all cases (Fig. 1E1,C,TD; $N=8$; mean direction, 359 deg; $r=0.909$; Rayleigh test, $P<0.001$; 1E2,C,TD; $N=8$; mean direction, 0.05 deg; $r=0.924$; Rayleigh test, $P<0.001$; Fig. 2E1,R,TD; $N=8$; mean direction, 5 deg; $r=0.930$; Rayleigh test, $P<0.001$; 2E2,R,TD; $N=8$; mean direction, 352 deg; $r=0.925$; Rayleigh test, $P<0.001$; Fig. 3E1,L,TD; $N=11$; mean direction, 359 deg; $r=0.900$; Rayleigh test, $P<0.001$; 3E2,L,TD; $N=8$; mean direction, 350 deg; $r=0.963$; Rayleigh test, $P<0.001$; Fig. 4E1,N,TD; $N=8$; mean direction, 3 deg; $r=0.923$; Rayleigh test, $P<0.001$; 4E2,N,TD; $N=8$; mean direction, 3.5 deg; $r=0.967$; Rayleigh test, $P<0.001$). When the four small local landmarks, the large distant landmark behind the old nest and the black cloth along the arena walls were added in experiment 2, the tandem starting points tended to cover a larger

area and the trajectories to run in parallel rather than fan out as in experiment 1 (comparison between TR panels for the two experiments, Figs 1–4). The trajectories of follower ants during tandem running were by no means straight: some included a few loops (Figs 1–4, TR panels) and one or two initially headed in the wrong direction (Fig. 2E1,R,TR, orange path, for example).

After the leader ants had been removed, the trajectories of former followers were very different from their trajectories as followers during the immediately preceding tandem runs. In both experiments, their paths were tightly convoluted in the interruption zone (Figs 1–4, FF panels). This suggests that in the aftermath of the interruption, they were searching for their lost leader, the new or the old nest. In the majority of cases such searches subsequently turned into trajectories heading in the general direction of the old nest. These return paths were more convoluted and contained more loops than those of the same individuals during the tandem run (Figs 1–4). Only two ants extrapolated forwards and found the new nest after searching (Table 1; Fig. 1E1,C,FF, orange and pink lines). This number is significantly lower (two out of 67 tandem runs; Table 1) than the number of former followers that were able to extrapolate forwards in our previous study (Franks et al., 2010) (eight out of 39 former tandem followers; Fisher's exact test, two-tailed, $P=0.005$). Another two ants apparently became lost because they neither found the new nest nor headed towards the old nest within the 10 min of observation after interruption (Table 1; Fig. 3E1,L,FF, orange and light blue lines). The remaining former followers headed towards the old nest but did not retrace their tandem run routes (Figs 1–4). Intriguingly, the number of those that returned to the old nest in experiment 1 (24 out of 31) was significantly greater than that in experiment 2 (11 out of 32; chi-squared test with Yates' correction, $\chi^2=10.144$, d.f.=1, $P<0.01$; Table 1; Figs 1–4, FF panels).

We were able to identify, through rigorous analysis, three different phases: a catch-up phase, a search phase and a return phase, in the former followers' paths after interruption by fitting a broken-stick regression model (see Search behaviour of former follower ants after interruption). The catch-up phase covers the first few seconds in the immediate aftermath of the interruption and is not visible in the trajectory plots (Figs 1–4). The subsequent, search phase, corresponds to the tightly convoluted section of the path, and the final, return phase, represents the last homeward part of the trajectory.

The distributions of the mean bearings for the former follower ants in the catch-up phase, which continued for up to approximately 5 s (supplementary material Table S1), were very scattered in both experiments and were pooled for each treatment. In each case, the follower ants' bearings during the first seconds after the leader had been removed were compatible with movement in a random direction (Rayleigh test, $P>0.05$ in all cases; Fig. 5). Hence, there is no evidence that former follower ants initially move in the same direction as the tandem run, in which they had been participating.

The tightly convoluted paths of former followers in the search phase look similar for the two experiments in the right and left treatments (Figs 2, 3, FF panels) but there are subtle differences between them in the other two treatments (Figs 1, 4, FF panels). In experiment 1 under the centre treatment, the tightly convoluted searching paths of three of the former followers are mostly beyond the interruption zone, close to the new nest (Fig. 1E1,C,FF; orange, pink and dark blue lines). Indeed, two of these ants found the new nest. By contrast, in experiment 1 under the no-landmark treatment, the tightly convoluted searching paths also extend beyond the interruption zone but in the opposite direction, towards the old nest (Fig. 4E1,N,FF; pink, black and green). When they were significantly orientated, the mean bearings of former followers

