**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, $\chi = -8.155$, $P < 0.0001$, 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, $\chi = 0.725$, $P = 0.468$). 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

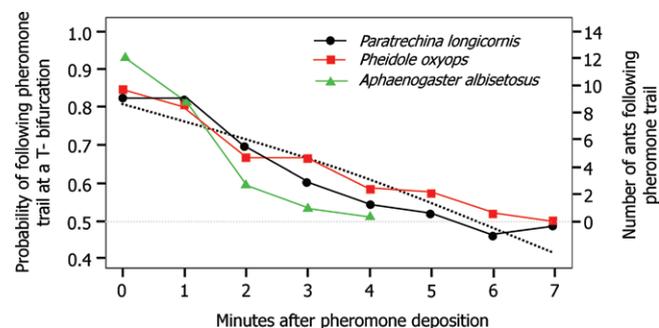


Figure 2

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:

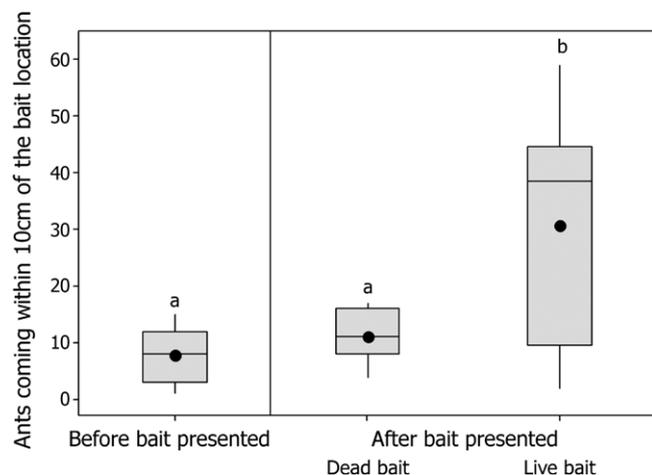


Figure 3

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 interquartile ranges, and whiskers signify the general extent of the data. Groups with the same letter above are not significantly different.

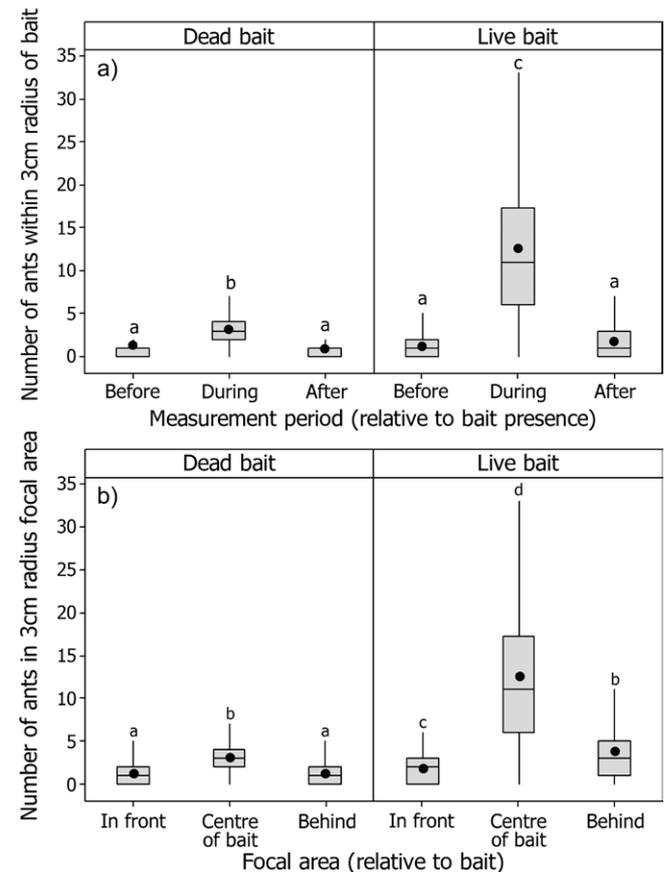


Figure 4

(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 interquartile ranges, and boxes signify interquartile ranges. Groups within each figure with the same letter above them are not significantly different.

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.

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