

Original Article

Prey escorting behavior and possible convergent evolution of foraging recruitment mechanisms in an invasive ant

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The longhorn crazy ant *Paratrechina longicornis* is a pest ant species with worldwide distribution. It tends honeydew-producing Hemiptera to obtain carbohydrates and is also an effective predator and scavenger. What strategies does *P. longicornis* use to aid in hunting and scavenging? Our results show that as well as long-term recruitment to stable food sources, this species uses specialized short-term recruitment pheromones to recruit nest mates to assist in the exploitation of large food items. By mass, 88% of externally carried food was retrieved cooperatively. Recruitment to large items is via a pheromone trail laid by the discovering ant. This trail is initially followed with few errors by naive recruits (82% correct choices at a T-bifurcation) but decays very rapidly (within 6 min). We also show that food-discovering ants can recruit nearby nest mates to a large food item without returning to the nest. These properties of the recruitment system are strikingly similar to 2 unrelated ant species, which also specialize in cooperative retrieval of large food items, suggesting convergent evolution. Lastly, we describe a novel "escort" behavior in which additional workers accompany a transported item but do not necessarily assist in carrying it. Both local recruitment and escorting are much more pronounced (3 and 4 times greater, respectively) when handling live prey. When an "escort" is present, live prey presented to the ants is 50% more likely to be captured and is captured 4 times sooner and 50% closer to the release point.

Key words: convergent evolution, cooperative transport, foraging, *Paratrechina longicornis*, recruitment, trail pheromones. [Behav Ecol]

INTRODUCTION

The longhorn crazy ant, *Paratrechina longicornis*, is perhaps the most widely distributed invasive ant in the world (Wetterer 2008). It effectively exploits honeydew-producing Hemiptera and is also a very effective hunter and scavenger (Kenne et al. 2005), rapidly recruiting workers to large prey items (Trager 1984; Kenne et al. 2005) and retrieving them using cooperative transport.

Cooperative transport, in which items are moved by 2 or more individuals, is common in humans and ants but rare in other animals (Czaczkes and Ratnieks 2013). In ants, one adaptation to cooperative transport is a specialized type of recruitment, in which a foraging ant that has discovered a food item it cannot move alone, such as a dead insect, lays a short-lived but accurately followed pheromone trail that nest mates can follow to the item in order to help with transport (Detrain and Deneubourg 1997; Cerdá et al.

2009). The trail needs to be accurately followed in order to guide nest mates to the specific location of the item, and strong, so that a followable trail can be laid by a single discoverer (Czaczkes and Ratnieks 2012). However, the trail need not be long lived as there is no need for ongoing recruitment to the food location because it is not a renewing food source such as a patch of honeydew-secreting aphids. Trail pheromone with these properties occurs in the ants *Aphaenogaster albisetosus* and *Pheidole oxyops*, both of which specialize in rapid recruitment to large food items, followed by cooperative transport back to the nest (Hölldobler et al. 1978; Czaczkes and Ratnieks 2012).

In addition, species that perform cooperative transport of large food items may use local recruitment. Local recruitment, also termed short-range recruitment, involves the attraction of ants that are already outside the nest to a specific location and begins before the recruiting ant has returned to the nest (Hölldobler et al. 1978; Traniello 1989; Schatz et al. 1997). Local recruitment may function in parallel to group or mass recruitment, in which nest mates are recruited from the nest itself (Hölldobler and Wilson 1990). Local recruitment often

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involves volatile attractant pheromones, either emitted into the air by the discoverer ant for point-source attraction (Hölldöbler et al. 1978) or by the recruitment trail laid by the discoverer having a dual effect—intercepting workers outside the nest in addition to recruiting ants from the nest itself, where the trail ends (Czaczkes and Ratnieks 2012). Local recruitment can also be achieved using stridulating (Markl and Hölldöbler 1978; Roces and Hölldöbler 1996), although this is usually over a very limited distance (Markl and Hölldöbler 1978; Baroni-Urbani et al. 1988). Local recruitment allows recruitment and retrieval to begin more rapidly and to sites too distant from the nest to allow a volatile trail to extend all the way from the nest entrance (Czaczkes and Ratnieks 2012).

The complexity of *P. longicornis* chemical communication (Witte, Attygalle, et al. 2007) suggests that it has convergently evolved a specialized recruitment behavior similar to that of *Ph. oxyops* and *A. albisetosus*. As *P. longicornis* is a very capable hunter and scavenger (Kenne et al. 2005), we predicted that it would have specialized adaptations to hunting live prey. A short-lived recruitment trail would allow *P. longicornis* to compete effectively for protein sources and would function alongside recruitment to long-lasting food sources. This study tested these predictions by examining the recruitment and foraging of *P. longicornis* and comparing it to ants specialized in recruiting to large, ephemeral food items. We find that *P. longicornis* indeed possess such a specialized recruitment system including both high-accuracy, short-lived recruitment and local recruitment, alongside long-lasting mass recruitment to semipermanent food sources. *P. longicornis* also demonstrates a unique escorting behavior for live prey items that helps prevent prey items from escaping. The multiple specialized recruitment and foraging strategies described here may help explain why *P. longicornis* is one of the most successful invasive ants (Wetterer 2008).