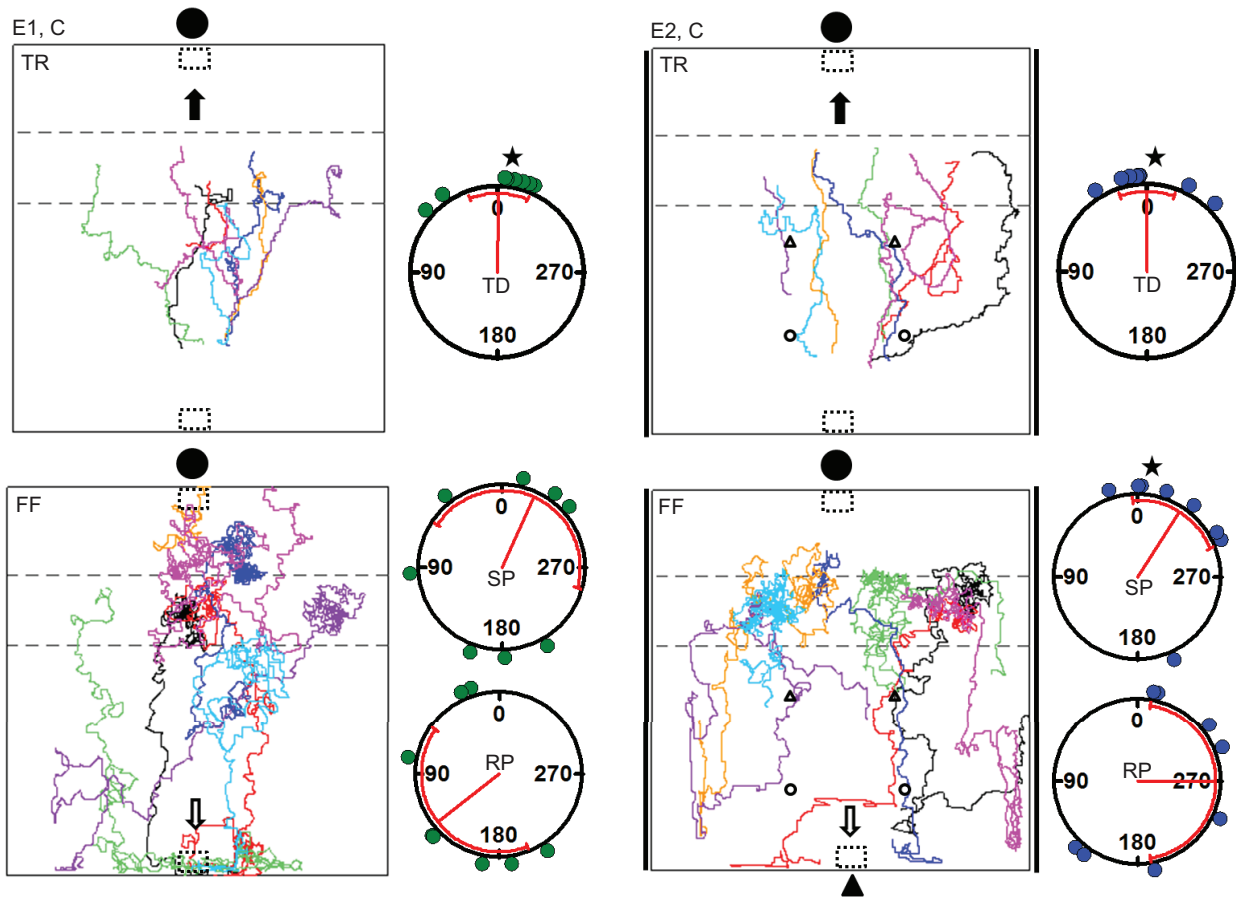


Fig. 1. The paths of follower ants during tandem runs and after interruption (in the absence of the leader ants) as recorded by the robotic gantry for experiment 1 (E1) and experiment 2 (E2) in the centre treatment. Top panels show the paths of follower ants during the tandem run (TR). Bottom panels show the former follower ants' paths after interruption (FF). A black circle above the TR or FF panel indicates the location of the large distant cylindrical landmark at the centre (C) position. Dashed lines indicate the interruption zone. Different line colours indicate different individual ants. The same line colour in the TR and FF panels indicates the same follower's path during the tandem run and after interruption. The path of E1,C, green, after interruption did not coincide exactly with the last point of its tandem run path because of the time it took to remove the leader (see Materials and methods for more details). Dashed-line rectangles depict the two nests. The filled arrow indicates the direction towards the new nest. The empty arrow indicates the direction towards the old nest. The two empty circles and two empty triangles inside the arena in E2 depict the additional small local landmarks. Paths going through a local landmark (e.g. in E2, C, FF, dark blue line) indicate that the ant climbed on the landmark. A triangle below the FF panel depicts the large distant landmark behind the old nest. All ants in the TR panel in both E1 and E2 moved forward in the direction of the new nest. Ants in the FF panel headed towards the old nest after interruption except two ants in the E1, C, FF, orange and pink lines, which moved towards the new nest after searching. The circle diagrams on the right of each panel (in this figure and Figs 2–4) show the mean directional bearings of the tandem runs (TD), search phase (SP) and return phase (RP). Mean bearings that are significantly different from random are marked with a star (Rayleigh test, $P < 0.05$).

during the search phase pointed towards the new nest or, in the case of the right treatment, to the right of the new nest. This suggests that at least some of the former followers were using the large distant landmark behind the new nest during their search. In the right and left treatments, the bearings during the search phase were significantly orientated only in experiment 1 (Fig. 2E1,R,SP; $N=8$; mean direction, 325 deg; $r=0.698$; Rayleigh test, $P < 0.05$; 95% CI to the right of 0 deg; Fig. 3E1,L,SP; $N=11$; mean direction, 0.9 deg; $r=0.583$; Rayleigh test, $P < 0.05$; 95% CI overlaps 0 deg). In the centre treatment, there was significant orientation only in experiment 2 (Fig. 1E2,C,SP; $N=8$; mean direction, 328 deg; $r=0.685$; Rayleigh test, $P < 0.05$; 95% CI overlaps 0 deg). In the no-landmark treatment the distribution of mean bearings was not significantly different from random in either experiment (Fig. 4, SP circle diagrams).

The trajectories of former followers during the return phase are spread over a wider area in experiment 2 than in experiment 1 under all treatments. This makes the return paths under the centre and no-landmark treatment, in particular, look less direct in experiment 2

than in experiment 1 (Figs 1–4, FF panels). Furthermore, as stated earlier, significantly fewer ants reached the old nest in experiment 2 within the 10 min observation period. This effect is at its most extreme in the no-landmark treatment, where only three former followers managed to move much beyond the interruption zone in the direction of the old nest (Fig. 4, FF panels). When they were significantly orientated, the mean bearings of former followers during the return phase pointed towards the old nest, or as in the case of the left treatment in experiment 2, to the left of the old nest (when facing it). In the left and right treatments, the return bearings were significantly orientated in both experiments or only in experiment 2, respectively (Fig. 3E1,L,RP; $N=11$; mean direction, 152 deg; $r=0.575$; Rayleigh test, $P < 0.05$; the 95% CI overlaps 180 deg; Fig. 3E2,L,RP; $N=8$; mean direction, 133 deg; $r=0.856$; Rayleigh test, $P < 0.001$; 95% CI to the left of 180 deg; Fig. 2E2,R,RP; $N=8$; mean direction, 169 deg; $r=0.872$; Rayleigh test, $P < 0.001$; the 95% CI overlaps 180 deg). By contrast, in the centre and no-landmark treatments, the distribution of mean

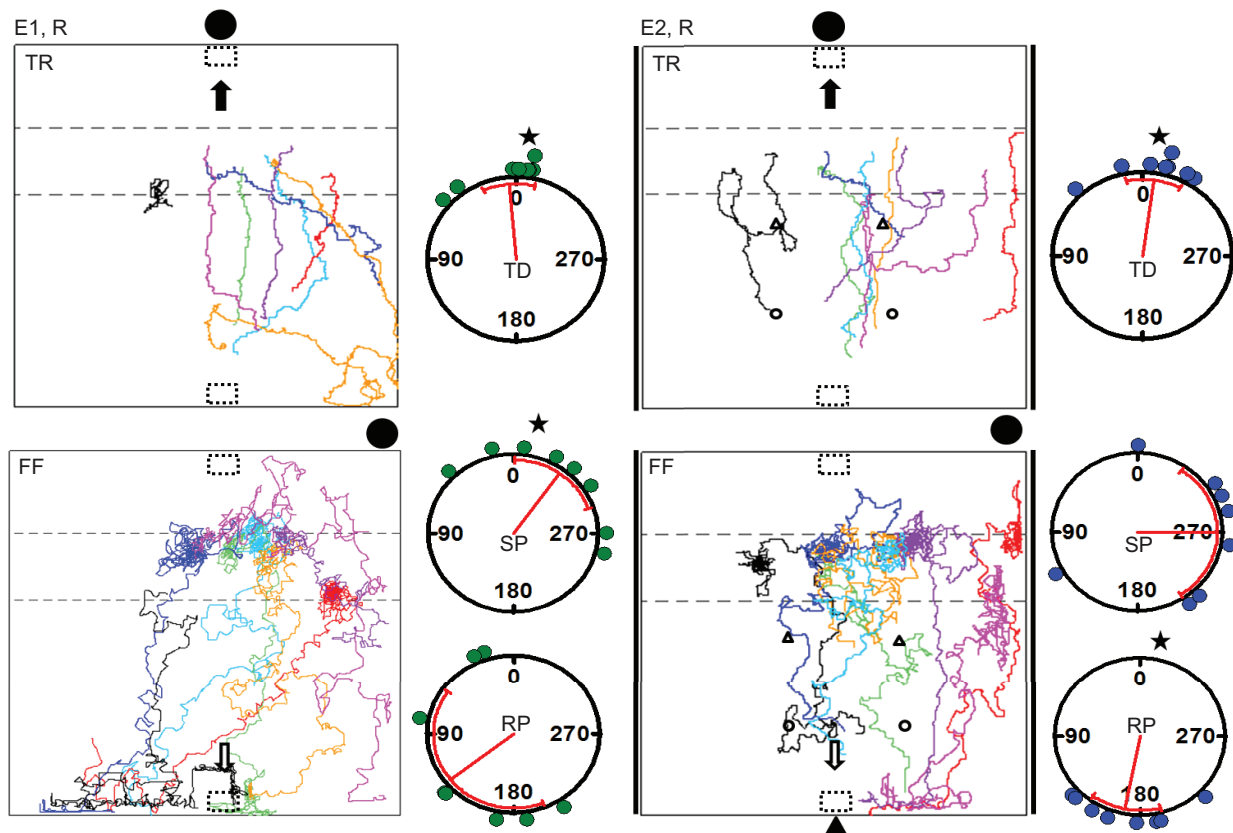


Fig. 2. The paths of follower ants during the tandem run and after interruption, together with mean directional bearings of their movements for the right treatment (R). A black circle above the FF panel indicates the location of the large distant cylindrical landmark that had been moved to the right position after interruption. All other symbols are the same as in Fig. 1. A black path on the left-hand side of the arena in E1, R, TR represents a short tandem run. The paths of E1, R, black, and E2, R, pink, after interruption did not coincide exactly with the last point of their tandem run path because of the time taken to remove the leader (see Materials and methods for more details). All ants in the TR panel in both E1 and E2 moved forward in the direction of the new nest. All ants in the FF panel headed towards the old nest after searching.

bearings was not significantly different from random in either experiment (Figs 1, 4, RP circle diagrams).

