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RESEARCH ARTICLE

Well-coordinated cooperative transport in the invasive ant *Anoplolepis gracilipes*

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Abstract

Cooperative transport, defined as the coordinated effort of multiple individuals to move a single item, enables social animals to retrieve resources more efficiently and reduces the risk of exploitation by competitors. In this study, we provide the first detailed description of such a coordinated prey retrieval behavior in the invasive ant *Anoplolepis gracilipes*. We examined the influence of prey and colony size on the success of cooperative transport and investigated whether chemical cues, such as footprints of nestmates or workers of competing *Diacamma rugosum* ants, affected foraging decisions. Our results, using a Y-maze experimental setup, revealed that *A. gracilipes* does not modify its foraging behavior based on the presence or absence of chemical footprints. However, colonies exhibited fine-tuned cooperative transport, only once exhibiting a deadlock in 144 transport processes. The ants adjusted the number of workers involved according to prey weight, increasing retrieval speed. Nevertheless, lighter prey was still transported faster than heavier prey, even though fewer workers were involved in the transport. Lighter prey items and those carried by larger groups were more likely to be transported successfully, with most failures caused by navigation errors rather than insufficient workforce. Workers joined a transport typically positioning themselves at the front, aligned with the nest, rather than at alternative locations. Our findings highlight the efficiency and plasticity of cooperative transport, a coordinated behavior that likely confers the invasive *A. gracilipes* a significant competitive advantage over species lacking such sophisticated cooperative strategies.

Keywords Group retrieval · Interspecies footprints · Foraging · Y-maze

Introduction

Foraging is an important fitness-relevant behavior in all animals and often under strong selection (Allen et al., 2017; Chen et al., 2012; Vijendravarma et al., 2012). Social insects employ complex strategies to optimize food intake at the colony level. In addition to diverse recruitment strategies, some ants have evolved cooperative transport, a behavior in which multiple individuals work together to move a single item that is too large for an individual ant to carry. Aside from humans, ants are the only animals that regularly engage in large-scale cooperative transport (Czaczkes and Ratnieks, 2013). This coordinated behavior has been documented in approximately 40 different ant genera (Czaczkes and Ratnieks, 2013; McCreery and Breed, 2014; Wilson, 1972). Given its widespread taxonomic distribution and the diverse, often complex strategies employed in carrying methods, cooperative transport is believed to have evolved multiple times independently within the family Formicidae (Hölldobler and Wilson, 1990; Moffett, 1987).

Some ant species exhibit slow, uncoordinated, and inefficient transport, while others transport food items quickly and directly to the nest, often with minimal ground contact. The level of coordination in cooperative transport can be classified into three general syndromes: uncoordinated transport, encircling coordinated transport, and forward-facing coordinated transport (Czaczkes and Ratnieks, 2013), and is distinguished by the frequency of deadlocks, the positioning of ants relative to the transported item, and their relative speed. Consensus in decision-making could be achieved by follow-the-leader strategy, as shown for *Paratrechina longicornis* (Feinerman, 2018; Feinerman et al., 2018; Gelblum et al., 2020, 2015) or via the wisdom-of-the-crowd, as seen

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in weaver ants (Carlesso et al., 2024). The latter strategy is considered more efficient for transporting live prey items, because the follow-the-leader strategy depends on sensing the forces exerted by the leaders, which can be disrupted by the prey's movements (Carlesso et al., 2024).

The reasons why some species engage in cooperative transport, and the observed differences in efficiency, remain unclear. It is possible that this type of transport originally developed to facilitate the transport of large brood objects such as queen or male pupae (Czaczkes and Ratnieks, 2013; Moffett, 1987). Nevertheless, the primary benefit of cooperative transport is the ability to retrieve large prey items that individual workers cannot carry, thereby increasing food availability for the colony (Czaczkes and Ratnieks, 2013). Many ant species that do not engage in cooperative transport forage on large food items by dissecting them, feeding on-site, or carrying smaller fragments back to the nest (Djieto-Lordon et al., 2001; Richard et al., 2001; Yamamoto et al., 2009). However, the dissection process can take several hours (Yamamoto et al., 2009), increasing the risk of competitors discovering and dominating the prey item. Therefore, cooperative transport offers a solution to avoid exploitative or interference competition by quickly retrieving items (Cerdá et al., 1998; Hölldobler et al., 1978; Traniello and Beshers, 1991). Further, transporting food items to the nest may facilitate task partitioning, allowing idle ants to perform the dissection task at the nest while foragers return to foraging or other tasks (Moffett, 1987).

Under laboratory conditions, we observed and documented cooperative carrying behavior in the yellow crazy ant, *Anoplolepis gracilipes*, providing here a detailed description of this coordinated behavior for the first time. This species is well-known for its efficient recruitment strategies, utilizing chemical trails with both short- and long-lasting signals, which allow it to rapidly monopolize resources by quickly recruiting workers to new food sources (Lizon À l'Allemand and Witte, 2010). Their fast distribution of resources among nests (Hoffmann, 2014), and their typically superior ability to monopolize them are key factors in their invasion success as supercolonial ants (Human and Gordon, 1996). When foraging, they exhibit sequential load transport by transferring food items ant-to-ant, forming a chain, or indirectly, by collecting items laid down by other workers (Hsu et al., 2006). Sequential load of small items (~2 mg flies) over short distances (< 30 cm) in *Anoplolepis gracilipes*, is up to five times slower and covers nearly twice the distance compared to individual foraging, indicating that this strategy may not always be efficient. Further, *A. gracilipes* nests in a variety of habitats, including soil, crevices, leaf litter, bamboo sections, and arboreal environments such as canopy hollows and coconut trees (Lee and Yang, 2022). While cooperative transport is very rare in arboreal species, as transporting groups are likely to fall off branches, *A. gracilipes* has been reported to exhibit cooperative retrieval of mealworms back to the nest (Yamamoto et al., 2009).

