



## Social learning of navigational routes in tandem-running acorn ants, *Temnothorax nylander*

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Tandem running in ants is a form of social learning that involves an informed leader guiding a naïve nestmate to a valuable resource, such as a nest site or a food source. Little is currently known about what tandem followers learn and how socially acquired navigational information affects future trips. While some studies suggest that tandem followers learn the resource position but not the route taken by the tandem pair to reach the resource, more recent evidence contradicts this view. We studied tandem running in foraging acorn ants, *Temnothorax nylander*, and provide evidence that tandem followers socially learn routes from their leaders and later use these routes when travelling between their nest and a food source. Followers that became tandem leaders themselves then guided their follower along the same routes in 90% of tandem runs, demonstrating that navigational information can spread in a forager population through sequential social learning. Ants increased their travelling speed, but not path straightness over successive trips. We also found that ants needed less time on subsequent trips if they experienced longer-lasting tandem runs, suggesting that longer-lasting tandem runs allow followers to learn routes more efficiently. Adding potentially salient visual cues did not affect most of the quantified variables, and we currently know little about the cues used by *T. nylander* during navigation. We discuss how the visual environment inhabited by different species might affect the importance of route learning during tandem running.

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Social learning shapes the behaviour of animals in a wide range of biological contexts as it allows animals to acquire behaviours that boost survival and reproduction (Heyes, 2012; Hoppitt & Laland, 2013; Kendal et al., 2018; Laland, 2004). Social insects frequently rely on social information, provided in the form of signals or incidental cues; for example, when foraging, during colony migrations and for nest defence (Grüter & Leadbeater, 2014; Leadbeater & Chittka, 2007; Leadbeater & Dawson, 2017).

A relatively well-studied example of social learning in social insects is tandem running in ants: after an ant has discovered a good food source or a suitable nest site, she returns to her nest to guide a fellow nestmate to the discovered resource (Franks & Richardson, 2006; Hingston, 1929; Kaur et al., 2012; Möglich et al., 1974; Silva et al., 2021; Wilson, 1959; reviewed by Franklin, 2014; Sasaki & Pratt, 2021). During foraging, tandem running may help colonies

visit food sources of better quality and defend them as a group against competitors (Glaser et al., 2021; Glaser & Grüter, 2023; Goy et al., 2021; Shaffer et al., 2013). During nest relocations, tandem running helps colonies assess the quality of new nest sites and migrate more efficiently (Mizumoto et al., 2023; Stuttard et al., 2015). Tandem running has been described in dozens of ant species and is likely to have evolved several times independently in phylogenetically distant ant groups belonging to at least four subfamilies (Glaser & Grüter, 2022; Mizumoto et al., 2023). A common feature of ant species that use tandem running is that they have relatively small colony sizes (Beckers et al., 1989; Glaser & Grüter, 2022).

To initiate a tandem run inside the nest, tandem leaders of some (and maybe all) species produce a short-range pheromone that alerts potential followers to the discovery of a resource and attracts them to the tandem leader ('tandem calling'; Möglich et al., 1974). Ant pairs then walk towards their goal, with the follower ant frequently touching the legs and abdomen of the leader to signal her presence, while the leader continues to release pheromone to help followers maintain contact with the leader (Basari,

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Laird-Hopkins, et al., 2014; Franks et al., 2022; Hölldobler & Traniello, 1980; Möglich et al., 1974; Stuttard et al., 2015). Because tandem leaders invest time to help a nestmate learn the location of a resource, tandem running is often considered a case of animal teaching (Franks & Richardson, 2006; Richardson et al., 2007). It is still unclear, however, what ants learn when they follow a tandem leader and how this information affects future navigation. Followers might learn the target location in relation to the nest location and return to their nest using path integration or environmental cues, such as landmarks or local odours. Ants can perform path integration by using a celestial compass, to gauge direction, in combination with a step-counting mechanism to estimate distances (Wittlinger et al., 2006). This type of navigational learning has been studied extensively in desert ants (especially *Cataglyphis* and *Melophorus*, reviewed in Collett et al., 2013; Knaden & Graham, 2016; Zeil, 2012). Alternatively, tandem followers could learn the route itself and later attempt to retrace their steps when returning home, relying on environmental or idiothetic (self-generated) cues (Knaden & Graham, 2016; Sasaki et al., 2020). It has been argued that tandem followers do not learn routes in the rock ant *Temnothorax albipennis*, the only ant species where this has been studied in more detail, as ants did not return to their nest using a similar path to the one taken by the tandem pair (Basari, Bruendl, et al., 2014; Franklin, 2014; Franklin & Franks, 2012; Franks & Richardson, 2006). Instead, these studies suggested that *T. albipennis* tandem followers learn target locations and subsequently use path integration and visual cues for homing using different routes. In contrast, Sasaki et al. (2020) recently provided the first quantitative analysis of paths of former tandem followers and found that tandem followers did learn routes from their leaders, which they subsequently used when walking from their nest to the food source, but not when they returned from the food source to the nest. The finding that the learning of specific routes in *T. albipennis* was only apparent when ants travelled towards the food, but not towards the nest, is consistent with the earlier observations that ants returning to their nest did not use the tandem route (Basari, Bruendl, et al., 2014; Franklin & Franks, 2012; Franks & Richardson, 2006). Visual cues seem to play an important role in *T. albipennis* ants navigating between nest and resource (McLeman et al., 2002; Pratt et al., 2001).

It is currently unknown if learning during tandem following is similar in other ant species. Here, we assessed learning during tandem following in the acorn ant *T. nylanderi*, a species inhabiting woodland habitats (Seifert, 2018), using two different experimental set-ups. First, we tested ants in a binary choice set-up that offered ants two alternative routes to a food source, mimicking a situation where the most direct path from nest to food is blocked by an obstacle. In the second set-up, ants navigated in an open area; that is, an environment without obstacles. Since visual cues have been shown to affect navigation in other *Temnothorax* species (*Temnothorax unifasciatus*: Aron et al., 1988; *T. albipennis*: McLeman et al., 2002; Pratt et al., 2001; *Temnothorax rugatulus*: Bowens et al., 2013) and in the tandem running *Diacamma indicum* (Mukhopadhyay & Annagiri, 2021), we tested ants in visually enriched and nonenriched environments. In addition, we also tested if former followers would teach routes they learned to naïve followers in later trips.

## METHODS

### Study Species and Maintenance

Twenty-six colonies were collected from small decaying branches. For experiment 1, 13 colonies were collected between September 2020 and December 2021; colony size (mean  $\pm$  SD) =  $121 \pm 20.0$ . For experiment 2, 13 colonies were collected between

November 2014 and April 2016; colony size =  $125 \pm 28.3$ . All colonies had a reproductive queen and brood. Colonies were kept in artificial nests made of two microscope slides (50 mm  $\times$  10 mm and 3 mm high) with a plexiglass slide in between the slides containing an oval living space and a nest entrance. Nests were covered with translucent red filter paper to reduce the light entering the living space. Each nest was stored in a nestbox (100 mm  $\times$  100 mm and 30 mm high). Colonies were fed twice a week with a drop of honey and fruit flies or crickets. Water was available ad libitum throughout the study in a 1.5 ml Eppendorf tube. Colonies were kept in a climate chamber at a temperature of 22 °C, 70% humidity and a 12:12 h light:dark cycle. Prior to a trial, colonies were starved of honey for 10 days to make sure ants were motivated to forage.

### Experiment 1

Focal colonies were placed in an experimental arena (20 cm  $\times$  30 cm and 1 cm high, cleaned with ethanol before adding a colony; Fig. 1a) on day 7 of the starvation period to allow ants to acclimatize to the experimental arena and chemically mark the territory (Bowens et al., 2013). The experimental arena presented a binary choice: ants could travel to a food source via a left or a right branch. The arena walls (0.8 cm height) were covered with paraffin oil to prevent the ants from escaping. Each colony was tested once in a visually enriched environment (blue and yellow paper on arena floor) and once without visual enrichment (white paper placed beneath the arena; however, lab equipment and furniture in the laboratory provided visual landmarks; Fig. 1a). Treatment order was randomized for each colony. Due to a malfunction of the climate cabinet, some colonies were damaged and only 21 of 26 possible trials could be performed.