Search behaviour of former follower ants after interruption

The broken-stick regression model (Muggeo, 2003) fitted to the \log_{10} -binned root mean square (r.m.s.) displacement helped identify three slopes (Fig. 6), representing: (1) a catch-up phase (first slope); (2) a search phase (second slope); and (3) a return phase (third slope). From a theoretical point of view, a slope of 0.5 for such a log–log relationship of r.m.s. displacement versus time represents movement compatible with a diffusive random search (e.g. in Brownian movement, a Brownian walker in 2D diffuses proportionately to the square root of time), whereas a slope greater or less than 0.5 represents super-diffusion or sub-diffusion, respectively, that is, displacement faster or slower than random (Franks et al., 2010).

The first slope of the r.m.s. displacement has a value significantly greater than 0.5 in both experiments and in all treatments (Fig. 6, all 95% CIs are above 0.5). This indicates that the movement of the ants was super-diffusive immediately after the leader had been removed. As we have already established, these short, fast movements are not significantly orientated. This suggests they represent a fast initial attempt to catch up with the lost leader. Then, during the search phase, the movement of the former followers changed from super-diffusive to either sub-diffusive (Fig. 6A, experiment 2; 6C, D, experiment 1) or diffusive (Fig. 6A, experiment

1; 6B, experiments 1 and 2; 6C, experiment 2) except when the large distant landmark behind the new nest was removed in experiment 2 (as indicated by the 95% CI of the second slope in Fig. 6D). In the latter experiment, the ants engaged in a super-diffusive movement throughout, from the catch-up phase until they returned to the old nest (Fig. 6D, experiment 2). The predominantly sub-diffusive or diffusive character of the former followers' movements during searching matches their tight convoluted trajectories for this phase (Figs 1–4, FF panels). In all treatments in both experiments, the ants performed a super-diffusive movement when returning to the old nest (this refers to the third slopes in Fig. 6A–D, all having 95% CIs above 0.5). In this return phase, the former followers diffused significantly more quickly in experiment 2 under the right treatment (Fig. 6A, 95% CIs of third slopes do not overlap). By contrast, they diffused significantly more quickly in the third phase of experiment 1 under the no-landmark treatment (Fig. 6D, 95% CIs of third slopes do not overlap). There was no significant difference between the third slopes for experiment 1 and 2 in the left and centre treatments {Fig. 6B, C, two-tailed, two-sample *t*-test for simple linear regression slopes assuming equal variances [see pp. 360–362 in Zar (Zar, 1998)] or unequal variances [see pp. 128–129 in Zar (Zar, 1998)], $P > 0.05$ in both cases}.

There was no significant difference in the first break point, i.e. the time when the former followers switched from the super-diffusive search of the catch-up phase to either the sub-diffusive or diffusive search of the search phase, between the two experiments in all

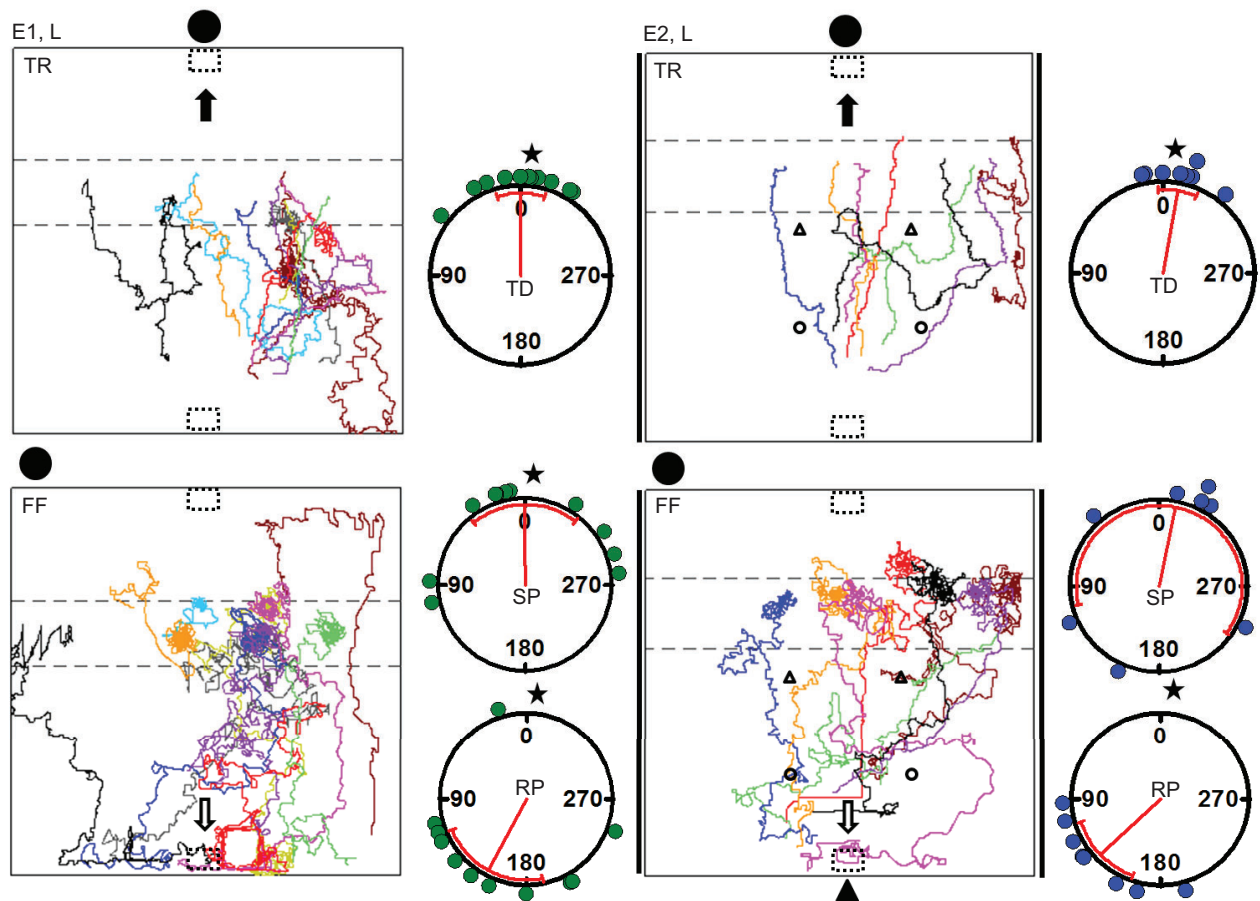


Fig. 3. The paths of follower ants during the tandem run and after interruption, together with mean directional bearings of their movements for the left treatment (L). A black circle above the FF panel indicates the location of the large distant cylindrical landmark that had been moved to the left position after interruption. All other symbols are the same as in Fig. 1. The path of E1, L, orange, after interruption did not coincide exactly with the last point of its tandem run path because of the time taken to remove the leader (see Materials and methods for more details). All ants in the TR panel in both E1 and E2 moved forward in the direction of the new nest. All ants in the FF panel headed towards the old nest after searching, except two ants in E1, L, FF, orange and light blue lines, which were lost (i.e. both ants failed to exhibit any directionality either towards the new nest or the old nest 10 min after interruption).