Group size can influence information transfer and expedite prey retrieval by increasing the number of available foragers (Kolay et al., 2020). More items can be retrieved individually or by joining forces in cooperative transport, when more carriers result in faster transport (Carlesso et al., 2024; McCreery et al., 2019). Larger transport groups could transport a load more successfully if the accuracy of the transport is increased by combining several opinions (wisdom-of-the-crowd). This can be done by a sharper distinction of subtle differences. For example, larger groups exhibit persistent sliding along boundaries, maintaining their motion over extended distances with minimal changes in direction, whereas smaller groups show less persistence, often losing speed and directionality upon collision (Dreyer et al., 2024). Nevertheless, the opposite could also occur, and the forces of foragers of larger transport groups could cancel each other out, making the transport slower and fixing the travel speed to that of the slowest member (Buffin and Pratt, 2016). Further, interpersonal variation on the applied force can make the next move non-evident and consensus mechanisms are needed for efficient motion (Dreyer et al., 2024). In ants, evidence of faster "superefficient" (Burchill et al., 2023; Carlesso et al., 2024), and slower cooperative transport compared to individual transport has been reported (Buffin and Pratt, 2016). To assess the efficiency of cooperative transport, we explored the relationship between colony size, transport group size and weight in relation to transport speed and success. We aimed to explore whether the likelihood of success depended on whether the group size is more or less dynamic. Therefore we recorded group size every minute of the transport to determine variation over time. Further, some ant species that perform cooperative transport were shown to attach themselves non-randomly to a load (Buffin and Pratt, 2016; Czaczkes et al., 2011). Therefore, we recorded the positions of attachment of the ants when a second ant joined the transporter group. Ants could initiate transport either by attaching themselves to the first position they find when they bump into a prey, or they could strategically search for an advantageous position relative to the desired direction of movement.

We also investigated whether foraging and cooperative transport behaviors were influenced by the likelihood of interspecific competition. Ants may adjust their foraging paths based on the context, potentially avoiding, being attracted to, or ignoring interspecific chemical cues such as footprints (Hölldobler et al., 1978; Traniello and Beshers, 1991; Wüst and Menzel, 2017), i.e., hydrocarbon microdroplets left passively by the insects arolium (Lenoir et al., 2009). We used footprints of *Diacamma rugosum* as interspecific cues as both species likely coexist in South East Asia (Chen, 2008). The outcome of encounters between the

two species remains unknown. *Diacamma rugosum* workers, which prey on small invertebrates and possess powerful mandibles, are larger than *Anoplolepis gracilipes* workers. This size and predatory advantage could influence the interactions and competition dynamics between the two species (Annagiri, 2021; Manting et al., 2013). We determined the number of items transported by *Anoplolepis gracilipes* in a Y-maze setup, to compare foraging arms covered with conspecific footprints, interspecific footprints or no footprints.

Material and methods

Colony collection and rearing

Anoplolepis gracilipes ants are recognized as one of the world's 'worst invasive species' (Wetterer, 2005), with evidence of having displaced several dominant ant species as *Monomorium minutula*, *Anochetus graffaei, Oecophylla smaragdina* and *Leptogenys processionalis* (Abbott, 2004; Abbott et al., 2007). They nest opportunistically and form extensive polygynous and polydomous supercolonies (Lizon À l'Allemand and Witte, 2010) extending by more than 100 ha and hosting up to 20-million ants per hectare (Abbott, 2005). Twelve colonies of *A. gracilipes* were used, bred from parental colonies collected in 2018 in Thailand, and kept under laboratory conditions in a climate room set at 24 °C and with 75% humidity. The colonies were fed twice a week with crickets, water, and honey. Data was collected in May and June 2024.

Experimental procedure

To study whether *A. gracilipes* ants visit a foraging area with *D. rugosum* footprints and how the presence of these footprints affected their decision to cooperatively transport food, a Y-maze was used. We opted for a Y-maze to simulate the arboreal conditions of the *A. gracilipes* habitat (Lee and Yang, 2022). The Y-maze consisted of arms and a stem, each 21-cm long and 3-cm wide, covered with paper overlays supported by two plastic cups coated with Fluon. At the end of each arm, a metal plate with a food reward was placed (Fig. 1). The paper overlay in each arm was marked with two lines: the first decision line was situated 4 cm from the start of the arm, and the second decision line 1 cm before reaching the food reward.

Each colony was subjected to three experiments, each composed of a 20-min training run and a 45-min test run. During the training run, workers of each colony were allowed to walk on a paper Y-maze devoid of footprints to familiarize themselves with the maze and the food reward at the end of each arm. Five small pieces of honey wheat cereal were offered for a 20-min interval. After the training, the paper strips were removed, and one of three arm

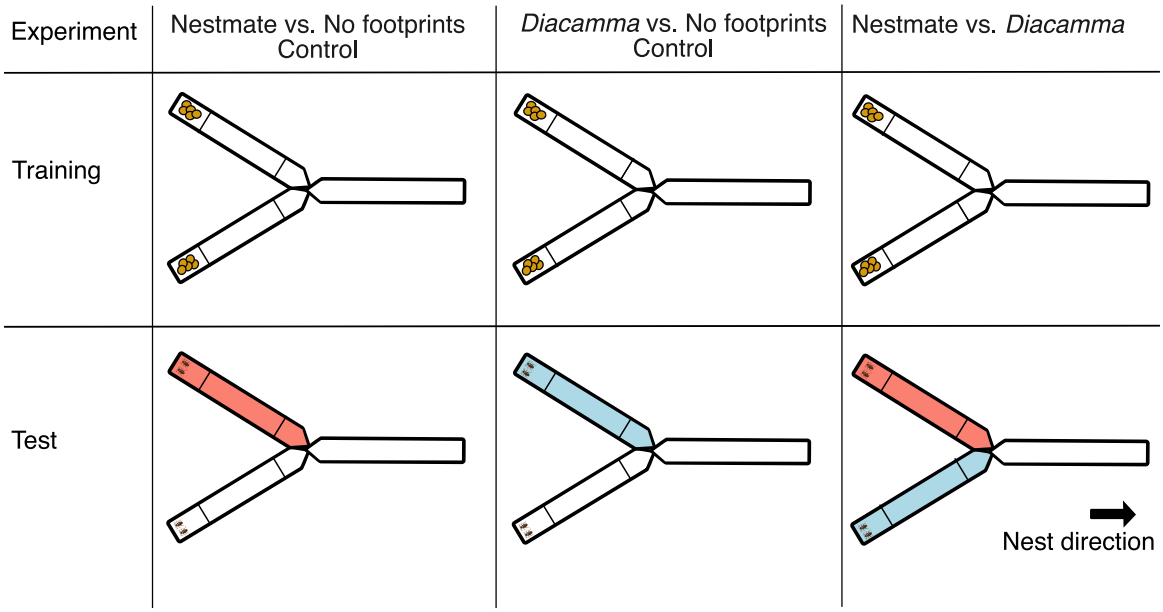

Fig. 1 Three arm combinations were tested using a Y-maze (21-cm long, 3-cm wide). The experiment consisted of a 20-min training phase followed by a 45-min footprint test. During the training phase, five honey wheat cereal pieces were placed on a metal plate at the end of each arm. For the test phase, two cockroaches of different weights were provided on a metal plate at each arm's end and replaced as workers removed them. Decision lines were marked on the paper strip: the first 4 cm from the bifurcation and the second 1 cm before the food plate. For graphical purposes, arms with nestmate *A. gracilipes* footprints are represented in red, while *Diacamma rugosum* competitor footprints in blue

combinations was tested: (1) one arm with a paper strip impregnated with conspecific footprints *(A. gracilipes*) vs. an arm free of footprints, (2) one arm with interspecific footprints (*D. rugosum*) vs. an arm free of footprints, and (3) conspecific footprints vs. interspecific footprints (Fig. 1).