METHODS

Study site and organism

Data were collected in March 2012 and in January and February 2013 on the campus of the University of São Paulo, Ribeirão Preto, Brazil. *Paratrechina longicornis* forage extensively on honeydew (Wetterer et al. 1999) (and see results) but also hunt and scavenge arthropod prey extensively (Kenne et al. 2005) (and see results). Large items are retrieved by coordinated encircling cooperative transport (Czaczkes and Ratnieks 2013). Wild, unmanipulated colonies were studied. Workers are small, and foragers not carrying liquids internally (i.e., without a distended abdomen) weighed on average 0.39 mg (standard deviation [SD] = 0.045 mg) (see results). As experimental prey items we used freshly caught termites (*Syntermes* sp.; mean weight 5.98 mg, SD = 1.3 mg). We frequently saw multiple workers retrieving dead and live insects, including leafcutter ant, *Atta*, and stingless bee, *Melipona*, workers.

Experiment 1—trail choice at a T-bifurcation

This experiment quantified the properties of a *P. longicornis* recruitment trail laid by a single food-discovering worker. We replicated the methods of Czaczkes and Ratnieks (2012) to determine the longevity of trails made by one discovering ant to an immovable food item and the accuracy with which recruits follow this trail. Recruitment to large food items by *P. longicornis* is similar to other

cooperatively transporting species (Detrain and Deneubourg 1997; Robson and Traniello 1998; Cerdá et al. 2009; Czaczkes et al. 2011). When an ant finds an item, it attempts to drag it back to the nest. If the item is too big for the discoverer to move, it returns to the nest depositing a pheromone trail. On entering the nest the recruiting ant triggers a surge of recruits, which leave the nest and follow the pheromone trail to the food item.

To initiate trail laying, a freshly freeze-killed termite was tethered to a piece of modelling clay and placed at the end of one arm of a T-maze. The T-maze head was a plastic platform, 20 × 220 mm, raised on stilts each placed in a container of water to prevent access via the stilts (Figure 1a). The stem of the T-maze was a 100-mm-long ramp, tapering from 50 mm in width at the base to 20 mm where it connected to the central section of the platform. The platform was overlaid with clean printer paper, with perpendicular decision lines marked 50 mm from the center of the overlay. The apparatus was placed within 30 cm of a *P. longicornis* nest entrance. The bait was typically discovered within circa 2 min by a single *P. longicornis* worker, which would then attempt to move the item, fail, and return to the nest depositing a pheromone trail and recruiting nest mates.

Trail laying in *P. longicornis*, as in many other formicine ants that lack stings, is a stereotyped behavior involving brief pauses to lay pheromone (e.g., *Lasius niger*, Beckers et al. 1993; Czaczkes et al. 2013) in which the abdomen is bent downward and dotted on the substrate (*P. longicornis* can also lay a continuous pheromone trail by dragging the abdomen along the substrate; Witte V, personal communication). The recruits would run up the ramp and onto the platform. Those passing one of the two decision lines on the head of the T (see Figure 1a) were considered to have chosen either left or right and then gently removed by brushing them from the platform with a piece of paper.

We continued monitoring choices for up to 7 min as new recruits left the nest. However, as the surge of recruits triggered by the discoverer normally lasts only 1–2 min, additional recruitment surges also had to be triggered. To do this, the bait was replaced at the center of the T-maze when the flow of recruits ceased. The bait would soon be found by an ant that would try to move it, fail, and return to the nest depositing more trail pheromone, thereby causing a further surge of recruits. In this way, we were able to send new recruits to the stem of the T without reinforcing the trail pheromone laid by the initial discoverer on one of the two branches at the head of the T.

Trials were videoed using a Sony HD XR520 camcorder. From the videos, the number of ants choosing left or right was determined and grouped into 1-min intervals. After every trial, the platform was cleaned with ethanol and the paper overlay replaced. All trials were conducted in the shade (minimum 28.3 °C, maximum 32.0 °C). Five colonies were tested. Colonies of *P. longicornis* are highly mobile and frequently relocate their nest. Due to frequent nest relocation, not all colonies could be tested an equal number of times. Three colonies were tested 6 times, one 3 times, and one twice ($n = 23$ trials in total).

Experiment 2—local recruitment to live and dead food items

To determine whether *P. longicornis* emits volatile recruitment pheromones known from its Dufour gland (Witte, Abrell, et al. 2007) when encountering large prey items, and whether the prey item being alive or dead affects this, we presented *P. longicornis* colonies with a termite that was tethered to the substrate circa 30 cm from a nest entrance. The substrate was white printer paper that had been