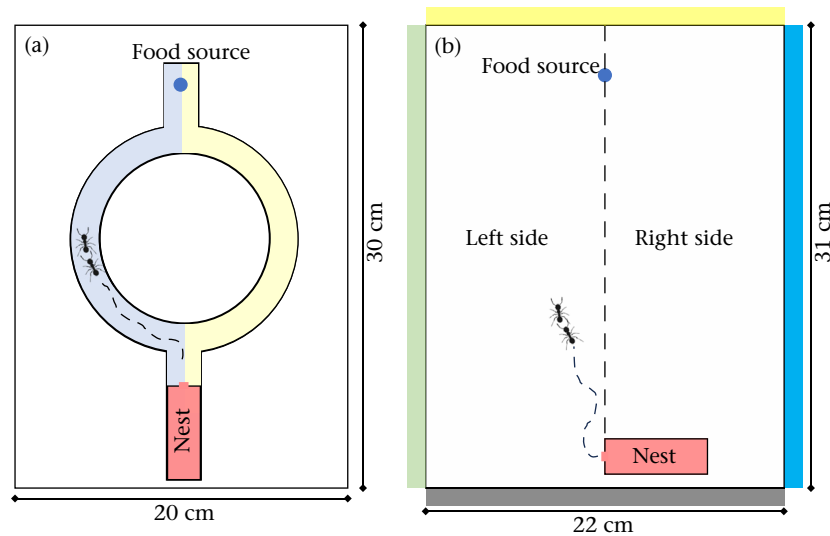
On a test day, a drop of unscented 1M sucrose solution was placed at the opposite end of the arena to the nest (25 cm from the nest; *T. nylanderi* typically forage less than 50 cm from their nest, see Heinze et al., 1996). A trial started when the first scout ant discovered the sucrose solution, and lasted 120 min. We video-recorded trials with a HC-V130 Panasonic camera ([www.panasonic.com](http://www.panasonic.com)) placed above the arena on a tripod. Followers of the first tandem runs (up to 10 followers per trial) were individually marked on the abdomen with different colour dots (Posca, Mitsubishi Pencil, U.K.; [www.mpuni.co.uk](http://www.mpuni.co.uk)) when they were feeding for the first time to allow individual ant identification throughout the trial. After a trial, colonies were returned to their nestboxes, provided with food and allowed a minimum of 7 days recovery before being starved again.

From the video recordings, we recorded the branch choices (left or right) and trip durations of the tandem pairs and of followers' subsequent eight journeys to the nest and the food source. Outward (returns to food) and homing (returns to nest) trip durations were measured as the time between leaving (or entering, in the case of a homing trip) the nest entrance and touching (or leaving, in the case of a homing trip) the sugar solution. We also recorded if former followers became leaders of tandem runs during a trial and we analysed the branch choice of these secondary tandem runs.

### Experiment 2

Focal colonies were placed in an open experimental arena on day 4 of the starvation period, to allow ants to explore and get used to their new surroundings. The experimental arena consisted of a transparent box, 31 cm  $\times$  22 cm and 5 cm high (cleaned with ethanol before adding a colony), with the walls partially covered with Fluon to prevent the ants from escaping (Fig. 1b).

Nests were placed on one side of the experimental arena, 25 cm from the food source location (Fig. 1b). A drop of 1M sucrose solution was again used as a food source. A trial started when the first scout ant discovered the sucrose solution, and lasted 90 min (trial duration was shorter than in experiment 1 because we focused on



**Figure 1.** Experimental arenas used for (a) experiment 1 and (b) experiment 2. (a) Ants could use two branches to reach the food source. (b) Ants navigated an open foraging arena. A 1M sucrose solution was used as a food source. Each experiment tested two conditions, with and without added visual cues. Visual cues consisted of (a) covering the floor with blue and yellow paper or (b) covering the sides of the arena (5 cm height) with blue, green, black and yellow paper (see also Fig. A1).

fewer trips of former followers in experiment 2). The experimental arena was video-recorded from above, again using a HC-V130 Full HD Panasonic camera. Tandem followers were individually marked as described above. After a trial, colonies were returned to their nestboxes, fed and allowed to recover as described above. Each colony was tested once with added visual cues (blue, green, black and yellow paper covering the arena walls; see Fig. A1 for a reconstruction of the ants' perspective) and once without added cues (white paper covering the arena walls). Treatment order was randomized for each colony.

We analysed the tandem runs of individually marked followers, their first return trip to the nest and the first return trip to the food source. We focused on these outward and homing trips because we assumed that they are most likely to be affected by experience gained during the tandem run. We measured trip duration, distance and speed (or rate of progress, cm/s) using the object detection and tracking software AnTracks (<http://www.antracks.org/>). To measure speed, we divided the total length of a trip (cm) by the duration (s) of the trip. Fig. A2 shows the combined trajectories of all studied ants during a 90 min trial.

To assess whether homing ants choose a route passing through the same areas in the arena as when they were tandem followers, we divided the arena into a left and right side (Fig. 1b). We measured the time ants spent on either side, arbitrarily dividing the time spent on the left side by the total travel time to calculate the percentage of time spent on the left side. This allowed us to test if the proportion of time spent on one side during the tandem run predicts the probability that an ant will walk on the same side when returning to the nest or the food source.

### Statistical Analyses

#### Experiment 1

Statistical analyses were carried out in R version 4.1.3 (R Core Team, 2021). To test if former followers used the same path on subsequent trips as during the tandem run, generalized linear mixed-effect models (GLMMs) with a binomial response (1 = same route as tandem run, 0 = different from tandem run) were used (lme4 package; Bates et al., 2015). Linear mixed-effects models (LMEs) with normally distributed response variables were used to analyse trip durations. Visual condition (enriched versus

nonenriched) and trip number were included as fixed effects to explore their effects on branch choice and trip duration. We tested the significance of interactions between fixed effects using likelihood ratio tests (LRTs) and removed nonsignificant interactions (Zuur et al., 2009). Colony ID and ant ID were included as random effects to control for the nonindependence of data points from the same ants and colonies. We checked our LMEs for normality and homogeneity of variance using the DHARMA package (Hartig, 2022) and used the box-cox method if necessary to optimally transform data (Crawley, 2007).

#### Experiment 2

We used GLMMs and LMEs to analyse the effects of visual condition (enriched versus nonenriched) and tandem characteristics (duration, distance, speed and arena side) on the duration, distance, speed and arena side of subsequent homing and outward trips. LMEs were used to test if the proportion of time that ants spend on one side during the subsequent nest return and first food return depended on the time spent on the same side during the original tandem run. Response variables in LMEs were transformed using the box-cox or the Logit transformation if necessary to comply with assumptions, as described above. We tested the significance of interactions between fixed effects as described above. When significant predictors had three levels, pairwise comparisons were performed using the multcomp package (Hothorn et al., 2008). We used the Tukey method to adjust for multiple comparisons by controlling the familywise error rate. When testing for relationships between continuous variables, we calculated marginal ( $R^2_m$ ) and conditional ( $R^2_c$ ) R-squared values using the MuMIn package (Bartoń, 2024). Marginal R-squared values indicate the proportion of variance explained by the fixed effects alone, while conditional R-squared values indicate the proportion of variance explained by both the fixed and random effects (Nakagawa & Schielzeth, 2013). Colony ID and ant ID were again used as random effects.

#### Ethical Note

No licences or permits were required for this research. During experiments the ants were not harmed and were taken care of as outlined in Study Species and Maintenance, above.