The experiment consisted of a 20-min training phase followed by a 45-min footprint test. During the training phase, five honey wheat cereal pieces were placed on a metal plate at the end of each arm. For the test phase, two cockroaches of different weights were provided on a metal plate at each arm's end and replaced as workers removed them. Decision lines were marked on the paper strip: the first 4 cm from the bifurcation and the second 1 cm before the food plate. For graphical purposes, arms with nestmate *A. gracilipes* footprints are represented in red, while *Diacamma rugosum* competitor footprints in blue.

To ensure the paper strips were thoroughly impregnated with footprints, they were placed inside a colony of *Diacamma rugosum* (~ 200 individuals) or their own *Anoplolepis gracilipes* colony (median size = 186, sd = 965, min = 134, max = 3266) for 50–60 min prior to the experimental run. We tested the displacement and cooperative transport of the ants in response to nestmate and heterospecific footprints. Control combinations, in which the ants were tested against an arm free of footprints, allowed us to determine whether *A. gracilipes* avoided *D. rugosum* footprints or exhibited increased attraction to their nestmate footprints. The order of the three possible combinations and the side on which the footprints of each species were positioned was randomized. Each experiment was conducted with a 2-day interval between trials.

Weight selection

Cockroaches (*Blaptica dubia*) of varying weights were offered in a pilot test to seven *A. gracilipes* colonies to estimate the maximum weight an individual ant could carry and induce cooperative transport in the Y-maze setup. Cockroaches weighing between 0.04 and 0.19 g (median = 0.16 g, $n = 19$ trials) were tested. Cooperative transport was elicited at a minimum weight of 0.04 g, while 0.14 g was the maximum weight an individual ant could carry successfully to the nest without failure (Supplementary Fig. 1). However, individual transport of items weighing 0.09 g failed in three cases. In all test runs, food rewards included two cockroaches, one larger and one smaller, each weighing between 0.09 and 0.20 g, placed at the end of each arm. Cockroaches on both arms were of similar weight and marked with paint for differentiation. When a colony removed a cockroach, it was replaced to ensure constant food availability, while carried cockroaches were removed before retrieval in the nest to prevent saturation. If ants dropped a cockroach during transport, it was returned to the metal plate, and the retrieval was recorded as a failed transport attempt due to load loss.

We recorded the retrieval time for each item, its weight, the number of ants involved in transport, their positions, and the frequency of deadlocks per retrieval. During test runs, ant positions while carrying food items were documented two minutes after transport began, allowing time for adjustments in the number of ants participating. Ant positions were classified as front (pulling in the direction of the nest while holding the item) or back, and the number of ants in the lateral positions. Similarly, the number of ants engaged in transport was recorded two minutes after transport began. To measure ant influx in each arm of the Y-maze, two lines were marked: one 4 cm from the bifurcation (first decision line) and another 1 cm before the food reward (second decision line). Each experimental run was video recorded to track the number of ants crossing each line toward the food reward. Ant crossings were reported in four 10-min intervals to analyze arm-specific influx. Colony size was determined by transferring ants into an empty box, photographing them, and counting individuals using ImageJ software (v. 1.54, Schneider et al., 2012).

To document the attachment positions of the first and second ants relative to the cockroach and the nest direction we analyzed in detail 91 transport events, of which 54 and 37 were successes and failures, respectively. The failure cases were selected after exclusion of four failures attempts because no second ant joined, and eight failure cases where ants were offered cereal instead of cockroaches. We randomly selected 96 successes (roughly twice the number of failure cases) from the remaining carry attempts. We noted the cockroach's orientation when the second ant joined, noting whether it was lengthwise (head or abdomen facing the nest) or crosswise (right or left side facing the nest). In the majority of cases, at the moment of the second ant attachment the cockroach's head faced the nest direction and the first ant pulled from it lengthwise (92.5%, $n = 74$), therefore we categorized the attachment positions as follows: 30°–330° angular distributions, setting 0° as the nest direction, for the area of the antennae, head, or head margin; 30°–90° and 270°–330° corresponding to the first pair of legs, right or left, including the pronotum; 90°–150° and 210°–270° corresponding to the second and third pairs of legs, including the meso- and metathorax; and 150°–210° for the abdomen. We recorded whether the cockroach was in motion when a second ant joined the transport. In addition, we calculated the participation rate, defined as the number of carrier ants relative to the total number of ants on the Y-maze per minute of transport.

Statistical analysis

Data were analyzed using generalized linear effect models (glmmTMB v. 1.1.9, Brooks et al., 2017) in R (v. 4.4.1, R Core Team, 2024). We tested for differences between the first and second decision lines and for side bias, as it has been reported that some ants have a left-preference (Hunt et al., 2014; Oberhauser et al., 2018), for each experiment using the model

$$N^{\circ}\text{ ants crossing} \sim \text{Line} + \text{Side} + \text{time}$$

The data was fitted using a nbinom1 distribution. We selected the second decision line as the final decision of the ant to continue to the end of the arm. We then described the ability of cooperative transport by testing if the number of ants engaged in the transport was affected by the weight of the item and/or the colony size fitting a model as

$$N^{\circ}\text{ ants carrying} \sim \text{weight} + \text{colony size} + (1|\text{colony id})$$

We tested if the variation in the transport group, represented as the standard deviation of the number of ants carrying across time, was influenced by the number of ants carrying, the colony size and the outcome of the carrying attempt as

$$\log(\text{sd } N^{\circ}\text{ ants carrying} + 1) \sim \text{median\_}N^{\circ}\text{ ants carrying} + \text{colony size} + \text{failure} + (1|\text{colony id \& date \& experiment})$$