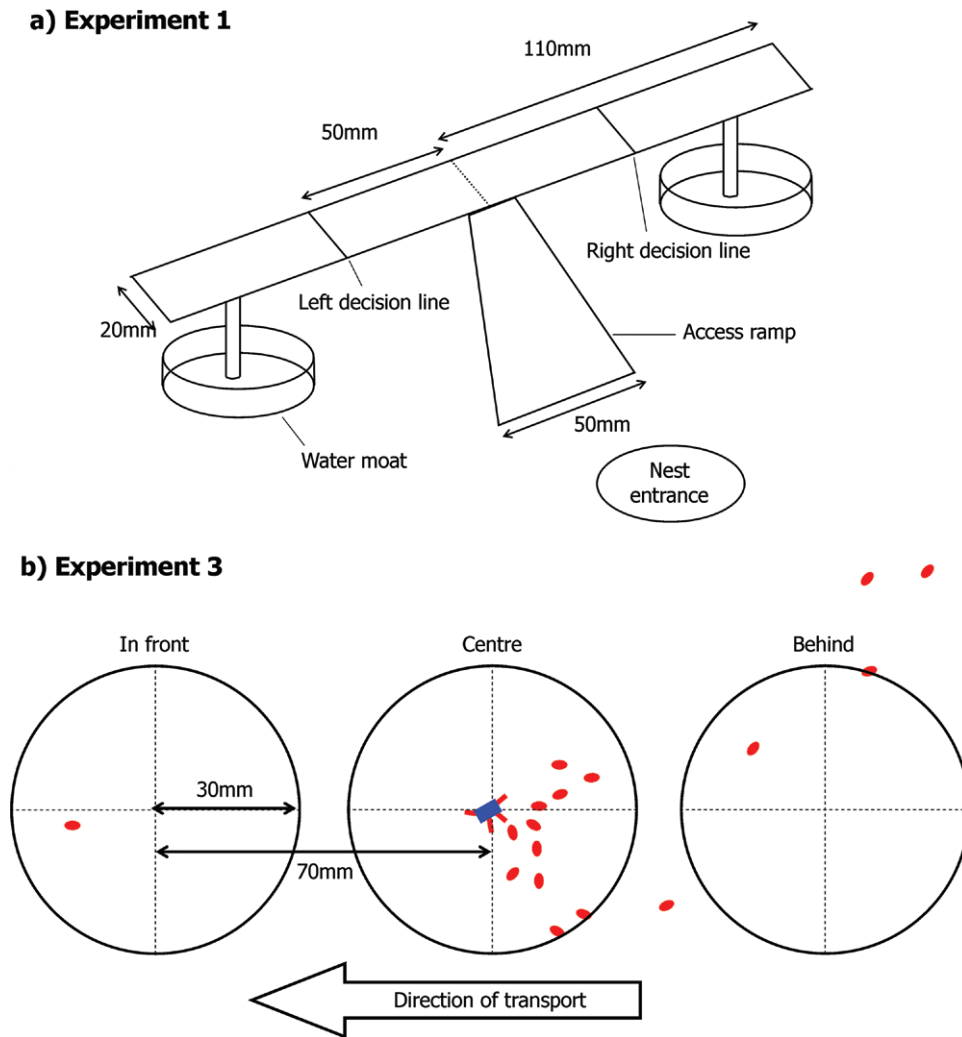


Figure 1

(a) T-maze apparatus used in experiment 1 (not to scale). A freeze-killed termite was placed at the end of one arm of the T. A *Pavlovichia longicornis* worker would find this and return to the nest, depositing a pheromone trail. Recruits emerge from the nest entrance almost immediately and enter the apparatus via the access ramp. Ants crossing a particular decision line were considered to have chosen either left or right. (b) Diagram based on video recordings representing a termite (blue rectangle) being cooperatively transported by 4 *P. longicornis* workers (red rectangles) from right to left. Other *P. longicornis* workers in the area are marked with red ovals. There are 11 workers in a 3 cm radius around the termite, 2 workers in a 3-cm radius circle 7 cm behind the item, and 1 worker in a 3-cm radius circle 7 cm in front of the item.

taped down on all sides to keep the edges flush with the concrete ground surface. The prey item was either alive or freshly freeze killed. As soon as an ant discovered the prey item, a circular plastic barrier, 1.5 cm radius and 1 cm high and coated in fluon, was placed around the bait plus ant to prevent the ant from returning to and recruiting workers from the nest. The area around the bait was videoed for 2 min before the bait was presented and then for 2 min after the bait was discovered. From the videos, the number of ants entering a 10 cm radius around the bait location was determined for both periods. At least 20 min was allowed between trials to allow the colony’s behavior to return to normal. Six colonies were tested, each once with a dead termite and once with a live termite.

Experiment 3—escorting behavior during cooperative transport

While studying cooperative transport in *P. longicornis*, we sometimes observed a conspicuous “escort” of ants following the carried item without assisting in carrying. In addition, it seemed that this escort

was only deployed when live prey items were being transported. To test whether *P. longicornis* specifically escort cooperatively transported food items, and whether this is affected by the item being alive or dead, we presented live or dead termites to active *P. longicornis* trails circa 2 m from the nest entrance. Live termites were gently held with soft forceps until a *P. longicornis* worker grasped it. The section of the trail where the termite was presented was videoed for 2 min before the termite was presented. The termite was then followed and the area around it videoed as it was transported to the nest. The section of the trail where the termite was presented was videoed for 2 min before the termite was presented, and the number of ants in a 3-cm radius circle around where termite was to be presented was counted every 30 s. After the termite had been retrieved, the original trail section was again videoed for a further 2 min. During transport, we counted the number of ants within a 3 cm radius of the prey item every 30 s, and an equal area centered 7 cm behind and 7 cm in front of the item, relative to the direction of transport (see Figure 1b). Ants actively carrying the termite were

counted separately. Before and after transport, similar counts were made in a 3-cm radius area centered on the trail segment where the termite was originally presented. Each colony was tested 3 times with live prey and 3 times with dead prey. Six colonies were tested in total.