treatments [Fig. 6A–D, two-tailed, two-sample *t*-test for unequal variances [see pp. 128–129 in Zar (Zar, 1998)]; $P > 0.05$ in all cases]. However, in all treatments, there was a significant difference between experiment 1 and 2 in the time when the ants stopped searching and began their return to the old nest. In the right treatment, the second break point, between the search phase and the return phase, in experiment 1 occurred significantly earlier (95% CI: 24.2–59.4 s) compared with experiment 2 (95% CI: 110.4–138.7 s; Fig. 6A). In the left treatment, the break point between the search and the return phase was also significantly earlier in experiment 1 (95% CI: 22.3–62.8 s; Fig. 6B) compared with experiment 2 (95% CI: 65.2–114.0 s; Fig. 6B). This was also the case in the centre treatment ($t_{36} = 2.410$, $P < 0.05$; Fig. 6C). By contrast, in the no-landmark treatment, the break point between the search and the return phase was significantly earlier in experiment 2 than in experiment 1 ($t_{34} = 2.642$, $P < 0.05$; Fig. 6D). Nevertheless, in experiment 2 under the no-landmark treatment, the 83 s mean value for this break point was still within the range of values for the other three treatments (70 s, centre treatment; 86 s, left treatment; and 124 s, right treatment; supplementary material Table S1). By contrast, in experiment 1, in the no-landmark treatment, the 151 s mean value of the same break point was four times longer than those for the other treatments (27 s, centre; 37 s, left; and 38 s, right; supplementary material Table S1).

DISCUSSION

We used a motorised gantry for the first time to track tandem running ants more accurately, over much larger distances, and in the presence of landmarks that were better controlled than has been achieved before. We then interrupted tandem runs by taking away the leader to determine what information follower ants may have obtained from the partial tandem run they had followed and how they behave after the tandem run has been interrupted by the removal of the leader.

Results show that in the immediate aftermath of the separation from her leader, a former follower makes a short quick movement probably in an attempt to re-establish contact. Then the ants search in either a sub-diffusive or a diffusive (random) movement. This might improve their initial chances of finding their lost leader or the new nest. Moreover, random search strategies can also be surprisingly effective, especially when animals have no information about the location of a target (Bartumeus et al., 2005) (see also Schultheiss and Cheng, 2011). After a random search, which was longer in the presence of local landmarks, but only if the large distant landmark behind the new nest was present, former follower ants typically headed towards the old nest and got there more swiftly when local landmarks were absent because their search phase was reduced. Even when all landmarks were removed, most former followers managed to find their way back to the old nest within 10 min (Fig. 4E1,N,FF). However, the fourfold

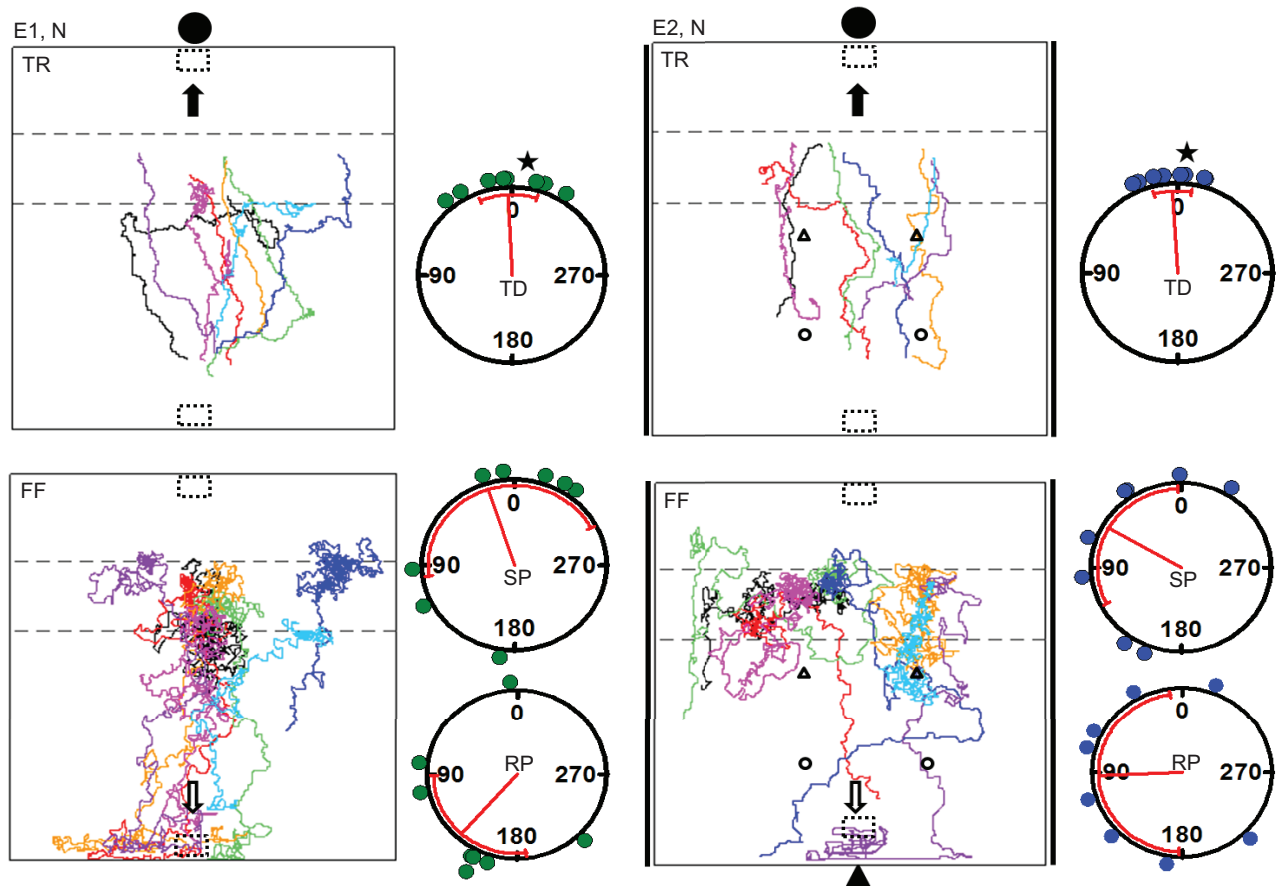


Fig. 4. The paths of follower ants during the tandem run and after interruption, together with mean directional bearings of their movements for the no-landmark treatment (N). All symbols are the same as in Fig. 1. All ants in the TR panel in both E1 and E2 moved forward in the direction of the new nest. All ants in the FF panel headed towards the old nest after searching.

increase in their search phase and the lack of consistent orientation in their mean bearings during both the search and the return phase (Fig. 4E1,N,SP,RP) indicate that the absence of landmarks is a hindrance to tandem follower ants separated from their leaders. Former followers also did not slavishly follow the route they had taken during tandem running. This indicates that, despite having engaged in a convoluted search, rock ants (*Temnothorax albipennis*) are able to find their own way back to the old nest even over environmental scales that are more than two times larger than those studied before (see Franks et al., 2010).