**Table 1**

Trips performed by former followers, showing the percentage of ants choosing the same branch as experienced during the tandem run

Trip	N	Same branch as tandem (%)	z	P
Nest return 1	115	87.8	6.93	<0.0001
Food return 1	72	80.6	3.77	0.0002
Nest return 2	71	76.1	2.62	0.0088
Food return 2	47	83.0	4.08	<0.0001
Nest return 3	43	81.4	2.61	0.009
Food return 3	30	83.3	3.21	0.0013
Nest return 4	30	66.7	1.79	0.07
Food return 4	17	82.4	2.38	0.017

## RESULTS

### Experiment 1

Overall, 125 tandem run followers were individually marked and observed. These tandem followers undertook a total of 599 trips to either the nest or the food. Tandem pairs had no significant preference for the left or right branch (GLMM,  $z = 0.77$ ,  $P = 0.44$ ). For the following analysis, we focused on the first four nest returns and the first four food returns after the initial tandem run because only very few ants performed more trips.

For all trips, except nest return trip 4, there was a strong, significant preference to choose the same branch as the one experienced during the tandem run (Table 1; Fig. 2) and even when ants returned to the food source the fourth time, they had >80% probability of choosing the same branch as the one taken during the tandem run. We tested if the strength of this branch preference depended on trip number, travel direction and visual enrichment, and found a significant interaction between trip number and travel direction (GLMM: LRT = 4.80,  $P = 0.028$ ). Therefore, we analysed branch choice in both directions separately. We found that the proportion of homing ants choosing the branch they experienced as tandem followers decreased with

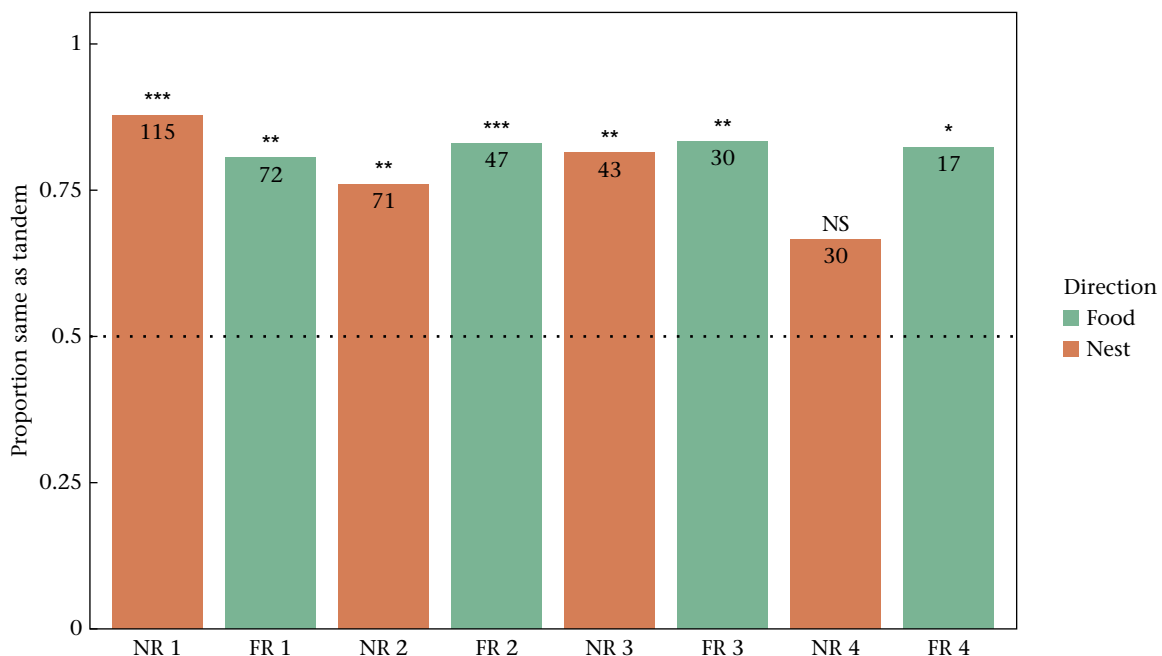
trip number ( $z = -2.73$ ,  $P = 0.006$ ; visual enrichment:  $z = -0.64$ ,  $P = 0.52$ ). There was no change in preference over successive trips for returns to the food source ( $z = -0.57$ ,  $P = 0.57$ ; visual enrichment:  $z = 0.39$ ,  $P = 0.70$ ; Fig. 2). We then tested if trip duration depended on trip number, travel direction and visual enrichment, and found a significant interaction between trip number and travel direction (LME: LRT = 30.3,  $P < 0.0001$ ). Fig. 3 reveals that trips to the nest were faster than trips to the food source, except the first return to the nest, which explains the significant interaction. Therefore, we analysed trip durations in both directions separately.

Trips to the nest decreased in duration with increasing trip number (GLMM:  $t = 13.81$ ,  $P < 0.0001$ ), but there was no effect of added visual cues ( $t = 0.92$ ,  $P = 0.36$ ). Similarly, trips to the food became shorter over successive trips ( $t = 2.99$ ,  $P = 0.003$ ), but no effect of visual cue enrichment was found on trip duration to food ( $t = 0.54$ ,  $P = 0.59$ ). Finally, we tested if trip durations depended on whether ants made 'correct' decisions (i.e. chose the branch they had experienced when they were tandem followers). However, trip duration was not affected by whether ants took correct decisions (LME:  $t = 1.44$ ,  $P = 0.15$ ).

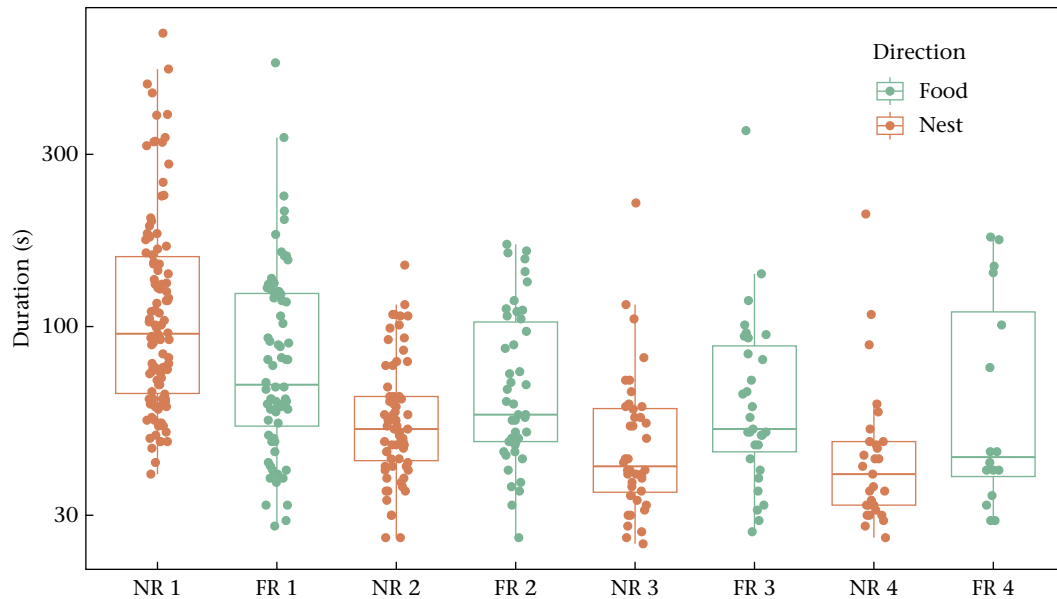
Of all observed followers, 14 went on to lead tandem runs themselves. They led 30 tandem runs, and they were significantly more likely to use the same branch as the one they had experienced when they were tandem followers (90% choosing same branch, GLMM:  $z = 2.29$ ,  $P = 0.022$ ).

### Experiment 2

In the second experiment, ants navigated in an open arena, with or without the presence of additional visual cues on the walls of the arena. Overall, we observed 246 individually marked ants; 200 were tandem-run followers and 46 were scouts that initially discovered the food source. Scouts were also studied to compare the homing time of social and individual learners (tandem followers versus scouts).