Experiment 4—testing the role of the “escorting” ants around a food item

A separate experiment was carried out to determine whether the “escort” reduces escape by live prey items. As before, we presented either live or dead termites to active *P. longicornis* trails circa 2 m from the nest entrance. The section of the trail where the termite was presented was videoed for 2 min to quantify baseline traffic levels on the trail before either a live or a dead termite was presented. The termite was then followed and the area around it videoed as it was transported to the nest. During transport, we counted the number of ants within a 3 cm radius of the prey item every 30 s as a measure of “escort” size. After 2 min, the transported termite was carefully removed using soft forceps, along with any ants that maintained their grasp of the item, and was replaced with a live termite. We then followed the live termite and noted whether or not the termite escaped and also, if the released termite was captured by the ants, how far from the release point the termite was captured, and how long this took. We defined a termite as being captured if it had been grasped by an ant and failed to free itself within 1 min. We considered a termite to have escaped when it either travelled 1 m from the release point or avoided capture for 2 min. Nine colonies were tested in total. In total, 24 trials were carried out using live termites and 22 using dead termites.

Experiment 5—the importance of cooperative versus individual transport in foraging

To ascertain the importance of individual versus cooperative retrieval of solid food items, we surveyed the entrances of 3 *P. longicornis* colonies for 4 h in total, collecting any items (excluding brood) being carried toward the nest. Items were weighed on a balance sensitive to 0.1 mg (Sartorius TE64) within 30 min of collection. The number of ants transporting each item was also recorded.

To ascertain the importance of liquid retrieval in *P. longicornis*, we observed active trails of 5 colonies for 10 min each, counting the number of ants returning with empty or distended abdomens. Where foraging trails could be traced, they were found to lead into trees presumably hosting colonies of honeydew-producing Hemiptera. As a comparison, we also collected data from 4 colonies of *Ph. oxyops* in the same location. *Pheidole oxyops* is a native neotropical species, which also specializes in cooperative retrieval of large food items (Czaczkes et al. 2011). We have never observed *Ph. oxyops* foraging above ground level. To get a measure of the amount of liquid being retrieved by each *P. longicornis* worker, we weighed 37 ants with nondistended abdomens and 16 with distended abdomens using a balance sensitive to 0.01 mg (Mettler Toledo UMT 2 balance).

Statistical analyses

We analyzed the data using generalized linear mixed-effect models (GLMM) (Bolker et al. 2009) and generalized linear models (GLM) using R2.15 (R Development Core Team 2009). Models were fitted using either the glm or the lmer functions (Bates et al. 2007). When colonies were tested multiple times, or multiple measurements were taken per trial, we added “colony” or “trial” or both as random

effects to control for the nonindependence of data points from these sources (Bolker et al. 2009; Zuur et al. 2009). Saturated models (containing all measured variables and random effects) were produced, and nonsignificant ($P > 0.05$) terms sequentially removed until a model containing only significant terms was arrived at (Zuur et al. 2009). Binomial data were analyzed as 0–1 responses (correct/incorrect decisions in experiment 1 and escaped/not escaped data from experiment 4) and were modelled using a binomial distribution family using the logit link function. Normally distributed data (experiment 2) were modelled using a Gaussian distribution family. Poisson distributed data (experiment 3 and escape time and distance measurements from experiment 4) were modelled using a Poisson distribution family.

RESULTS

Experiment 1—trail choice at a T-bifurcation

In every trial, ants that discovered the bait returned to the nest depositing a pheromone trail that immediately caused a surge of recruits to leave the nest and follow the trail. As Figure 2 shows, trail choice is initially very accurate with 82% correct choices in the first minute. But this rapidly drops, with the modelled decay curve intersecting random choice (50% correct) in less than 6 min. We found that the minute in which a recruit was observed making a choice following trail laying was a strong predictor of whether it chose the correct branch (GLMM, $\chi^2 = -8.155$, $P < 0.0001$, see Figure 2). These results are almost identical to data collected using the same protocol on *Ph. oxyops* (Czaczkes and Ratnieks 2012). Indeed, when data from both species are combined in a single statistical analysis, ant species is not a significant factor (GLMM, $\chi^2 = 0.725$, $P = 0.468$). Colony and trial were added as random effects in this analysis. In addition, the trail pheromone decay characteristics of these 2 species are very similar to those

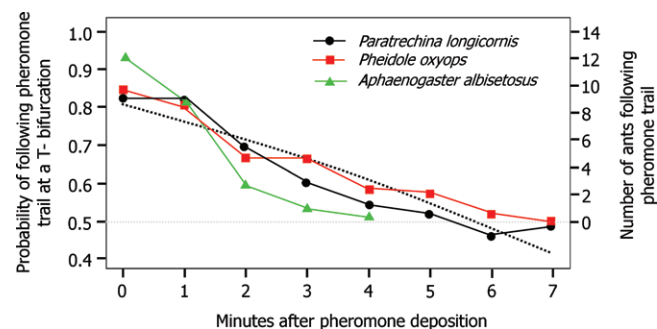


Figure 2

Trail pheromone decay rates in 3 ant species. In all 3 species, a single discoverer ant recruits nest mates to a large food item by laying a pheromone trail to assist in cooperative transport. The data from *Paratrechina longicornis* (circles) and *Pheidole oxyops* (squares) refer to the proportions of ants making a correct decision at a T-bifurcation on which a single discoverer ant had deposited a pheromone trail and were gathered using identical protocols using a T-maze. The data from *Aphaenogaster albisetosus* (triangles) refer to the number of ants following a pheromone trail out of the nest. The trail was made using extract from a single poison gland and had been aged a varying number of minutes before being presented (right axis). Data on *A. albisetosus* are from Hölldobler et al. (1978). Data on *Ph. oxyops* are from Czaczkes and Ratnieks (2012). The curved dotted line is the modelled pheromone decay rate for *P. longicornis* (this study). The horizontal dashed line marks 0.5 probability (H_0) for trail choice and 0 ants for trail following. That is, random trail choice due to the trail having no behavioral effect.

of *A. albisetosus* reported by Hölldobler et al. (1978) (see Figure 2). However, statistical comparison between the 2 data types is not possible because Hölldobler et al. (1978) use a different bioassay to ours.