In earlier work, with much shorter interrupted tandem runs and without controlled landmarks, former followers sometimes extrapolated forward towards the new nest site (Franks et al., 2010). In the present study, extrapolation occurred just two times in the first experiment and not once in the second experiment. This goes against

our expectation that the number of ants that are able to extrapolate forward towards the new nest will be higher in the second experiment, where, in addition to the large landmark behind the new nest, there were small local landmarks inside the arena together with a large landmark behind the old nest. Previous studies have shown that cues from a conspicuous object may draw animals to it and thus help them in their navigation (reviewed by Rodrigo, 2002). In our case, the ants chose not to continue their journey after an interrupted tandem run. Losing their leader part-way before reaching the goal might inform a former follower ant that it is not safe to continue her journey, thus it may be a much safer option to head home rather than to proceed forwards into *terra incognita*.

Even though the ants did not extrapolate forward towards the new nest or the large landmark behind it, results from the present experiments do show that the large distant landmark behind the new

Table 1. Numbers of former follower ants in each treatment of each experiment with a breakdown according to whether they found the new nest, headed towards the old nest, or apparently became lost (neither found the new nest nor headed towards the old nest) within 10 min after the separation from the leader

Experiment	Treatment based on position of landmark behind the new nest				Extrapolated towards the new nest		Headed towards the old nest	
	R	L	C	N	Found new nest	Lost	Returned	Not
	1	8	11	8	8	2 (in C)	2 (in L)	24
2	8	8	8	8	0	0	11	21

R, landmark moved to the right; L, landmark moved to the left; C, landmark picked up and replaced; N, landmark removed. We defined a former follower as having returned if it had reached the line described by the proximate wall of the new nest within the 10 min of observation. The number of former followers that returned in experiment 1 was greater than that in experiment 2 for all four treatments (R: 7 and 4, L: 7 and 2, C: 5 and 3, N: 5 and 2, respectively).

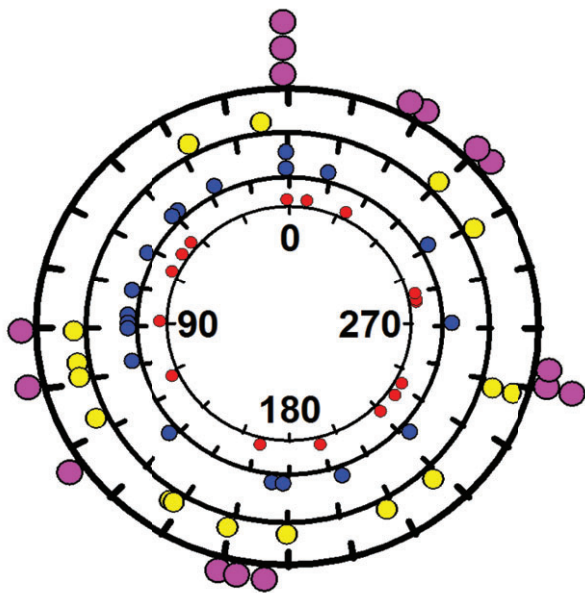


Fig. 5. Mean directional bearings during the catch-up phase. Mean bearings for experiment 1 and experiment 2 were pooled together. None of the results differ from randomness, indicating that the ants did not show any directional preferences (Rayleigh test, $P > 0.05$ in all cases); from the outermost circle, pink: no landmark; yellow: centre; blue: left; red: right treatment.

nest and the small local landmarks together with the large landmark behind the old nest interact in a way that affects the former follower ants' navigation. This is because in experiment 1 the ants searched briefly when the large landmark behind the new nest was maintained even if it had been displaced, whereas in the no-landmark treatment, that is in the absence of any landmarks, the ants searched four times longer. This suggests that in the absence of landmarks, the ants need longer to become orientated. When more landmarks were added in experiment 2, the search phase was significantly longer than in experiment 1 except for the no-landmark treatment. In the latter, search duration was still within the range of the rest of the treatments in experiment 2 but movement was super-diffusive instead of sub-diffusive or diffusive. Perhaps in an environment rich with information (in the presence of the large landmark behind the new nest and the local landmarks together with the large landmark behind the old nest), former follower ants are less at risk of becoming lost and can therefore search for longer (e.g. Bühlmann et al., 2011; Schultheiss et al., 2013). However, when not all landmarks are present (experiment 2, no-landmark treatment), this second phase is of a similar duration but involves a more orientated movement than a search.

The way the local and distant landmarks interact and affect the orientation of former followers is evident not only during the search phase but also during the return phase of their movements after interruption. The super-diffusive, orientated movement in the return phase is faster in experiment 2 than in experiment 1 for the right treatment but the opposite is true for the no-landmark treatment (Fig. 6A,D). Furthermore, the regression line for experiment 2, particularly in the centre and no-landmark treatments, finishes below the line for experiment 1, indicating that displacement in experiment 2 does not reach as far from the interruption point as in experiment 1. This matches the less orientated appearance of the return paths in experiment 2, particularly for the centre and no-landmark treatments (Figs 1, 4, FF panels). Why might there be such an apparent

slowing-down effect of the additional landmarks introduced in experiment 2 on the return of former followers to the old nest? Under the right, left and centre treatments, this might be due to the delay associated with the longer search phase. Furthermore, the ants could be using the local landmarks as beacons (McLeman et al., 2002) to help them recognize the paths they have taken during tandem running. Previous work has shown that some ants aim directly towards small local landmarks in the arena and move near to such landmarks before shifting their paths towards the new nest (McLeman et al., 2002). This may have made the paths in experiment 2 spread over a wider area and appear less orientated than in experiment 1. Therefore, our results strongly suggest that former follower ants learn landmarks during tandem running.

Alternatives to learning landmarks are unlikely. One possible cue that the ants might rely on in the absence of landmarks is pheromonal chemical trails. However, with only minimal traces of chemical trails, because we removed all other ants from the arena (see Materials and methods) and judging by the ants' return paths, which clearly do not overlay their tandem run routes, it seems that they are not following their own chemical trails on their return journeys. It could be that they can calculate a home vector, but over a very large scale this might be very difficult because of the accumulation of errors in their estimations. Thus they might take more time to reorient themselves before heading back towards the old nest. Even for well-trained outbound ants, finding the original goal (i.e. the new nest site) could be a difficult task without the help of any conspicuous cues because they have to make a precise estimation of the distance from their nest to the goal to avoid missing it (Schwarz et al., 2012).

By learning landmarks, former followers could not only maximize their chances of finding the new nest but also of recognizing their way home more easily and thus returning home faster. Hence, spending more time searching for the new goal in landmark-rich environments may well be a fair trade-off for the ants. Indeed, our results, and those for the right treatment in particular, suggest that there is a positive association between the length of the search phase and the diffusivity of the return path. The third slope tends to be steeper when the second phase is longer (Fig. 6A). This suggests that longer searching facilitates a faster return home.