We calculated the median participation rate across the whole transport attempt, and modeled if it changed over time and with the weight of the item fitting a model as

$$\text{median\_participation\_rate} \sim \text{weight} + \text{Relative time} + (1|\text{colony id, date, experiment})$$

To test for preferences in engaging at the front or back of the item during transport, we fitted a model with position as a categorical fixed effect, comprising two levels: the ant is pulling the cockroach on the front (in the direction of the nest) or the back as

$$\text{Number of ants} \sim \text{Position} * \text{Total\_ants\_carrying (including in lateral positions)} + (1|\text{cockroach transported}) + (1|\text{colony id, experiment})$$

We used the time to traverse the arm (20 cm distance) to calculate an approximative speed to successfully retrieving items to the nest. Then we tested if the speed of transport was affected by the weight of the item and the number of ants cooperating (nbinom2, link = log) as

$$\text{Speed carrying} \sim \text{weight} + N^{\circ}\text{ ants carrying} + \text{colony size} + (1|\text{colony id})$$

A similar model was fitted for the probability of success in carrying the item across the second line assuming a binomial distribution of the variable "Success carrying item" using the model

$$\text{Success} \sim \text{weight} + N^{\circ}\text{ ants carrying} + (1|\text{colony id})$$

We tested if the time to start the transport was dependent on colony size with a model

$$\text{Time to start} \sim \text{colony size} + (1|\text{colony id})$$

To test the effect of interspecific and conspecific footprints (*D. rugosum* vs. *A. gracilipes, D. rugosum* vs. no footprints and *A. gracilipes* vs. no footprints) in the choice of visiting ants (i.e., attractiveness or repellant effects of footprints), we tested if the number of ants visiting the arm (log transformed) was influenced by the colony size (log transformed) and the odor of the arm for each comparison using the model

$$\log(\text{total ants}) \sim \text{odor} + \text{scale}(\log(\text{colony size})) + (1|\text{colony id, experiment})$$

using a gaussian distribution, and having "Experiment" as run in which the two arms are offered. To test if the odor of the path had an effect in the effectiveness of the cooperative transport, we used a model to test if the number of retrieved items (log transformed) was dependent on the odor of the arm, and the colony size, with random effects fitted as before as

$$\log(\text{carried items} + 1) \sim \text{odor} + \text{scale}(\log(\text{colony size})) + (1|\text{colony id, experiment})$$

In all cases, DHARMa (Diagnostics for HierArchical Regression Models, v.0.4.6, Hartig, 2020) was employed to check the residuals of the fitted models.

Results

Description of the cooperative transport

Anoplolepis gracilipes workers successfully transported cockroaches from the Y-maze arm tip to the nest (42 cm) in 96 of 144 attempts (66.7%). Failures, defined as the inability to cross the first decision line, occurred in 48 cases (33.3%). Of these, 32 cases (66.7%) resulted from workers losing the load by falling out of the setup, 10 cases (20.8%) involved workers descending from the setup and failing to return to the bridge while carrying the load, and 6 cases (12.5%) were due to workers abandoning the load and

ceasing transport. The transport groups that felt, predominantly did it from the inner margins of the Y-arm, the farthest edge from the nest, with 72%, while 28% of the groups felt outer margins, the nearest edge of the nest. The number of ants engaged in transport (measured 1 min after initiation) significantly increased with both prey weight (glmmTMB: z-value = 7.25, p = 4.13e-13; Fig. 2A) and colony size (glmmTMB: z-value = 2.53, $p < 0.05$; Fig. 2B). However, the decision to initiate transport was not influenced by colony size (z-value = − 1.45, $p = 0.15$) and typically occurred after a median of eight minutes (SD = 8.27) from the start of the experiment.

A detailed analysis of 91 transport attempts revealed a non-linear relationship between the number of ants on the setup and the number actively engaged in transport. The number of cooperating ants increased significantly (gam model, F-value = 12.19, $p < 2e\text{-}16$, Supplementary Fig. 2) until reaching an apparent saturation point at transport groups of four ants (Fig. 2C). Consequently, the participation rate—the ratio of ants transporting to ants present on the setup—increased slightly but significantly from 17.6