**Figure 2.** Proportion of trips where ants chose the same branch as the one taken when they were followers of a tandem run. NR = Nest return; FR = Food return. Numbers indicate sample sizes. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.0001$ .



**Figure 3.** Trip duration (s) for different trips and travel directions. NR = Nest return; FR = Food return. Note that the Y axis is in  $\log_{10}$  scale. Box plots show median (thick horizontal line), 25% and 75% quartile (box) and 5% and 95% percentile (whiskers). Dots represent individual data points.

### Trip Duration

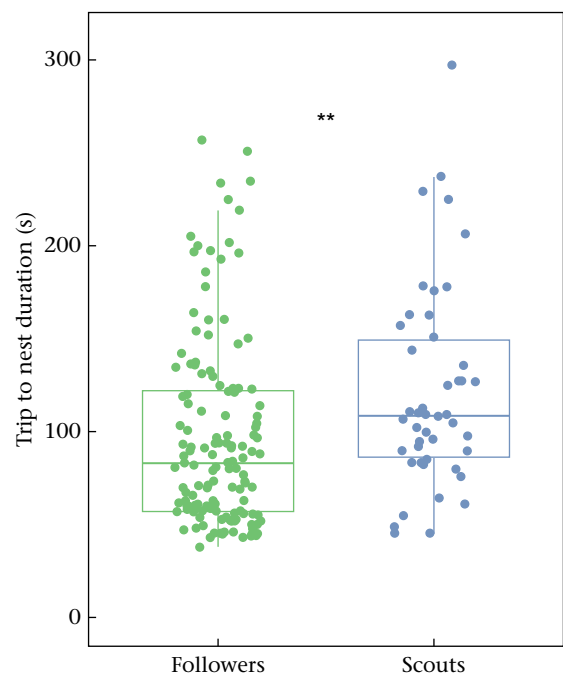
We first compared the homing efficiency of ants that found the food source either by themselves (scouts; individual learners) or by following a tandem run (recruits; social learners) and found that former followers needed significantly less time than scouts ( $-20.2\%$ ;  $121.5 \pm 54.6$  s versus  $97.0 \pm 51.2$  s; LME:  $t = 3.24$ ,  $P = 0.0014$ ; visual cues:  $t = -1.25$ ,  $P = 0.21$ ; interaction: LRT = 0.96,  $P = 0.33$ ; Fig. 4) to return to the nest. We then analysed the characteristics of trips over the course of a trial and found that travel durations decreased (by 55.3% from tandem run to first food return; LME:  $t = 17.65$ ,  $P < 0.0001$ ; pairwise comparisons: to nest versus to food,  $z = -9.69$ ,  $P < 0.0001$ ; tandem run versus first food return,  $z = -17.67$ ,  $P < 0.0001$ ; tandem run versus first nest return,  $z = -8.66$ ,  $P < 0.0001$ ; Fig. 5a). Surprisingly, trips took longer when added visual cues were present ( $t = 4.75$ ,  $P < 0.0001$ , interaction: LRT = 0.9,  $P = 0.34$ ). The distance walked by ants did not change-over time ( $t = 1.62$ ,  $P = 0.11$ ; Fig. 5b), but trip distances were longer when visual cues were present ( $t = 5.21$ ,  $P < 0.0001$ ; interaction: LRT = 0.42,  $P = 0.51$ ). The walking speed doubled during the trial ( $t = 27.34$ ,  $P < 0.0001$ ; pairwise comparisons: to nest versus to food,  $z = -9.57$ ,  $P < 0.0001$ ; tandem run versus first food return,  $z = -27.05$ ,  $P < 0.0001$ ; tandem run versus first nest return,  $z = -19.0$ ,  $P < 0.0001$ ; Fig. 5c), independently of the presence of additional visual cues ( $t = 0.85$ ,  $P = 0.40$ ; interaction: LRT = 0.29,  $P = 0.59$ ).

### Tandem Run Effects

Tandem pairs had no significant preference for the left or right side of the arena (44.4% of time on left side, 55.6% on right side; binomial GLMM:  $z = -1.4$ ,  $P = 0.16$ ). The proportion of time a tandem pair spent walking on one side of the arena significantly affected the proportion of time that ants would walk on the same side when they returned to their nest (LME:  $t = 4.39$ ,  $P < 0.0001$ ; Fig. 6a), independently of visual enrichment ( $t = 0.11$ ,  $P = 0.91$ ; interaction: LRT = 2.86,  $P = 0.091$ ;  $R^2_m = 0.10$ ,  $R^2_c = 0.14$ ). To explore whether this could be caused by chemical marks left on the ground by tandem pairs, we tested if homing ants were more likely to choose the side that was used by the previous tandem pair (i.e.

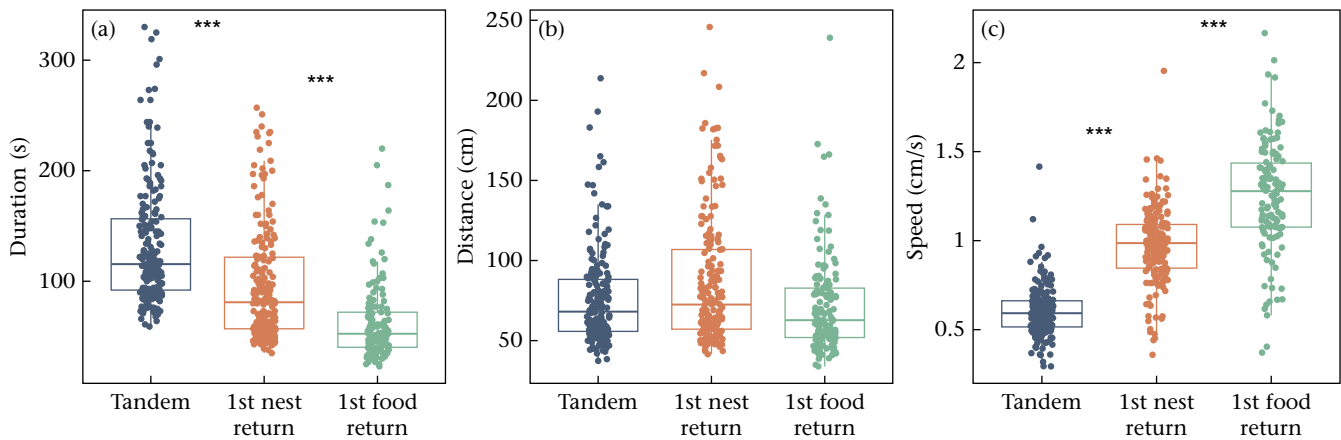
the tandem run that happened just before the ant returned to the nest). Homing ants were not more likely to choose the side that was used by the previous tandem pair (binomial GLMM:  $z = 0.55$ ,  $P = 0.58$ ). Visualizing all trajectories by ants during a typical trial further shows that colonies did not form a pheromone trail during a trial (Fig. A2; see also Sasaki et al., 2020).