Experiment 2—local recruitment to live and dead food items

As expected, before the termite prey item was presented, the subsequent treatment (termite live or dead) had no significant effect on the number of ants entering the observed area (GLM, $\zeta = -0.069$, $P = 0.945$). After the prey item was presented, however, on average 3 times more ants entered the area around a live versus a dead food item (GLM, $\zeta = 3.077$, $P = 0.00419$, see Figure 3. Interaction term, $\zeta = 2.923$, $P = 0.0139$). Similarly, as expected, there was no significant difference in the number of ants entering the area before or after a dead termite was presented (GLM, $\zeta = 1.005$, $P = 0.315$), but significantly more ants entered the area after a live versus dead termite was presented ($\zeta = 3.696$, $P = 0.0004$, see Figure 3). As each colony was only tested once in each treatment, no random effects were used.

Experiment 3—escorting behavior during cooperative transport

We found a significant interaction between treatment (termite live or dead) and the area observed (in front, behind, or centered on the transported termite) on the number of ants counted (GLMM, $\zeta = -5.051$, $P < 0.0001$).

As Figure 4b shows, when a dead termite was presented, slightly but significantly more ants (excluding those grasping the termite) were found around the item than either 7 cm in front (GLMM, $\zeta = 6.83$, $P < 0.0001$) or behind (GLMM, $\zeta = 8.986$, $P < 0.0001$, see Figure 4b). There was no difference between the number of ants in front of the item versus behind the item (GLMM, $\zeta = -0.445$, $P = 0.656$, see Figure 4b). However, when a live termite was

presented, many more ants (excluding those grasping the termite) were found in the area surrounding the termite (mean = 12.64, SD = 9) than in front (mean = 1.92, SD = 2, GLMM, $\zeta = -16.43$, $P < 0.0001$) or behind (mean = 3.90, SD = 4.9, GLMM, $\zeta = -12.49$, $P < 0.0001$) the item (see Figure 4b). In addition, more ants were found behind the item than in front of it (GLMM, $\zeta = 3.673$, $P = 0.0002$, see Figure 4b) and more were found in the equivalent locations than in trials with a dead termite. We also found a significant interaction effect between the number of ants in a 3 cm radius around where the termite was to be placed on the trail (henceforth “traffic level”) and whether the termite was alive or dead on the number of ants counted (GLMM, $\zeta = 2.64$, $P = 0.0111$). When the termite carried was alive, the number of ants around the transported termite (excluding ants carrying the termite) was strongly correlated with traffic level (GLMM, $\zeta = 6.823$, $P < 0.0001$). When the termite carried was dead, there was still a relationship between traffic level and the number of ants around the item during carriage, but the relationship was weaker (GLMM, $\zeta = 3.00$, $P = 0.008$). The effect of traffic level on the number of ants either in front or behind the transported termite was not affected by whether the termite was alive or dead (in front: GLMM, $\zeta = -1.75$, $P = 0.08$; behind:

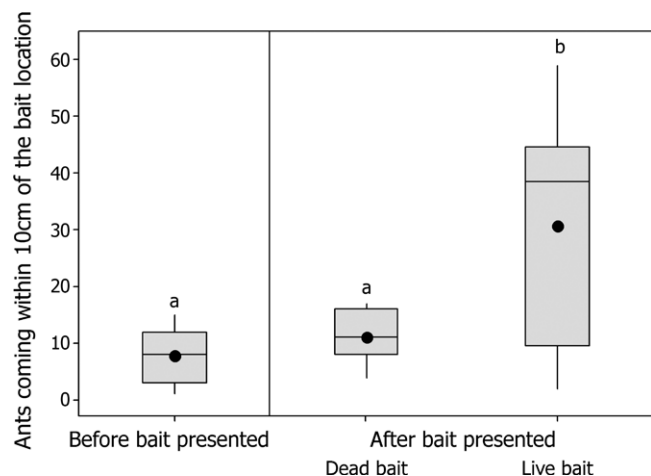


Figure 3

Local recruitment to live and dead baits versus control (before bait presented). A live or dead termite is placed by a colony entrance, and the first ant to find the bait is confined with the bait, preventing it from returning to the nest and recruiting workers by means of its pheromone trail. The number of ants entering a 10 cm radius around the location of the bait is counted for 2 min before and after the bait was found by the discovering ant. Dots signify means, horizontal lines signify medians, boxes signify interquartile ranges, and whiskers signify the general extent of the data. Groups with the same letter above are not significantly different.