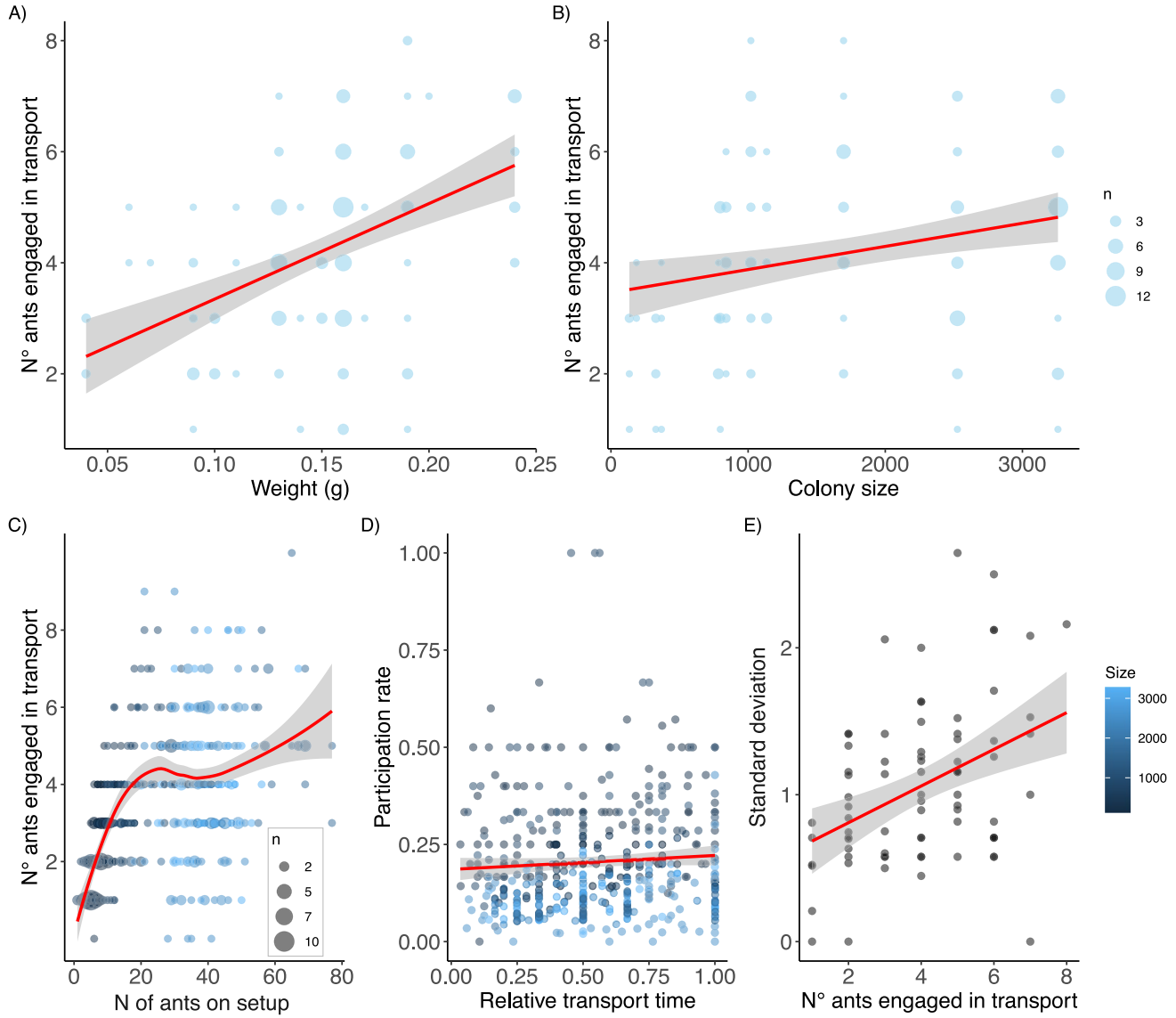

Fig. 2 Cooperative transport in *Anoplolepis gracilipes*. The number of ants engaged in cooperative transport increased with (**A**) the weight of the transported item and (**B**) colony size ($n = 144$). Dot size represents the number of samples for each value. In addition, the number of ants engaged in transport also increased with (**C**) the number of ants present on the setup ($n = 91$), with dot size again indicating sample counts. **D** The participation rate (number of ants engaged in transport relative to the number of ants on the setup) significantly increased over time ($n = 91$), while (**E**) variability in the number of ants engaged (higher standard deviation) increased as transport group size grew ($n = 91$)

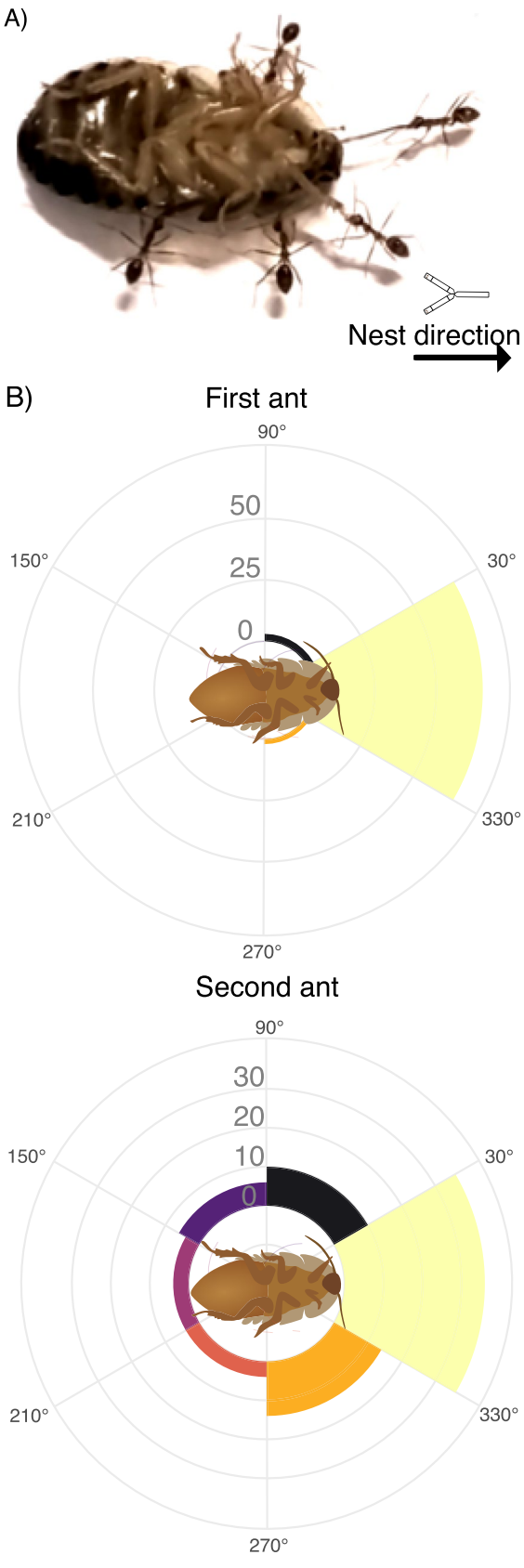

Fig. 3 Ant positions during cooperative transport. **A** An example of the location of ants during carrying behavior, and **B** the positions of the first ant initiating transport and the second ant joining cooperative transport when the cockroach was pulled lengthwise with the head facing the nest direction ($n = 74$). Positions were categorized based on an approximation of angular distributions setting 0° as the nest direction as: 30°–330° for the area of the antennae, head, or head margin; 30°–90° and 270°–330° corresponding to the first pair of legs, right or left, including the pronotum; 90°–150° and 210°–270° corresponding to the second and third pairs of legs, including the meso- and metathorax; and 150°–210° for the abdomen. The relative size of the wedge indicates the frequency with which ants attached to the cockroach at this position

to 18.9% during transport (glmmTMB, z-value = 5.11, $p = 4.8e\text{-}7$, Fig. 2D). Transport groups were more dynamic, with increased forager turnover (ants joining and/or leaving), when more ants participated in transport (glmmTMB, median number of ants, z-value = 2.89, $p < 0.01$, Fig. 2E) and in larger colonies (z-value = 2.10, $p = 0.04$). However, group dynamism was independent of the transport outcome (z-value = 0.25, $p = 0.80$).