We then tested if the proportion of time a tandem pair spent walking on one side also affected the proportion of time that an ant walks on the same side when she returned to the food source alone for

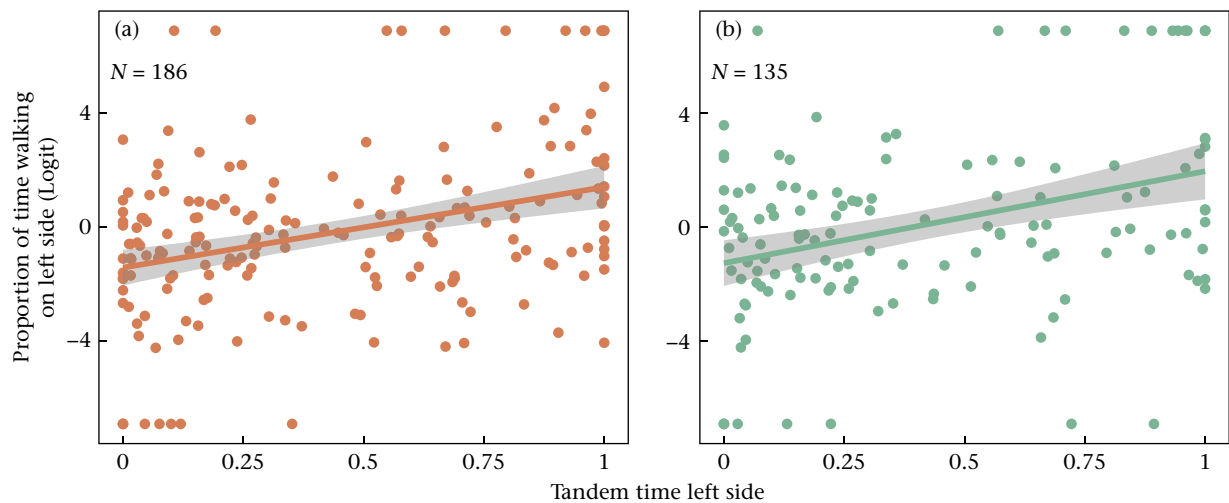


**Figure 4.** Duration of trips back to the nest of tandem followers and scouts. Box plots show median (thick horizontal line), 25% and 75% quartile (box) and 5% and 95% percentile (whiskers). Dots represent individual data points.





**Figure 5.** Characteristics of analysed trips during a trial. (a) The duration of trips decreased during a trial. (b) The distance walked by ants did not change over time. (c) The speed of ants increased during a trial. Box plots show median (thick horizontal line), 25% and 75% quartile (box) and 5% and 95% percentile (whiskers). Dots represent individual data points.



**Figure 6.** Relationship between proportion of time spent (probability of) walking on left side on first return trips and proportion of time spent on left side during tandem run. (a) First return trip to the nest and (b) first return trip to the food source are shown. Dots represent individual ants; the shaded area represents the 95% confidence interval.

the first time, and found a significant positive relationship (LME:  $t = 4.2$ ,  $P < 0.0001$ ; Fig. 6b), independently of visual enrichment ( $z = -1.3$ ,  $P = 0.19$ ; interaction: LRT = 0.005,  $P = 0.94$ ;  $R^2_m = 0.13$ ,  $R^2_c = 0.14$ ).

We tested if the duration of the tandem run predicted the time the former follower needed to return to the nest after drinking, and found a significant negative relationship between tandem run duration and nest return duration (LME:  $t = -2.45$ ,  $P = 0.015$ ; Fig. 7a), but no effect of visual landmark presence ( $t = 0.84$ ,  $P = 0.40$ ; interaction: LRT = 1.39,  $P = 0.24$ ;  $R^2_m = 0.043$ ,  $R^2_c = 0.094$ ). Similarly, the duration of the first return trip to the food source by an ant was negatively associated with tandem run duration (LME:  $t = -2.38$ ,  $P = 0.019$ ; Fig. 7b). The presence of added visual cues significantly increased the time ants needed to return to the food source for the first time, by 29% ( $t = 3.97$ ,  $P = 0.0009$ ; interaction: LRT = 1.24,  $P = 0.27$ ;  $R^2_m = 0.13$ ,  $R^2_c = 0.21$ ).

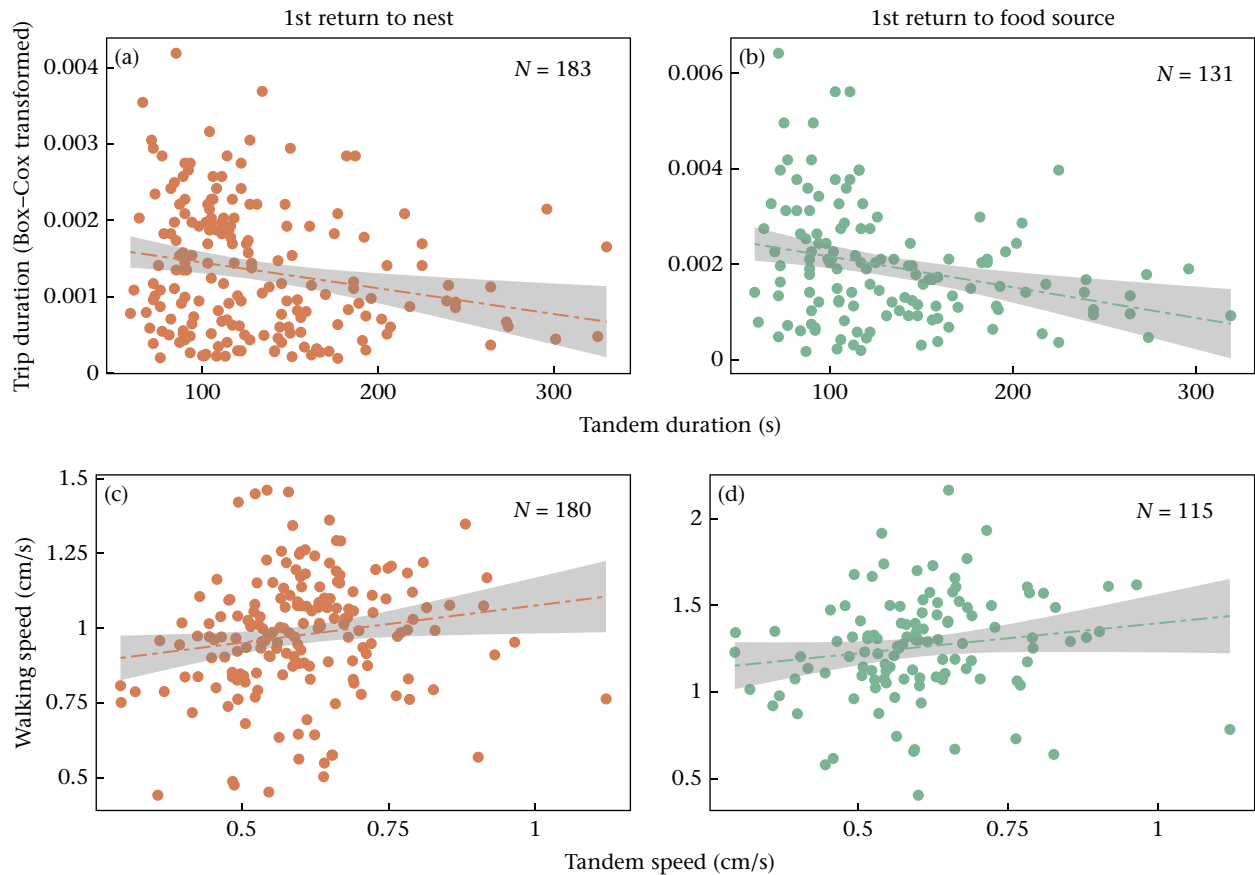
The distance walked by ants returning to their nest did not depend on the distance walked by the tandem pair ( $t = -1.53$ ,  $P = 0.127$ , visual cues:  $t = 0.99$ ,  $P = 0.33$ ; interaction: LRT: 0.0,  $P = 0.98$ ;  $R^2_m = 0.02$ ,  $R^2_c = 0.05$ ). Likewise, the distance walked by ants returning to the food source was unaffected by the distance walked by the tandem pair ( $t = -1.38$ ,  $P = 0.17$ ); however, the presence of additional visual cues increased the walked distance

by 25% ( $t = 4.32$ ,  $P < 0.0001$ ; interaction: LRT = 0.44,  $P = 0.51$ ;  $R^2_m = 0.16$ ,  $R^2_c = 0.28$ ). The walking speed of former followers was weakly linked to tandem speed: faster tandem runs predicted a faster walking speed of ants returning to their nest (LME:  $t = 2.08$ ,  $P = 0.039$ ; visual cues:  $t = 1.18$ ,  $P = 0.24$ ; interaction: LRT = 0.96,  $P = 0.33$ ;  $R^2_m = 0.03$ ,  $R^2_c = 0.09$ ; Fig. 7c) and those returning to the food source ( $t = 1.72$ ,  $P = 0.088$ ; visual cues:  $t = 0.68$ ,  $P = 0.50$ ; interaction: LRT = 0.028,  $P = 0.87$ ;  $R^2_m = 0.03$ ,  $R^2_c = 0.03$ ; Fig. 7d).