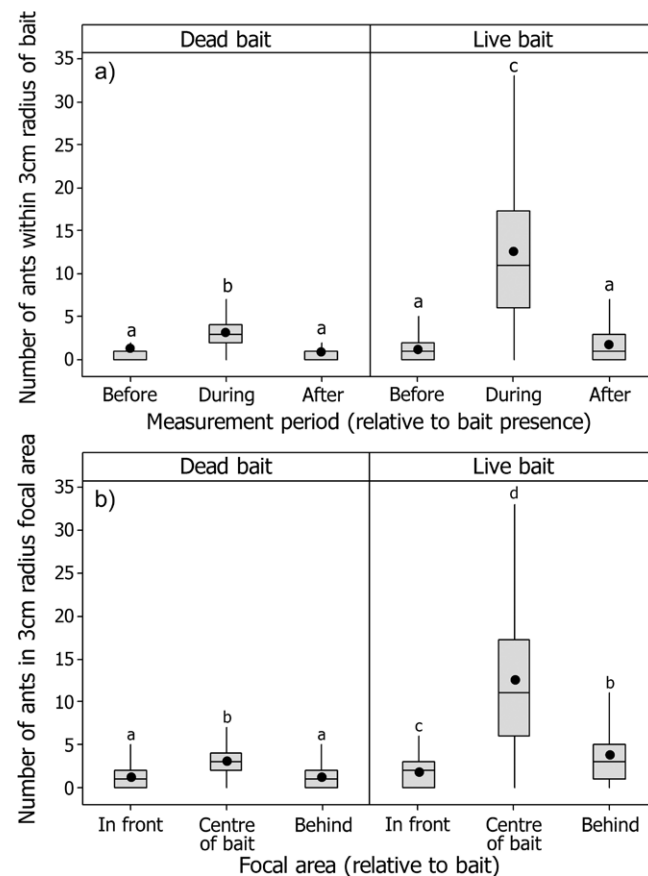


Figure 4

(a) Number of ants within a 3 cm radius around a transported bait item before, during, and after transport of the item in time. This number does not include ants carrying the bait. The bait item was either a live or a dead termite. (b) Numbers of ants in 3-cm radius areas around a transported bait item and in equal areas 7 cm in front of or behind the item. This number does not include ants carrying the bait. The bait item was either a live or a dead termite. In both figures, dots signify means, horizontal lines signify medians, and boxes signify interquartile ranges. Groups within each figure with the same letter above them are not significantly different.

GLMM, $\chi = 1.75$, $P = 0.120$). In these models, trial and colony were added as random effects. Although calculation of a coefficient of determination for mixed-effect models (R^2) is nontrivial (Orelien and Edwards 2008) and is the subject of current statistical research, it is noteworthy that an equivalent GLM model to our GLMM models, predicting the number of ants around the termite during transport using an interaction between traffic level and treatment (live or dead termite) has an R^2 of 73.5%. Such a model considering treatment alone has an R^2 of 56.1%, and a model considering traffic level alone has an R^2 of 21.3%.

When comparing the 3-cm radius area surrounding the transported item with the same area before the item was presented and after transportation had ended, we found significant interactions between whether the termite was alive or dead and whether the measurement was taken before the item was presented, during transportation or after transportation ended (GLMM, $\chi = 4.529$, $P < 0.0001$).

As Figure 4a shows, when a dead termite was presented, more ants were found in the observed area during transportation than either before (mean = 1.33, SD = 2.46, GLMM, $\chi = -5.33$, $P < 0.0001$) or after (mean = 1.0, SD = 1.36, GLMM, $\chi = -6.635$, $P < 0.0001$). There was no difference in the number of ants before and after transportation (GLMM, $\chi = -0.591$, $P = 0.555$). An identical but stronger pattern was found when live termites were presented: More ants were found in the observed area during transportation than either before (mean = 1.24, SD = 1.59, GLMM, $\chi = -11.94$, $P < 0.0001$) or after (mean = 1.81, SD = 2.2.1, GLMM, $\chi = -10.18$, $P < 0.0001$) (see Figure 4a). There was no difference in the number of ants in the area before and after transportation (GLMM, $\chi = -1.335$, $P = 0.273$, see Figure 4a). There was no significant difference between treatments in which a live or dead termite was presented either before presentation (GLMM, $\chi = 0.74$, $P = 0.46$) or after transport had ended (GLMM, $\chi = 1.468$, $P = 0.178$), whereas there were significantly more ants near a live than dead termite during transport (live termite mean = 12.64, SD = 9.0; dead termite mean = 3.16, SD = 2.81, GLMM, $\chi = 5.736$, $P < 0.0001$, see Figure 4a).

Experiment 4—testing the role of the “escorting” ants around a food item

A larger “escort” recruited to live prey items did indeed play a role in preventing prey items from escaping. Higher mean numbers of ants within 3 cm of the carried item resulted in significantly fewer prey escaping (GLMM, $\chi = -2.297$, $P = 0.0433$). Of the termites that were captured, the capture time was significantly lower with larger escort sizes (GLMM, $\chi = -2.695$, $P = 0.00703$). When analyzed in terms of treatment, when live termites were transported, capture time was lower (live termite treatment mean = 8.35 s, SD = 9.37 s; dead termite treatment mean = 33.93 s, SD = 24.82 s, GLMM, $\chi = -3.519$, $P = 0.000434$, see Figure 5). As treatment and escort size are so highly correlated (see experiment 4), when both terms are added to the model, escort size becomes nonsignificant (GLMM, $\chi = -0.735$, $P = 0.462$). This merely demonstrates that escort size and treatment explain the same variation in the data. Capture distance was also significantly lower when live termites were transported (live termite treatment mean = 2.52 cm, SD = 2.78 cm; dead termite treatment mean = 5.29 cm, SD = 5.69 cm, GLMM, $\chi = -2.93$, $P = 0.00337$, see Figure 5). Colony was added as a random effect in all the analyses.