We analyzed 91 transport attempts in detail and found that *Anoplolepis gracilipes* workers exhibited a non-random distribution around the cockroach when initiating transport. Workers predominantly pulled or dragged the cockroach lengthwise (92.0%, $n = 80$) with the head facing the nest direction (92.5%, $n = 74$, example in Fig. 3A). The first ant typically pulled from the head or antennae (93.2%, $n = 69$; Fig. 3B) while walking backward. The second ant preferentially joined at the head position (49.0%, $n = 36$) or the first thoracic segment, including the first pair of legs (32.4%, $n = 24$; Fig. 3B, Supplementary Video 1). Only 5.4% of second ants joined at the back position (abdomen). In 73.6% ($n = 64$) of cooperative transport cases ($n = 87$), the load of the first ant was stationary when the second ant joined transport. Crosswise dragging occurred in only 8% of cases, with the first ant pulling from the left side (2.3%) or the right side (5.7%) while the cockroach faced dorsally. One minute after transport began, more ants were positioned at the front of the cockroach in the direction of movement (toward the nest), while fewer ants were at the back (glmmTMB: z-value = 4.96, $p < 6.9e\text{-}7$, Supplementary Fig. 3A, $n = 134$). The number of ants at the front further increased as additional ants joined the transport (z-value = 4.21, $p < 2.6e\text{-}5$, Supplementary Fig. 3B). Only one deadlock was observed, involving three ants pulling in opposite directions for two minutes. Workers were observed to deposit pheromones on their way back from the food to the nest by touching the ground with the gaster at regular intervals.

We found that transport speed decreased 25.9% with increasing 0.06 g of load weight (glmmTMB, z-value = -5.41, $p = 6.23e\text{-}8$, Fig. 4A) and increased 28.7% when approximately two more ants joined the transport group (z-value = 3.92, $p = 9.0e\text{-}5$, Fig. 4A). Colony size

did not significantly affect transport speed (z-value = 1.76, p = 0.07). An outlier test for the transport speed model was not significant, indicating no need to exclude any data points (DHARMa *testOutliers*, observations = 96, p = 0.54). For transport success, the probability of successfully carrying the prey item to the nest increased with the number of ants engaged (glmmTMB, z-value = − 4.25, p = 2.11e-5, Fig. 4B) and decreased with increasing load weight (z-value = 3.39, p < 0.001, Fig. 4B). Outlier testing for the success model also showed no significant results (DHARMa *testOutliers*, n = 144, p = 1). For visualization, cockroach weight was categorized as light for weights < 0.14 g, medium > = 0.14 & < 0.18 g, and heavy as > = 0.18 g, as weight data deviated from a normal distribution (Shapiro–Wilk test, z-value = 0.94, p = 7.2e-6).

A. gracilipes do not regulate foraging in the presence of interspecific footprints

We observed a significant decrease in ant foraging activity over time (glmmTMB, z-value = − 16.58, p < 2e-16, Supplementary Fig. 4). Contrary to previous findings in other ant species, *A. gracilipes* did not exhibit a side preference across the three experimental conditions (lm A vs. N, t-value = − 1.5, p = 0.13; D vs. N, t-value = − 0.59, p = 0.56; glmmTMB, D vs. A, z-value = − 1.44, p = 0.15, Supplementary Fig. 5). Given that the second decision line was crossed less frequently than the first line (lm A vs. N, t-value = − 2.22, p = 0.02; D vs. N, t-value = − 3.22, p < 0.001; glmmTMB, D vs. A, z-value = − 2.74, p < 0.01, Supplementary Fig. 4–5) and its proximity to the food reward, we focused on the number of crosses of the second decision line, as an indication of the ants exhaustively foraging through the whole arm.

Workers of *A. gracilipes* showed no significant preference for an arm free of odor compared to one marked with conspecific footprints (glmmTMB A vs. N, z-value = − 1.09, p = 0.28, Fig. 5A), interspecific footprints (glmmTMB D vs. N, z-value = − 0.59, p = 0.55, Fig. 5B), or when both conspecific and interspecific footprints were simultaneously present (glmmTMB D vs. A, z-value = − 0.40, p = 0.67, Fig. 5C). Colony size was positively correlated with the number of ants in each arm across all comparisons, indicating that larger colonies have more foragers available (A vs. N, z-value = 3.21, p = 0.001; D vs. N, z-value = 4.55, p = 5.4e-06; A vs. D, z-value = 5.9, p = 5e-09).

Similarly, there were no significant differences in the number of items carried by the colonies based on arm odor (glmmTMB A vs. N, z-value = -0.36, p = 0.72; D vs. N, z-value = − 0.26, p = 0.80; D vs. A, z-value = − 0.25, p = 0.80, Fig. 5D–F). However, larger colonies transported more items in some comparisons (glmmTMB D vs. N, z-value = 2.71, p = 0.01; D vs. A, z-value = 2.30, p = 0.02, Fig. 5E, F), except when comparing conspecific footprints to an odor-free arm (A vs. N, z-value = 0.74, p = 0.46,