One very strong message from our results is that the effects of large distant and small local landmarks can interact in the way they affect the navigation of former followers. In addition, this effect depends on whether the distant landmark behind the new nest was moved to the right, to the left, picked up and replaced in the centre or removed altogether. The effects we found decreased in strength progressively: (1) the right; (2) the left; (3) the centre; and (4) the no-landmark treatments, respectively. Indeed, we found that *T. albipennis* seem to be biased towards their right visual field and that as a consequence they exhibit behavioural lateralization. That the ants' right visual field is dominant in their navigation is suggested because deviation to the right is significant during the search phase when the large landmark was moved to the right in experiment 1. In addition, when they return to the old nest, the deviation to the left is significant when the large landmark was moved to the left side in experiment 2. This is what would be expected if the ants use their right vision to navigate because their right eye would see the large landmark placed on the left side of the arena as they move back towards the old nest. As a result, mean bearings were biased to the left. Lateralization is typical of the behaviour of many vertebrates and also some invertebrates. In various studies, researchers have found that vertebrates use a different brain hemisphere for specific tasks (Letzkus et al., 2008; Zucca and Sovrano, 2008; Bonati et al.,

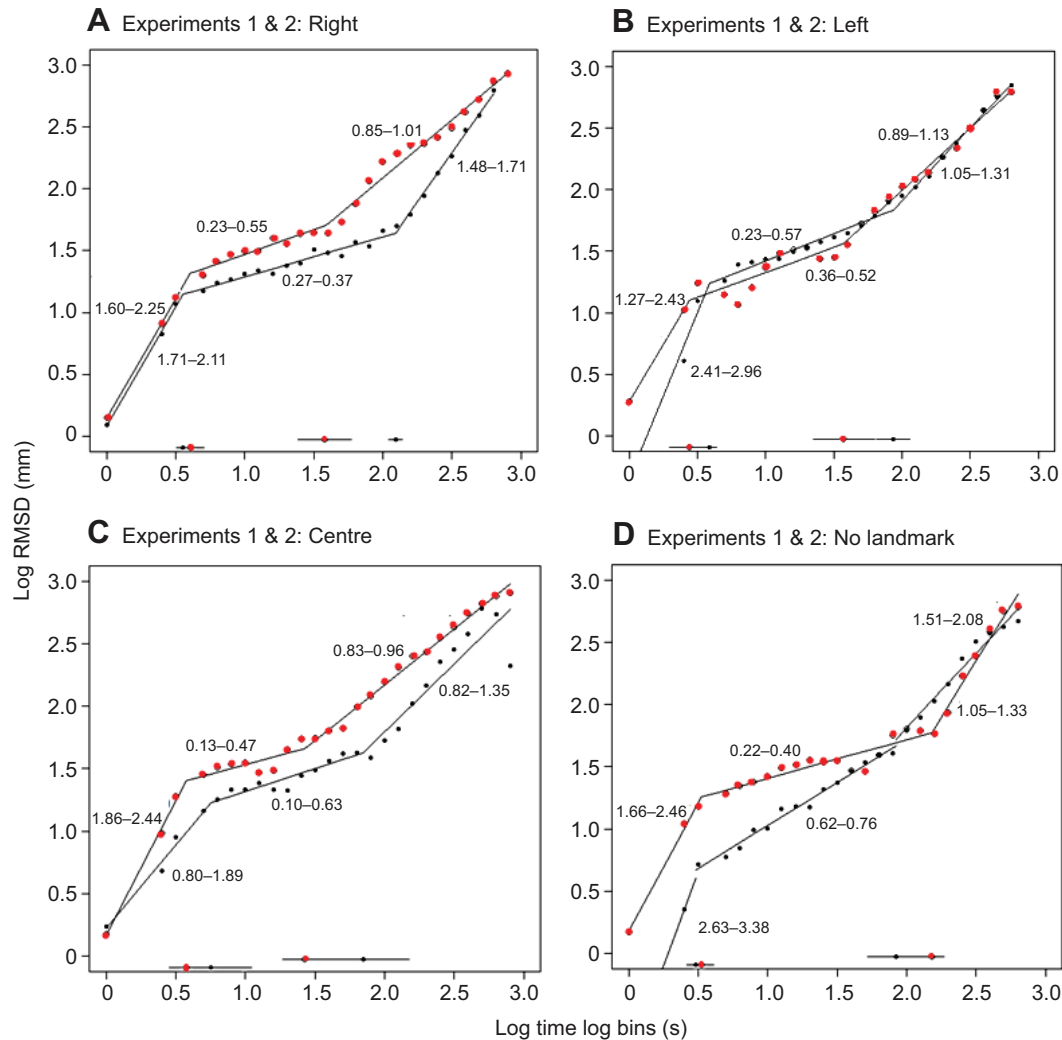


Fig. 6. Log-binned root mean square (r.m.s.) displacement for the trajectories of former tandem followers as a function of the time since the leader was removed in experiment 1 (red dots) and experiment 2 (black dots). See Materials and methods for details of calculation. Numbers on plots represent the lower and upper limit of the 95% CI for each slope of the log–log relationship between r.m.s. displacement and time. See supplementary material Table S2 for slope estimates and goodness-of-fit statistics. Lines at the bottom of each graph represent the 95% CI for the break points of the broken-stick linear regression model (see supplementary material Table S1 for exact values).

2010; Rogers, 2010). Several studies have shown evidence that the right eye/left hemisphere is involved in predatory behaviour and food searching, whereas the left eye/right hemisphere seems to control predator monitoring, making lateralized individuals able to carry out both tasks simultaneously (Bonati et al., 2010). Certain invertebrates such as bees (*Apis mellifera*), were found to use their right eye more often for learning or for the purpose of detecting objects (Letzkus et al., 2008). Hence, the results from our experiments suggest that ants use their right eye to detect landmarks (here, the large landmark behind the new nest), which could help them to remember the paths they had explored. However, further studies should be conducted to validate this possible lateralization behaviour in ants.

In summary, the results of our experiments suggest that the former follower ants gain useful information even from incomplete tandem runs. Even after a long convoluted search and over a much larger environmental scale, many former follower ants managed to return to the old nest. The information derived either from the large distant landmark behind the new nest (experiment 1) or the small local landmarks together with the large landmark behind the old nest

(experiment 2) probably helped the ants to compute the general bearing of the path they took during tandem runs. Even though the ants did not extrapolate forward towards the new nest or towards the large landmark behind the new nest, information derived from the landmarks was important in helping ants in their navigation on their way home and also in their search strategies. This is supported further by the evidence that ants have a bias towards the right visual field even on their way back home. We show that individual ants are able to make strategic decisions by changing their behaviour after weighing benefits and risks depending on both their current situation and in the context of the spatial scale of their environment.

MATERIALS AND METHODS

The 16 colonies of *Temnothorax albipennis* (Curtis) used for these experiments were collected in September 2011, from the Dorset coast, England, UK. In the laboratory, each colony was housed in an artificial nest, consisting of a cardboard perimeter, sandwiched between two microscope slides (75×50 mm). The cardboard formed an inner nest cavity measuring (50×35×2 mm), with a 2 mm wide entrance. The colonies were provided with water, honey and *Drosophila* flies *ad libitum*.

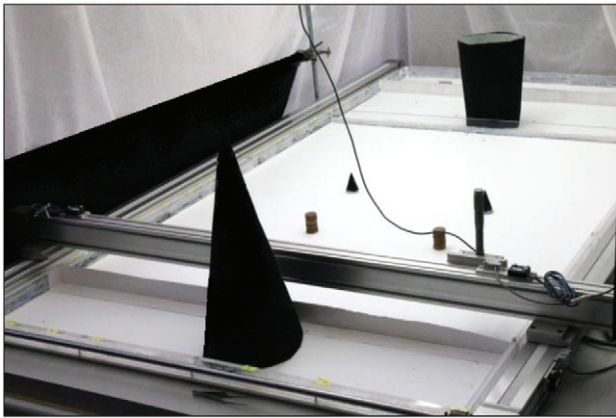


Fig. 7. Experimental arena setup with the motorized gantry. A cylindrical large landmark was placed behind the new nest in experiment 1. In experiment 2, the following additional elements were introduced: four small landmarks were placed inside the tandem run area, a large cone-shaped landmark was placed behind the old nest and a black stripe was provided along both sides of the arena to help keep the ants in the centre of the arena. The two parallel sliders to the left and right were used for the y-axis and the slider going across for the x-axis. A USB camera was attached to the element moving along the x-axis. The whole setup was surrounded with white muslin cloth. For more information, see Materials and methods.

Arena setup and the robotic gantry

An arena measuring 1×1 m and with a 40 mm wall height was used in the two experiments. The inner wall of the arena was coated with FLUON® (polytetrafluoroethylene, also known as PTFE) to prevent the ants escaping.