## DISCUSSION

We found that acorn ants of the species *T. nylanderi* socially learn routes during tandem running and use this information later when travelling in both directions between a food source and their nest. This contrasts with findings from another ant species, *T. albigipennis*, where navigational learning during tandem running only affected routes of ants walking from the nest to the food source (Sasaki et al., 2020), but not when returning to the nest (Basari, Bruendl, et al., 2014; Franklin & Franks, 2012; Franks & Richardson, 2006; Sasaki et al., 2020).

In experiment 1, we found that the branch taken by the tandem leader affected subsequent branch choices of their tandem followers, as 67–88% of former followers continued to use the same



**Figure 7.** Relationship between tandem run duration (s) and speed (cm/s) with the duration and speed of (a, c) the first return trip to the nest and (b, d) the first return trip to the food source. Dots represent individual ants; the shaded area represents the 95% confidence interval.

branch during the next trips to either the nest or the food source (Fig. 2). Branch preference remained strong over successive outward trips but weakened slightly over successive homing trips. Further evidence for route learning during tandem running was found in experiment 2, which used an open foraging arena with a featureless floor. The proportion of time spent on one arena side by the tandem pair significantly predicted the proportion of time that former followers spent on the same side on subsequent trips, both towards the nest and towards the food source (Fig. 6). This social learning seems to be remarkably efficient given that ants that discovered a food source by following a tandem run needed ca. 20% less time to return to their nest than scouts that discovered the food source by themselves (i.e. through individual learning; Fig. 4). When former followers became tandem leaders themselves, they used the path they were taught by their tandem leaders in 90% of instances, suggesting that social learning of foraging routes spreads to the wider forager population.

These findings suggest that *T. nylanderii* foragers navigated using memorized routes, rather than path integration. Path integration is particularly useful in landscapes that do not offer many visual features, such as landscapes experienced by desert ants (Collett et al., 2013; Knaden & Graham, 2016; Müller & Wehner, 1988; Zeil, 2012), but might be less useful when travelling on forest floors covered in physical obstacles that force ants onto tortuous paths. Retracing your steps (e.g. using idiothetic cues such as leg or body movement) might be a better strategy in such an environment. However, path integration might be important in other contexts in *T. nylanderii* and further experiments are needed to explore if ants

fall back on path integration when they find themselves in unfamiliar surroundings (e.g. through the use of displacement experiments; Müller & Wehner, 1988; Zeil, 2012).

Differences in habitats could explain why route learning seems to be more dominant in these acorn ants, *T. nylanderii*, than in rock ants like *T. albipennis*. The more open grasslands inhabited by *T. albipennis* (Seifert, 2018) are likely to favour a more important role of visual cues combined with path integration, especially during homing when views experienced by ants differ from those seen during the tandem run (Franklin, 2014; McLeman et al., 2002; Pratt et al., 2001; Sasaki et al., 2020). Indeed, *T. albipennis* do not follow the tandem route when returning to their nest (Sasaki et al., 2020). Sasaki et al. (2020) used an open arena with small obstacles, and it would be interesting to assess *T. albipennis* in a binary choice set-up, as in our experiment 1, to further compare these two species. Studying two different *Temnothorax* species, Alleman et al. (2019) found a species-specific upregulation in the expression of genes linked to learning in tandem followers, and future studies could compare brain gene expression in *T. nylanderii* and *T. albipennis* to test if different cognition-linked genes are upregulated in the brains of tandem followers and leaders in these two species.

Trip durations decreased over time in both experiments (Figs. 3 and 5a). This was not due to tandem paths becoming more direct (Fig. 5b), but due to an increase in walking speed over successive trips (Fig. 5c). This is consistent with the observation that the paths of tandem leaders did not change in straightness over successive trips (Glaser & Grüter, 2018S. Glaser & Grüter, 2018), but again

contrasts with findings in *T. albipennis*, where paths of tandem leaders and individual ants became more direct over successive trips (Franklin & Franks, 2012; Franks & Richardson, 2006; Sasaki et al., 2020). We speculate that a stronger reliance on target location learning and path integration in *T. albipennis* could again explain this difference between the two species. We also found that followers experiencing longer-lasting tandem runs needed less time to travel between nest and food source on subsequent trips (Fig. 7a and b). Longer tandem runs might allow followers to acquire better route information, which in turn reduces trip durations on later trips. The walking speeds of ants travelling alone between nest and food source correlated positively, albeit weakly, with the speed of the tandem run (Fig. 7c and d). Individual differences in walking speed could explain such correlations; for example, caused by differences in body size, which have been shown to affect walking speed in this species (Wagner et al., 2021).

Little is currently known about the importance and identity of environmental cues for navigation in *T. nylanderii*. Our additions of visual cues did not affect most of the measured parameters, but we found that visual enrichment (coloured paper along the foraging arena walls in experiment 2) caused trips back to the food source to be longer in both distance and duration (25% and 29%, respectively). Buehlmann et al. (2018) similarly found that desert ants walked more sinusously and slowly when encountering unexpected visual cues. In contrast, *Lasius niger* foragers travelled faster between their nest and a food source when additional visual cues were present (Grüter et al., 2015). It is possible that the visual cues we used in our experiments might not be particularly salient for *T. nylanderii* foragers and future research could test different visual cues; for example, mimicking a high-contrast shape of a tree.

An alternative to route learning could be navigation by following pheromone trails deposited by the tandem pair. While chemical signals are known to be important during tandem running, they are short-lived and function to maintain contact between tandem partners (Basari, Laird-Hopkins, et al., 2014; Franks et al., 2022; Möglich, 1979; Möglich et al., 1974; Traniello & Hölldobler, 1984). Furthermore, chemical cues left on the ground (e.g. in the form of footprints) seem to be important for territorial marking (Bowens et al., 2013), while visual cues have been shown to dominate navigation in *T. albipennis* and *T. rugatulus* (Bowens et al., 2013; Pratt et al., 2001; Sasaki et al., 2020). We explored whether colony-specific or individual-specific (Mallon & Franks, 2000) pheromone trails could explain path choice in *T. nylanderii*. Homing ants were not more likely to walk on the side that was used by the preceding tandem run (i.e. the tandem run that happened just before an ant left the food source to return to the nest) and the return trip distance of an ant did not correlate with the distance of her tandem run, suggesting that ants used neither colony-specific nor individual-specific pheromone trails laid by tandem pairs (see also Fig. A2).

Taken together, our results suggest that tandem followers socially learn routes and that this learning affects routes taken in both directions over several successive foraging trips. We found nuanced, but noteworthy, differences between *T. nylanderii* and *T. albipennis* and speculate that these are linked to differences in the habitats used by these two species. More research is needed to understand the importance of idiothetic and environmental cues for navigation in *T. nylanderii*. We also encourage the study of navigational learning in other tandem running species, especially phylogenetically more distant species with better vision and inhabiting more varied habitats, such as *Diacamma* (Kaur et al., 2012; Mukhopadhyay & Annagiri, 2021; Mukhopadhyay et al., 2019) or *Pachycondyla* (Grüter et al., 2018; Silva et al., 2021).

## Author Contributions

**Aina Colomer-Vilaplana:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christoph Grüter:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Simone M. Glaser:** Writing – review & editing, Methodology, Conceptualization. **Tara Williams:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization.

## Data Availability

The data set used for this study is available in the Supplementary Material.

## Declaration of Interest

We declare no conflict of interest for this study.