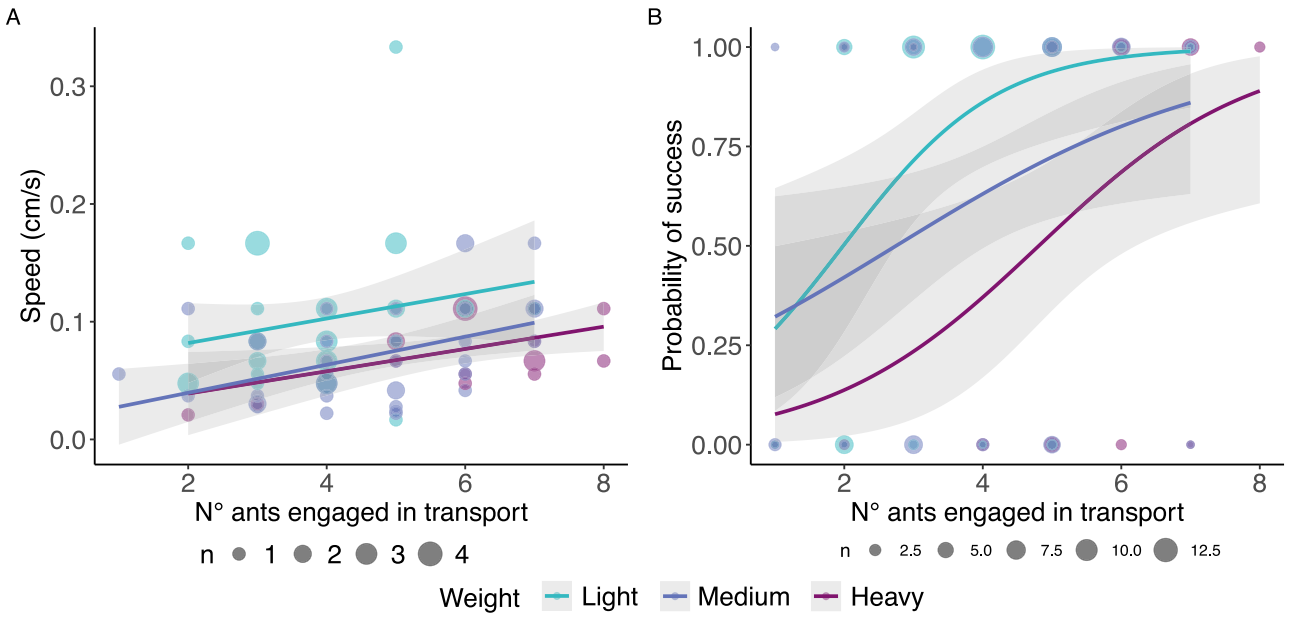

Fig. 4 Cooperative retrieval of prey to the nest. **A** The speed (cm/s) of successful transports increased with the number of ants cooperating but decreased with the weight of the prey item (n = 96). **B** The probability of successfully carrying an item to the nest increased with the number of ants involved in the transport and decreased with the weight of the prey item (n = 144). For visualization purposes, items were classified into three categories as "Light" for weights < 0.14, "Medium" weights between > = 0.14 & < 0.18, and "Heavy" for weights > = 0.18 g. Dot size represents the number of samples for each value

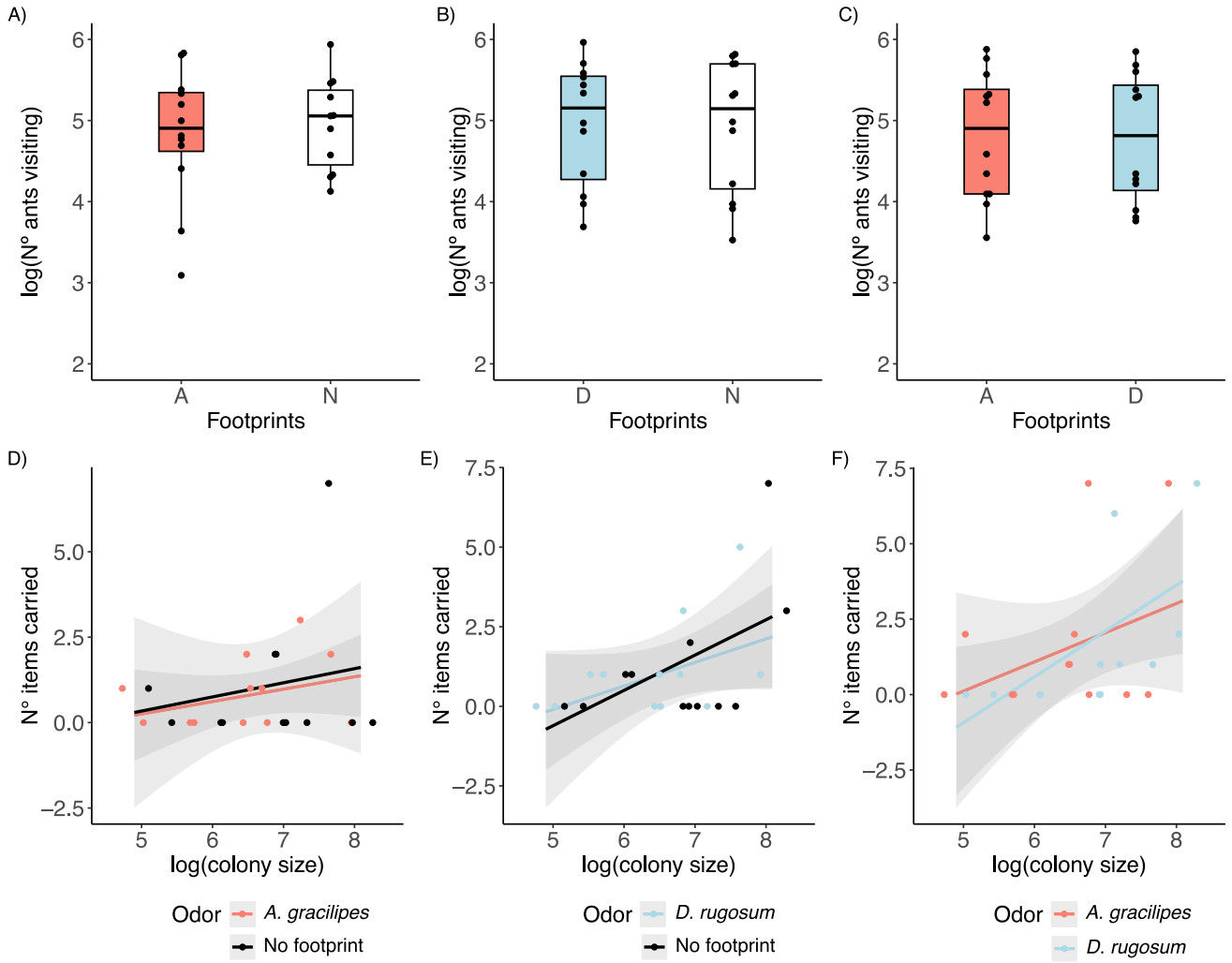

Fig. 5 Number of visits and items retrieved for each comparison. The number of *A. gracilipes* workers (log-transformed) per arm for comparisons **A** "A" for conspecific footprints of *A. gracilipes*, vs. "N" for no footprint. **B** "D" for interspecific footprints of *D. rugosum* vs. N, **C** A vs. D, and **D** the total items retrieved for each comparison depending on the colony size (log transformed) for the comparisons A vs. N. **E** D vs. N, and **F** A vs. D

Fig. 5D). The total number of items carried was similar across different experimental conditions (glmmTMB, AN vs. DA, z-value = 0.81, $p = 0.42$; AN vs. DN, z-value = 0.48, $p = 0.63$).

Discussion

Here, we provide the first detailed account of cooperative transport behavior in the invasive *Anoplolepis gracilipes*, highlighting several key findings. First, the number of workers involved in cooperative transport increased with the weight of the load, highlighting the adaptive allocation of labor based on task difficulty. Second, larger transport groups were more efficient, exhibiting a higher probability of successfully transporting items and a higher transport speed. Although larger groups increased transport speed, they could not fully compensate for the negative effect of prey weight. Heavier items were not transported faster than lighter items, even with more ants involved in the transport. Lighter items and larger transport groups increased the probability that the items were successfully transported back to the nest. Furthermore, the size of the transport group was affected not only by the weight of the item but also by the colony size, suggesting a scaling effect in resource mobilization. The position of the prey item during transport was not random, and the item was predominantly transported lengthwise, dragged from the head to the nest. Remarkably, workers did not change their foraging or transport behavior in response to the presence of competitors or the footprints of nestmates, suggesting that they do not rely on these cues in transport dynamics.