A robotic X-Y motorized gantry was positioned above the experimental arena (Fig. 7). The main frame of the X-Y gantry was constructed from power supply unit (PSU2) linear sliders (HepcoMotion® Lower Moor Business Park, Tiverton Way, Tiverton, Devon, UK). Two parallel sliders were used for the 1.5 m y-axis; and one 1 m unit was used between them as the x-axis (during the experiment, the gantry was set to move along 1×1 m for the x- and y-axes). The drive used SA28 system stepper motors (SmartDrive Ltd, The Old School, Earith, Cambridge, UK). A universal serial bus (USB) camera (Logitech® Webcam, Slough, Berkshire, UK) mounted on the gantry was used to follow the paths of individual or multiple moving ants, where the position of the gantry and thus the camera was controlled manually by the experimenter with an analog joystick. Movement speeds of the gantry (and thus the camera) were controlled between 0.5 and 40 mm s⁻¹ and positive and negative accelerations were controlled precisely between 0.2 and 60 mm s⁻². An in-house LabVIEW™ based program (v10.0 National Instruments, Austin, TX, USA) interfaced with the gantry motion-control system using standard active-X controls. Within the software we developed the capability also to view the camera image and gantry position to record the paths of the ants and input behavioural observer keystroke data. Specifically, images of each ant or group of ants were recorded at 20 frames s⁻¹ and were converted in real time from the red, green, blue (RGB) output format of the camera into a hue, saturation, luminance (HSL) colour space format. Previous trials had allowed the experimenter to manually optimize the image contrast level. The luminance channel of each frame was then thresholded and the following LabVIEW Vision Development Module image processing routines were then used to record the position of the centre of mass of each of the ants: fill-holes; remove-boarder-objects; particle-analysis-report. By combining the calibrated position of the ant within the image and the real world position of the gantry, the x-y coordinates of the moving ants were recorded with a resolution of 0.05 s and with an accuracy of ±0.1 mm.

Experimental procedures and data collection

The two experiments were conducted one after the other from 24 October 2011 to 2 February 2012. Data were collected 4 days per week between 13:00 h and 18:00 h. Each of the 16 colonies involved in the experiments

had more than a 100 workers, a single queen and more than 100 brood items. Thirty-five tandem runs were recorded in experiment 1 and 32 in experiment 2. Each of the 16 experimental colonies was used at most twice in each experiment 1 and 2. There was a period of at least 6 days before any colony was used again in an experiment. This was to ensure the ants had forgotten the previous experience of emigration (Langridge et al., 2004), during which tandem running could have occurred.

Experiment 1: large distant landmark behind the new nest

During each trial, the test ant colony in its nest was placed in the arena together with a new better quality nest. The inner cavity and entrance size of the new nest were the same as those of the old nest (50×35×2 mm cavity size and a 2 mm wide entrance) but the top slide was covered with cardboard to make it dark and more attractive. The new good quality nest was placed 1000 mm away from the old nest (distal wall to distal wall). *T. albipennis* workers are 2 mm long, and 1000 mm is a considerable distance for them to cover. A cylindrical large landmark measuring 594 mm in height and 250 mm in diameter was placed centrally behind the new nest at the start of each trial. To induce emigration, the old nest was destroyed by removing its top slide. The ants were allowed to search for and then investigate the new nest. As soon as the first tandem run had begun, all other scouts wandering in the arena were removed carefully using an aspirator and were returned to the old nest. The old nest was covered with a Petri dish to prevent ants, other than the target tandem pair, from wandering in the arena. This was done to prevent the follower from receiving information from another scout after the leader ant had been removed. This also allowed the operator of the joystick to track the tandem pair without the risk of confusing the tracked individual with other ants in the arena.

Once the tandem run had begun, the pair was followed with the gantry and the coordinates of the follower ant were recorded. This was done to compare the route taken by the follower before and after the leader was taken away. For each tandem pair, the gantry coordinates were set to (0,0) at the start of data collection. Once the ants started a tandem run, the gantry, controlled by the joystick, tracked the follower until the pair had reached the interruption zone. When the tandem pair arrived in the interruption zone, which was between 600 and 800 mm from the old nest, the gantry was stopped and the leading ant was removed. The leader was removed gently with softly sprung entomological forceps when a natural separation occurred between the leader and the follower. We did this so that the follower was not disturbed directly by the removal of the leader. After the leader had been removed, the gantry tracked the former follower ant and continued recording her coordinates from the point where the leader ant had been removed, except in four cases, for up to 10 min. In each of these four cases, after the follower entered the interruption zone and the gantry was stopped, it took approximately a minute to remove the leader (namely: 71 s for E1, C, green path; 57 s for E1, R, black path; 83 s for E2, R, pink path; 85 s for E1, L, orange path). Hence, for these followers, the start of the track after separation from the leader does not coincide with the end of the track during the tandem running. If the former follower ant found the old nest in less than 10 min, data recording stopped when she entered the old nest. Each removed leader was kept in a separate Petri dish to prevent it from sharing any information with other colony members. After a trial with a colony was completed, the arena floor was cleaned with 70% alcohol and water to remove any traces of pheromone trails. To minimize the effect of polarised light cues, the laboratory windows were shaded. The whole experimental set-up was surrounded with white muslin cloth to obscure permanent landmarks in the laboratory (e.g. computers, lamps, etc.) and to minimize any potential biases from external influences (Fig. 7).

To investigate whether the large landmark behind the new nest might act as a beacon that could aid the former follower ant in her navigation, the former follower ant was left to search for the new nest (after the leader had been removed) under four conditions (which are referred to as treatments): (1) in the presence of the large landmark, which was picked up and re-placed centrally behind the new nest; (2) with the large landmark moved to the right; (3) with the large landmark moved to the left; and (4) with the large landmark removed (Fig. 8A). In treatments 2 and 3, the large landmark was moved to its respective position (right or left) and in treatment 4 it was removed from the arena. Each of these treatments was carried out

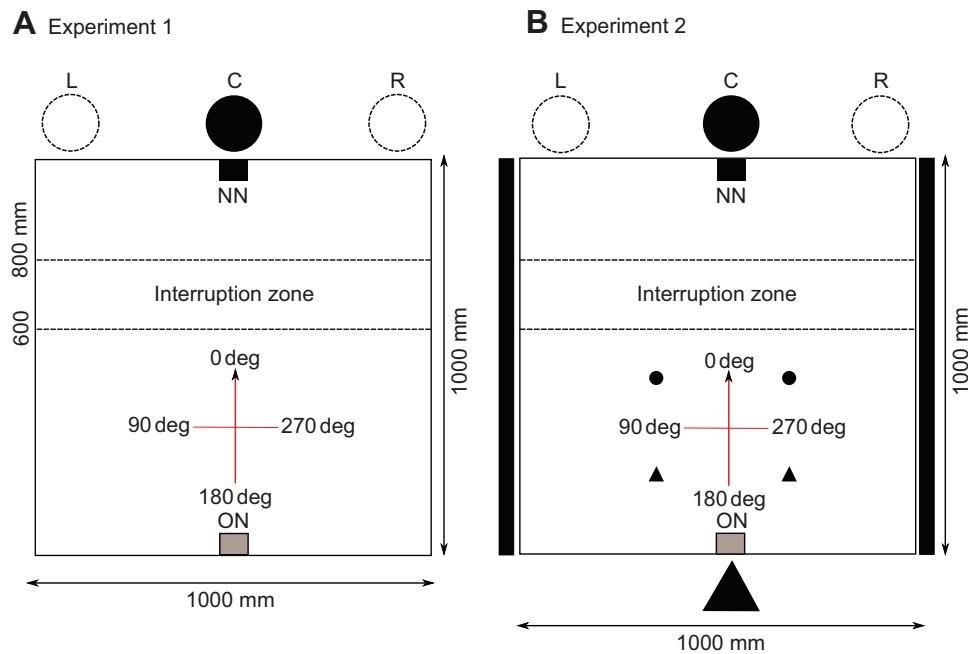


Fig. 8. The top view of the arena setup. (A) Experiment 1; (B) experiment 2 (not to scale). Rectangles represent the nests: ON, old nest; NN, new nest. The black circle behind the new nest indicates the large cylindrical landmark at the centre position (C). Circles with dashed lines and the letter L or R indicate the location of the large cylindrical landmark for the left and right treatments. The two triangles and the two circles inside the arena represent the four small local landmarks. The thick black lines on both sides of the arena indicate the black stripes added in experiment 2. The two horizontal dashed lines represent the interruption zone. The red arrow indicates the direction from the new nest (0 deg) to the old nest (180 deg).