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## Supplementary Material

Supplementary material associated with this article is available at <https://doi.org/10.1016/j.anbehav.2025.123107>.

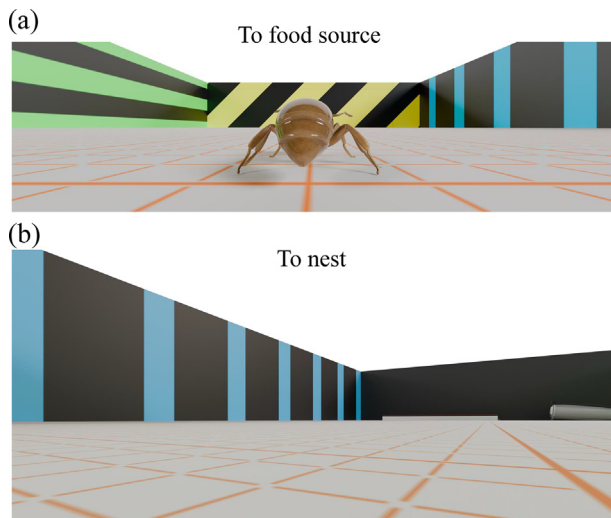
## References

- Alleman, A., Stoldt, M., Feldmeyer, B., & Foitzik, S. (2019). Tandem-running and scouting behaviour are characterized by up-regulation of learning and memory formation genes within the ant brain. *Molecular Ecology*, 28(9), 2342–2359. <https://doi.org/10.1111/mec.15079>
- Aron, S., Deneubourg, J. L., & Pasteels, J. M. (1988). Visual cues and trail-following idiosyncrasy in *Leptothorax unifasciatus*: An orientation process during foraging. *Insectes Sociaux*, 35, 355–366. <https://doi.org/10.1007/BF02225811>
- Bartoň, K. (2024). *MuMIn: Multi-model inference. R package version 1.48.4*. <https://CRAN.R-project.org/package=MuMIn>.
- Basari, N., Bruendl, A. C., Hemingway, C. E., Roberts, N. W., Sendova-Franks, A. B., & Franks, N. R. (2014a). Landmarks and ant search strategies after interrupted tandem runs. *Journal of Experimental Biology*, 217, 944–954. <https://doi.org/10.1242/jeb.087296>
- Basari, N., Laird-Hopkins, B. C., Sendova-Franks, A. B., & Franks, N. R. (2014b). Trail laying during tandem-running recruitment in the ant *Temnothorax albipennis*. *Naturwissenschaften*, 101, 549–556. <https://doi.org/10.1007/s00114-014-1191-1>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Beckers, R., Goss, S., Deneubourg, J.-L., & Pasteels, J. M. (1989). Colony size, communication and ant foraging strategy. *Psyche: Journal of Entomology*, 96, 239–256. <https://doi.org/10.1155/1989/94279>
- Bowens, S. R., Glatt, D. P., & Pratt, S. C. (2013). Visual navigation during colony emigration by the ant *Temnothorax rugatulus*. *PLoS One*, 8(5), Article e64367. <https://doi.org/10.1371/journal.pone.0064367>
- Buehlmann, C., Fernandes, A. S. D., & Graham, P. (2018). The interaction of path integration and terrestrial visual cues in navigating desert ants: What can we learn from path characteristics? *Journal of Experimental Biology*, 221(1), jeb167304. <https://doi.org/10.1242/jeb.167304>
- Collett, M., Chittka, L., & Collett, T. S. (2013). Spatial memory in insect navigation. *Current Biology*, 23(17), R789–R800. <https://doi.org/10.1016/j.cub.2013.07.020>
- Crawley, M. J. (2007). *The R Book*. J. Wiley.