Experiment 5—the importance of cooperative versus individual transport in foraging

We collected and weighed 76 items being transport to the nest. Of these, 18 (25%) were being cooperatively transported versus 54 by single ants. However, the cooperatively transported items accounted for 87.9% of the total mass (193 mg of a total of 219 mg), being on average 21 times heavier.

We counted 1246 ants, of which 488 (39.2%) had a visibly distended abdomen. By comparison, of 284 *Ph. oxyops* minor workers returning to 4 nests (excluding those returning from the refuse pile), only 5 (1.8%) had a visibly distended abdomen.

We weighed 37 *P. longicornis* with an apparently empty abdomen and 16 with a visibly distended abdomen. “Empty” ants weighed on average 0.39 mg (SD = 0.045) and “full” ants 0.86 mg (SD = 0.13). Thus, on average, a “full” ant carried 0.49 mg of liquid, circa 120%

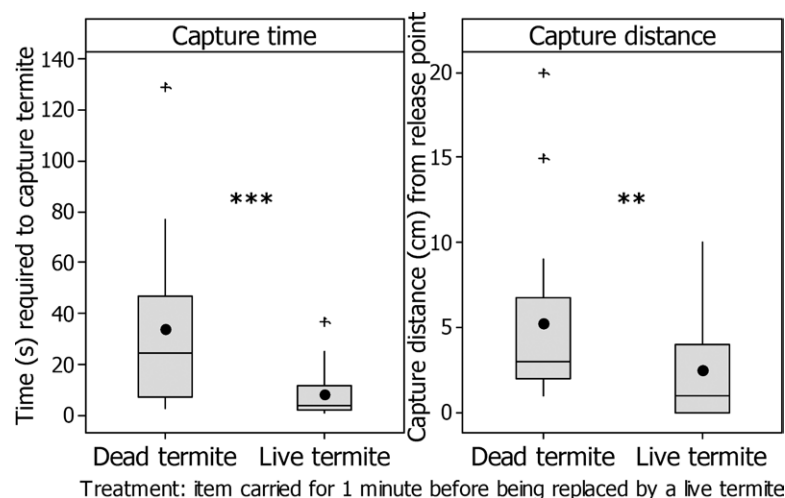


Figure 5

Either live or dead termites were presented to the ants and allowed to be cooperatively transported toward the nest for 2 min. The transported item was then replaced by a live termite. The left and right panels show the time required (in seconds) to capture the termite and capture distance (in centimeter from the release point), respectively. In both figures, dots signify means, horizontal lines signify medians, boxes signify interquartile ranges, and crosses signify outliers. *** $P < 0.001$, ** $P < 0.01$.

of its body weight. On average, each colony was retrieving 4.78 mg of liquid and 0.9 mg of solid food per minute.

DISCUSSION

Our results show that alongside long-term mass recruitment to semipermanent food sources, *P. longicornis* perform short-term recruitment to large, ephemeral food items. Food item size is assessed according to tractive resistance. If the item cannot be moved, the discovering forager returns to the nest depositing a pheromone trail that recruits and directs nest mates to the discovery site to aid in transport. Such a decision-making system is common in hunting and scavenging ants, ranging from primitive ponerine ants to *Oecophylla* weaver ants (Hölldobler and Wilson 1978; Déjean et al. 1993).

Our results clearly show that a *P. longicornis* forager that discovers a food item that it cannot move alone emits a recruitment signal, presumably a volatile pheromone and possibly the same pheromone used to lay a short-lived trail, that recruits nearby nest mates to a food item it had discovered. Similar local or short-range recruitment was described in *A. albisetosus* (Hölldobler et al. 1978) and *Lasius neoniger* (Traniello 1989). However, it is also possible that the observed local recruitment was achieved via stridulation. *Pheidole oxyops* has also been shown to perform local recruitment of nearby ants by means of the pheromone trail laid by a discovering ant, as this trail intercepts workers outside the nest and directs them toward the food source (Czaczkes and Ratnieks 2012). Indeed, any mass-recruiting species in which trail pheromones alone are sufficient to draw foragers from the environment will likely also have local recruitment via interception by pheromone trails. It is possible that all 4 species use both types of recruitment. Our results can be attributed to recruitment, as *P. longicornis* do not seem to sense prey via volatile odors, relying instead on physical antennal contact (Kenne et al. 2005). This may explain their unusually long and widely splayed antenna (Kenne et al. 2005).

Our data also clearly show that worker *P. longicornis* provide an escort of noncarrying ants to a food item being cooperatively transported, particularly live prey. As described in experiment 3, this seems to be related to local recruitment behavior, with escorting and local recruitment being elicited more to live prey. Escorting behavior is also absent from cooperatively transported brood items (personal observation). This strongly suggests that local recruitment and the presence of the escort around live prey items are adaptations to handling live prey items, which may escape or require more ants to transport or kill, rather than for defense against predators or competitors. If the latter were true, we would also expect escorts for both dead prey and brood. In experiment 4, we demonstrate that larger escorts, formed when transporting live prey, are more effective at capturing live prey items released around the transported prey item. Prey items are also captured sooner, and closer to the release point, when large escorts elicited by transporting live prey are present. This local recruitment to live prey items only is in contrast to local recruitment in *Aphaenogaster cockerelli*, which occurs strongly to dead food items (Hölldobler et al. 1978). The members of the escort are drawn from the passing ant traffic on the trail, and thus larger escorts are formed when ant traffic is greater. Escort membership does not seem to be stable, but rather involves members joining and leaving continuously.