immediately after the leading ant had been taken away from its follower. Each of the four treatments was repeated eight times except for the left treatment, which was replicated 11 times.

Experiment 2: additional small local landmarks and a large distant landmark behind the old nest

The arena setup in this experiment was the same as in experiment 1 except that four small local landmarks were placed in a regular pattern inside the tandem run area (Fig. 8B). Two of the small landmarks were cone-shaped (50×35 mm, height × diameter of base) and two were cylindrical (40×25 mm, height × diameter). In addition, one large cone-shaped landmark (370×170 mm, height × diameter) was placed behind the old nest and a long black cloth (315×1000 mm; height × length) was placed horizontally along the two sides of the arena in front of the white muslin cloth to help keep the ants in the central part of the arena (Pratt et al., 2001). The four treatments and experimental procedures as described in experiment 1 were repeated again. Each of the four treatments was repeated eight times in experiment 2.

Statistical analysis

We performed coarse graining to minimize the spurious 0, 90, 180 and 270 deg bearing angles, which are inevitable because of digitization at very short time intervals when paths are recorded with high time precision (0.05 s here). The raw path coordinates recorded by the gantry were smoothed by four iterations of two times (or altogether 16 times) coarse graining. Four iterations of smoothing were chosen in preference to 1, 2 or 3 because they reduced digitization substantially. Each iteration of two times coarse graining consisted of calculating the weighted mean (x', y') of two successive coordinates (x_i, y_i) and (x_{i+1}, y_{i+1}), at time $t' = (t_i + t_{i+1})/2$. Thus, $x' = (x_i \Delta t_i + x_{i+1} \Delta t_{i+1}) / (\Delta t_i + \Delta t_{i+1})$; $y' = (y_i \Delta t_i + y_{i+1} \Delta t_{i+1}) / (\Delta t_i + \Delta t_{i+1})$, where x_i and y_i were the coordinates of the ant's position at time t_i , and $\Delta t_i = t_{i+1} - t_i$.

Heading directions and trajectories of follower ants during tandem running and after interruption

The compass bearing of each former follower was calculated on the 16-times coarse-grained data as:

$$\alpha_i = \cos^{-1} \frac{(x_i - x_{i+1})}{\sqrt{(x_i - x_{i+1})^2 + (y_i - y_{i+1})^2}}, \quad (1)$$

where x_i and y_i are the coordinates of the ant's position at time t_i . Then for each former follower we calculated the mean bearing angle during the tandem running and for each of three phases after interruption. We determined the first and the last positions of the ants in each phase using the break points (supplementary material Table S1) given by a broken-stick regression model fitted to the root mean squared (r.m.s.) displacement of

former followers (see later). The compass bearing calculations were carried out in Minitab® v. 15 (www.minitab.com). The counter-clockwise mean compass bearings (0–360 deg) of tandems and former followers with 95% CIs were calculated and Rayleigh tests (Batschelet, 1981) were carried out in Oriana, v. 4 [Kovach Computing Services (Kovach, 2011)] circular statistics software. The direction of the new nest was at 0 deg and the direction of the old nest was 180 deg. To test whether angle distributions were significantly different from random and hence whether it was meaningful to use 95% CI for their means, we used the Rayleigh test. Based on such results, we pooled the bearings data for the 'catch-up' phase after interruption (see later) for the two experiments for each treatment. This is because in both experiments and all treatments the former follower ants did not show any directional preference during the 'catch-up' phase ($P > 0.05$, Rayleigh test in each case).

Search behaviour of former follower ants after interruption

To test for different phases in the movement of the former followers after interruption, we fitted a broken-stick linear regression model to the \log_{10} – \log_{10} relationship of r.m.s. displacement against time using the 'segmented' R-package v. 0.2-9.1 (Muggeo, 2003; Muggeo, 2008) in R (R Development Core Team 2012). We calculated the r.m.s. displacement on the 16 times coarse grained data according to the method described by Franks et al. (Franks et al., 2010) using Minitab® Statistical Software v. 15. We calculated the distance of the position of the former follower from the point of separation from the leader. Then we calculated the mean over all ants for each time point and took the square root of this mean. Time was binned logarithmically to improve the reliability of the means at long times and the subsequent regression analyses.

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Competing interests

The authors declare no competing financial interests.

Author contributions

The experiments were designed by N.B., N.R.F. and A.B.S.-F.; the experiments were conducted by N.B., A.C.B. and C.E.H.; N.W.R. and N.R.F. helped to

construct the gantry; N.B., A.B.S.-F. and N.R.F. analysed the data; and N.B., A.B.S.-F., N.R.F. and N.W.R. contributed to writing the paper.

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Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.087296/-/DC1>

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Table S1. Breakpoints between the catch-up and search phase, and between the search and return phase for the former follower ants for each experiment and treatment

Treatment (landmark position)	Experiment	Breakpoint	Estimate value (s)	95% CI lower value (s)	95% CI upper value (s)
Right	1	1	4.05	3.23	5.07
	2	1	3.57	3.19	4.00
	1	2	37.93*	24.21	59.43
	2	2	123.88*	110.41	138.68
Left	1	1	2.77	1.97	3.89
	2	1	3.89	3.46	4.39
	1	2	37.41*	22.34	62.81
	2	2	86.10*	65.16	114.03
Centre	1	1	3.75	3.17	4.44
	2	1	5.61	2.83	11.12
	1	2	26.67	18.49	38.37
	2	2	70.47	33.04	150.31
No landmark	1	1	3.33	2.73	4.07
	2	1	3.04	2.62	3.53
	1	2	151.01	123.03	185.78
	2	2	83.37	52.60	132.43

*Significant difference between experiment 1 and 2 at 5%.

The second breakpoint is an estimate of mean search time; 'Landmark position' refers to the large distant landmark behind the new nest.

Table S2. Slope estimates and multiple R^2 -values for the broken-stick linear regression model for each of the three slopes for each experiment and treatment

Treatment (landmark position)	Experiment	Slope	Estimate value	Multiple R^2
Right	1	1	1.9250	0.9936
		2	0.3899	
		3	0.9331	
	2	1	1.9100	0.9963
		2	0.3201	
		3	1.5960	
Left	1	1	1.8500	0.9872
		2	0.4044	
		3	1.0130	
	2	1	2.6800	0.9959
		2	0.4394	
		3	1.1800	
Centre	1	1	2.1480	0.9948
		2	0.3008	
		3	0.8936	

	2	1	1.3400	
		2	0.3671	0.9635
		3	1.0890	
No landmark	1	1	2.0580	
		2	0.3105	0.9872
		3	1.7960	
	2	1	3.0020	
		2	0.6900	0.9969
		3	1.1890	