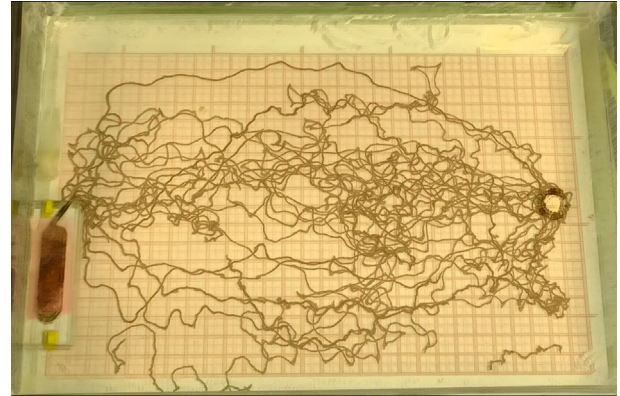


- Franklin, E. L. (2014). The journey of tandem running: The twists, turns and what we have learned. *Insectes Sociaux*, 61, 1–8. <https://doi.org/10.1007/s00040-013-0325-3>
- Franklin, E. L., & Franks, N. R. (2012). Individual and social learning in tandem-running recruitment by ants. *Animal Behaviour*, 84, 361–368. <https://doi.org/10.1016/j.anbehav.2012.05.002>
- Franks, N. R., Podesta, J. A., Jarvis, E. C., Worley, A., & Sendova-Franks, A. B. (2022). Robotic communication with ants. *Journal of Experimental Biology*, 225(15), Article jeb244106. <https://doi.org/10.1242/jeb.244106>
- Franks, N. R., & Richardson, T. (2006). Teaching in tandem-running ants. *Nature*, 439, 153. <https://doi.org/10.1038/439153a>
- Glaser, S. M., Feitosa, R. M., Koch, A., Goß, N., do Nascimento, F. S., & Grüter, C. (2021). Tandem communication improves ant foraging success in a highly competitive tropical habitat. *Insectes Sociaux*, 68, 161–172. <https://doi.org/10.1007/s00040-021-00810-y>
- Glaser, S., & Grüter, C. (2018). Ants (*Temnothorax nylanderi*) adjust tandem running when food source distance exposes them to greater risks. *Behavioral Ecology and Sociobiology*, 72, 40. <https://doi.org/10.1007/s00265-018-2453-2>
- Glaser, S. M., & Grüter, C. (2022). Ancestral state reconstruction suggests repeated losses of recruitment communication during ant evolution (Hymenoptera: Formicidae). *bioRxiv*. <https://doi.org/10.1101/2022.05.18.492496>
- Glaser, S. M., & Grüter, C. (2023). Social and individual learners use different pathways to success in an ant minisociety. *Animal Behaviour*, 198, 1–9. <https://doi.org/10.1016/j.anbehav.2023.01.014>
- Goy, N., Glaser, S. M., & Grüter, C. (2021). The adaptive value of tandem communication in ants: Insights from an agent-based model. *Journal of Theoretical Biology*, 526, Article 110762. <https://doi.org/10.1016/j.jtbi.2021.110762>
- Grüter, C., & Leadbeater, E. (2014). Insights from insects about adaptive social information use. *Trends in Ecology & Evolution*, 29(3), 177–184. <https://doi.org/10.1016/j.tree.2014.01.004>
- Grüter, C., Maitre, D., Blakey, A., Cole, R., & Ratnieks, F. L. W. (2015). Collective decision making in a heterogeneous environment: *Lasius niger* colonies preferentially forage at easy to learn locations. *Animal Behaviour*, 104, 189–195. <https://doi.org/10.1016/j.anbehav.2015.03.017>
- Grüter, C., Wüst, M., Cipriano, A. P., & Nascimento, F. S. (2018). Tandem recruitment and foraging in the ponerine ant *Pachycondyla harpax* (Fabricius). *Neotropical Entomology*, 47, 742–749. <https://doi.org/10.1007/s13744-017-0571-6>
- Hölldobler, B., & Traniello, J. (1980). Tandem running pheromone in ponerine ants. *Naturwissenschaften*, 67(7). <https://doi.org/10.1007/BF01106596>, 360–360.
- Hartig, F. (2022). *DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.6*. <https://CRAN.R-project.org/package=DHARMA>.
- Heinze, J., Foitzik, S., Hippert, A., & Hölldobler, B. (1996). Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderii*. *Ethology*, 102(3), 510–522. <https://doi.org/10.1111/j.1439-0310.1996.tb01143.x>
- Heyes, C. M. (2012). What's social about social learning. *Journal of Comparative Psychology*, 126(2), 193–202. <https://doi.org/10.1037/a0025180>
- Hingston, R. W. G. (1929). *Instinct and intelligence*. Macmillan.
- Hoppitt, W., & Laland, K. N. (2013). *Social learning: An introduction to mechanisms, methods, and models*. Princeton University Press.
- Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3), 346–363. <https://doi.org/10.1002/bimj.200810425>
- Kaur, R., Anoop, K., & Sumana, A. (2012). Leaders follow leaders to reunite the colony: Relocation dynamics of an Indian queenless ant in its natural habitat. *Animal Behaviour*, 83(6), 1345–1353. <https://doi.org/10.1016/j.anbehav.2012.02.022>
- Kendal, R. L., Boogert, N. J., Rendell, L., Laland, K. N., Webster, M., & Jones, P. L. (2018). Social learning strategies: Bridge-building between fields. *Trends in Cognitive Sciences*, 22(7), 651–665. <https://doi.org/10.1016/j.tics.2018.04.003>
- Knaden, M., & Graham, P. (2016). The sensory ecology of ant navigation: From natural environments to neural mechanisms. *Annual Review of Entomology*, 61, 63–76. <https://doi.org/10.1146/annurev-ento-010715-023703>
- Laland, K. N. (2004). Social learning strategies. *Learning & Behavior*, 32, 4–14. <https://doi.org/10.3758/BF03196002>
- Leadbeater, E., & Chittka, L. (2007). Social learning in insects – from miniature brains to consensus building. *Current Biology*, 17(16), R703–R713. <https://doi.org/10.1016/j.cub.2007.06.012>
- Leadbeater, E., & Dawson, E. H. (2017). A social insect perspective on the evolution of social learning mechanisms. *Proceedings of the National Academy of Sciences*, 114(30), 7838–7845. <https://doi.org/10.1073/pnas.1620744114>
- Möglich, M. (1979). Tandem calling pheromone in the genus *Leptothorax* (Hymenoptera: Formicidae): Behavioral analysis of specificity. *Journal of Chemical Ecology*, 5(1), 35–52. <https://doi.org/10.1007/BF00987686>
- Möglich, M., Maschwitz, U., & Hölldobler, B. (1974). Tandem calling: A new kind of signal in ant communication. *Science*, 186(4168), 1046–1047. <https://doi.org/10.1126/science.186.4168.1046>
- Müller, M., & Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proceedings of the National Academy of Sciences*, 85(14), 5287–5290. <https://doi.org/10.1073/pnas.85.14.5287>
- Mallon, E. B., & Franks, N. R. (2000). Ants estimate area using Buffon's needle. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 267(1445), 765–770. <https://doi.org/10.1098/rspb.2000.1069>
- McLeman, M. A., Pratt, S. C., & Franks, N. R. (2002). Navigation using visual landmarks by the ant *Leptothorax albipennis*. *Insectes Sociaux*, 49(3), 203–208. <https://doi.org/10.1007/s00040-002-8302-2>
- Mizumoto, N., Tanaka, Y., Valentini, G., Richardson, T. O., Annagiri, S., Pratt, S. C., & Shimoji, H. (2023). Functional and mechanistic diversity in ant tandem communication. *iScience*, 26(4), Article 106418. <https://doi.org/10.1016/j.isci.2023.106418>
- Mukhopadhyay, S., & Annagiri, S. (2021). Importance of vision in tandem running during colony relocation in an Indian ant. *Ethology*, 127(9), 739–750. <https://doi.org/10.1111/eth.13213>
- Mukhopadhyay, S., Pathak, M. K., & Annagiri, S. (2019). Path minimization in a tandem running Indian ant in the context of colony relocation. *Journal of Experimental Biology*, 222(22), Article jeb206490. <https://doi.org/10.1242/jeb.206490>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining  $R^2$  from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Pratt, S. C., Brooks, S. E., & Franks, N. R. (2001). The use of edges in visual navigation by the ant *Leptothorax albipennis*. *Ethology*, 107(12), 1125–1136. <https://doi.org/10.1046/j.1439-0310.2001.00749.x>
- R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Richardson, T. O., Sleeman, P. A., McNamara, J. M., Houston, A. I., & Franks, N. R. (2007). Teaching with evaluation in ants. *Current Biology*, 17(17), 1520–1526. <https://doi.org/10.1016/j.cub.2007.08.032>
- Sasaki, T., Danczak, L., Thompson, B., Morshed, T., & Pratt, S. C. (2020). Route learning during tandem running in the rock ant *Temnothorax albipennis*. *Journal of Experimental Biology*, 223(9), Article jeb221408. <https://doi.org/10.1242/jeb.221408>
- Sasaki, T., & Pratt, S. C. (2021). Tandem running recruitment by *Temnothorax* ants as a model system for social learning. In A. B. Kaufman, J. C. Kaufman, & J. Call (Eds.), *The Cambridge Handbook of Animal Cognition* (pp. 472–485). Cambridge University Press.
- Seifert, B. (2018). *The ants of central and north Europe*. Lutra Verlags und Vertriebsgesellschaft.
- Shaffer, Z., Sasaki, T., & Pratt, S. C. (2013). Linear recruitment leads to allocation and flexibility in collective foraging by ants. *Animal Behaviour*, 86, 967–975. <https://doi.org/10.1016/j.anbehav.2013.08.014>
- Silva, J. P., Valadares, L., Vieira, M. E. L., Teseo, S., & Châline, N. (2021). Tandem running by foraging *Pachycondyla striata* workers in field conditions vary in response to food type, food distance, and environmental conditions. *Current Zoology*, 67(5), 541–549. <https://doi.org/10.1093/cz/zoab050>
- Stuttard, J. P., Gottlieb, D., & Franks, N. R. (2015). Ants incommunicado: Collective decision-making over new nest sites by ants with reduced communication. *Behavioral Ecology and Sociobiology*, 70, 145–155. <https://doi.org/10.1007/s00265-015-2033-7>
- Traniello, J. F., & Hölldobler, B. (1984). Chemical communication during tandem running in *Pachycondyla obscuricornis* (Hymenoptera: Formicidae). *Journal of Chemical Ecology*, 10(5), 783–794. <https://doi.org/10.1007/BF00988543>
- Wagner, T., Bachenberg, L., Glaser, S. M., Oikonomou, A., Linn, M., & Grüter, C. (2021). Large body size variation is associated with low communication success in tandem running ants. *Behavioral Ecology and Sociobiology*, 75(1), 4. <https://doi.org/10.1007/s00265-020-02941-x>
- Wilson, E. O. (1959). Communication by tandem running in the ant genus *Caridiodicondyla*. *Psyche*, 66, 29–34. <https://doi.org/10.1155/1959/29093>
- Wittlinger, M., Wehner, R., & Wolf, H. (2006). The ant odometer: Stepping on stilts and stumps. *Science*, 312(5782), 1965–1967. <https://doi.org/10.1126/science.1126912>
- Zeil, J. (2012). Visual homing: An insect perspective. *Current Opinion in Neurobiology*, 22(2), 285–293. <https://doi.org/10.1016/j.conb.2011.12.008>
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer.

## Appendix



**Fig. A1.** Reconstructions of the perspectives of (a) a tandem follower and (b) an ant returning to her nest, in experiment 2. Walls were covered with coloured paper. Note that these illustrations show reconstructions of the ants' perspectives, not their actual views (reconstructions by Oriol Colomer Delgado).



**Fig. A2.** Lines show the combined paths that marked ants travelled between the nest (left side) and a sucrose solution food source (right side), after they followed a tandem run, during a 90 min trial. Photo: Aina Colomer-Vilaplana.