Paratrechina longicornis has a complex chemical communication system involving at least 3 orientation pheromones, derived from 3 separate glandular sources (Witte, Attygalle, et al. 2007).

In laboratory experiments using extracts from specific glands, Witte, Abrell, et al. (2007) demonstrated that hindgut extract elicited strong, long-lasting trail following and weak point-source attraction, poison gland extract showed intermediate trail following, persistence and point-source attraction, and Dufour's gland extract (mainly undecane and tridecane; Witte, Abrell, et al. 2007) elicited low trail following and persistence but high point-source attraction. Both local recruitment and the escorting behavior are likely mediated by a pheromone emitted from the Dufour's gland, which elicits a strong point-source attraction (Witte, Abrell, et al. 2007; Witte, Attygalle, et al. 2007). However, it is also possible that these behaviors are elicited by, or modulated by, stridulation, which occurs in many ants. Stridulation on solid ground by *A. cockerelli* is not perceptible over distances greater than 2 cm and does not attract nearby nest mates, but rather increases the amount of time ants remain at a food source (Markl and Hölldobler 1978). However, in *Atta* leafcutter ants, stridulation during leaf cutting does attract nearby workers (Roces and Hölldobler 1996).

Paratrechina longicornis possess a dual recruitment system. Firstly, workers demonstrate similar specialized adaptations to foraging for large food items to *Ph. oxyops* and *A. albisetosus*. Indeed, *P. longicornis* also demonstrates an escort behavior for live prey items, which has not been previously described in ants. *Paratrechina longicornis* also displays recruitment consisting of long-lasting trail pheromones specialized for exploiting semipermanent carbohydrate sources (Witte, Attygalle, et al. 2007). Unlike *Ph. oxyops*, which appears to rely almost entirely on retrieving large food items, *P. longicornis* is very effective at exploiting long-lasting liquid carbohydrate sources, with almost 40% of worker returning to the nest with a filled crop. *Paratrechina longicornis* is thus also likely to possess a well-developed route memory, as demonstrated by other Hemiptera-tending ants (Salo and Rosengren 2001; Grüter et al. 2011).

There is a striking similarity between the short-term recruitment mechanism of *P. longicornis* and that of both *Ph. oxyops* and *A. albisetosus* (Figure 2). In all 3 species, an ant that discovers a food item that it cannot move always returns to the nest laying a pheromone trail, which always immediately results in a brief (1–2 min) surge of recruits exiting the nest then following the trail toward the item (Hölldobler et al. 1978; Czaczkes and Ratnieks 2012). In all 3 species, the pheromone is initially followed with considerable accuracy, with circa 85% of recruits choosing the correct branch in *P. longicornis* and *Ph. oxyops*, but the bioactivity of the pheromone falls rapidly, so that after 5–7 min, the pheromone has no more effect. This similarity is suggestive of convergent evolution, adapting the recruitment system to the task of rapid recruitment to large individual items. By contrast, when tested on an identical apparatus to that used here and in Czaczkes and Ratnieks (2012), a trail laid by a single *L. niger* worker was only followed by 62% of naive ants (Grüter et al. 2011), and such a trail was estimated to last up to 47 min (Beckers et al. 1993). *Lasius niger* relies heavily on semipermanent food sources and does not perform cooperative transport. We predict that other ant species that use tractive resistance in deciding whether to recruit to large food items, and then recruit nest mates to assist in cooperative transport, will also possess pheromone trails with similar properties to those of *P. longicornis*, *Ph. oxyops*, and *A. cockerelli*. The item must be located rapidly and the recruits sent to a precise location, which favors a volatile trail.

Our results show that *P. longicornis* has evolved a specialized system of recruiting nest mates to large food items, which are cooperatively transported and form the major part of the nonliquid food

brought back to the nest. This recruitment system works alongside long-lasting mass recruitment to long-lasting food sources, and no doubt gives *P. longicornis* an advantage over other species with a less flexible recruitment system. But to what extent this flexible recruitment system contributes to the crazy ant's success as an invader and how important it is relative to other common characteristics of invasive ants, such as unicoloniality, polygyny, and flexible nesting habits (Holway et al. 2002) remain open questions.

FUNDING

T.J.C. was funded by a Biotechnology and Biological Sciences Research Council (BBSRC) studentship (BB/D526888/1). A.V.-N. was funded by a São Paulo Research Foundation (FAPESP) PhD studentship. F.L.W.R. was supported by a travel grant from the São Paulo Research Foundation (FAPESP).

We would like to thank prof. Fabio Nascimento for inviting us to work with him in Brazil, Dr Benjamin Czaczkes for help with data management, prof. Marlene Sofia Arcifa for the use of her microbalance, and prof. Volker Witte and 2 anonymous referees for comments on previous versions of the manuscript.

Handling editor: Paco Garcia-Gonzalez

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