Using the three syndromes of cooperative transport previously defined (Czaczkes and Ratnieks, 2013), we classify *A. gracilipes* within the category of coordinated encircling transport. The cooperative transport exhibited a low number of deadlocks, and a quite high success rate when transporting a cockroach back to the nest through a tight (3 cm) paper branch. When the transport failed, 88% of the cases were due to navigation failure (falling from the setup or walking down the bridge and being unable to climb again) and only 12% due to workforce failure (workers giving up on the transport), suggesting that the biggest challenge during the transport is the coordination of forces. In this category, workers at the leading edge of the item pull, while those at the rear either push or lift the item with some species exhibiting specific behavioral adaptations. For instance, *Pheidole oxyops* workers preferentially grasp objects at corners, which increases transport speed, and they tend to hold the front and back of an object rather than the middle (Czaczkes et al., 2011). Here we showed that *A. gracilipes* preferentially transports the cockroach lengthwise with the head in the direction of movement, grabbing the cockroach by the edge of the head or from the antennae. Notably, the food item was never fully lifted off the ground during transport, aligning with the observation that the second and subsequent ants joining the effort did not lift from the rear but instead contributed to pulling from the front.

We observed that the number of ants engaged in transport increased with the weight of the item. While the transport speed increased with the number of ants, it decreased with heavier loads. This pattern is consistent with findings in other ant species, where group size increased with load weight, but transport speed declined accordingly (Carlesso et al., 2024; McCreery et al., 2019). When the item is heavier more carriers are present; nevertheless the load carried per capita remains higher, ultimately reducing the overall transport speed (Carlesso et al., 2024). Further, we observed that while *A. gracilipes* ants adjusted the number of carriers during transport, they appeared to reach a saturation point beyond which no additional ants joined regardless of the total number of ants present on the setup. Because the ants were not individually marked, we could not precisely determine the identity of ants joining or leaving the transport group or the duration of their participation. Nevertheless, larger transport groups significantly improved the likelihood of successfully carrying the item to the nest, with success rates being higher for lighter objects. These results suggest that having more workers in the transport group than necessary may lead to wasted effort (McCreery and Breed, 2014). It is unknown if the foragers can assess the ability of their nestmates to move a load, or if joining the transport group is influenced by the size of the item and the number of ants already engaged in transport. These findings are consistent with previous studies on cooperative transport in various ant species such as *Formica incerta* (Robson and Traniello, 1998), *Eciton burchellii*, and *Dorylus wilverthi* (Franks et al., 2001), where the group size of ants engaged in transport matches the size of the food item. In *E. burchellii* and *D. wilverthi*, large workers leave the transport group when no longer needed, ensuring that the group size remains optimal (Franks et al., 2001; McCreery and Breed, 2014). Further, *Paratrechina longicornis* ants succeed transporting a load by matching the group size to the object being carried, suggesting that ant move only after reaching a certain mass per ant threshold (McCreery et al., 2019). It is proposed that groups may evolve to function near a critical point, as this confers certain advantages such as increased responsiveness and the ability to process incoming information. In the case of collective transport by ants, the critical point appears to correspond to a specific group size (Gelblum et al., 2015).

Active recruitment was observed, as pheromone trails were deposited. *A. gracilipes* is known for its efficient recruitment system based on mass communication via chemical trails (Lizon À l'Allemand and Witte, 2010). The transport of heavy food items that require cooperative transport is an all-or-nothing event (McCreery and Breed, 2014). The colony either successfully transports the entire item or gets nothing. Therefore, eliciting rapid, strong recruitment to heavy items requiring cooperative transport, as we see in *A. gracilipes,* could be an adaptation to cooperative transport. *A. gracilipes* also exhibit sequential transport, a strategy associated with longer retrieval times and greater distances to the nest. Moreover, the presence of "robbers" near the nest entrance suggests that loaded workers actively avoid these individuals, providing evidence of non-cooperative behavior (Hsu et al., 2006). The lack of observed cooperation in that study may be attributed to the relatively light load (~2 mg flies) provided. In contrast, we offered significantly heavier items (the lightest being 45 times heavier), effectively promoting cooperative transport. Our findings demonstrate a clear benefit of cooperation, as it enhanced transport efficiency.

Our study revealed that *A. gracilipes* does not modulate their foraging behavior on a Y-maze with *D. rugosum* footprints. While the two species are likely to be sympatric in southern Asia, the colonies studied here had not previously encountered *D. rugosum*. It is possible that the footprints were below the threshold of perception or that they were perceived but did not elicit a response. It is known that the quantity of cues present plays a role to elicit or not avoidance behavior (Binz et al., 2014). All colonies retrieved a similar number of prey items from each arm, meaning *A. gracilipes* did not show attraction to arms marked with nestmate footprints. This lack of preference suggests no adjustment in foraging behavior in response to the likelihood of encountering a food source or nestmates.

Cooperative behavior is particularly intriguing, as it requires coordination of movements and efforts among multiple individuals. Such behavior likely arises from simple rules: informed individuals lead by pulling the load, while uninformed individuals align their efforts with the current direction of motion (Gelblum et al., 2016). Forces exerted by workers cause vibrations and small-scale deformations in the object, transmitting information about the direction of force through the object, even if it remains stationary (McCreery and Breed, 2014). Future research should investigate the mechanisms underlying the stability of transport groups, focusing on the rules that determine when ants join or leave and how they overcome navigation failures. Experimental setups that mimic complex environmental challenges, such as navigating a load across a tree branch, would provide valuable insights.

Regarding exploitative competition, further studies should examine whether *A. gracilipes* adjusts its behavior in the presence of competitors such *D. rugosum* or in response to higher concentrations of chemical cues, such as dense footprints indicative of interference competition. Confirming these results could provide evidence of how this invasive species avoids actively responding to competitor cues, leveraging its ability to monopolize resources and displace native species.

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Data availability statement All data used in this paper are available in tables 1-6 as supplementary material S2. One example video is included as supplementary material S3, and the script to recreate the analyses is provided as supplementary material S4.

Declarations

Conflict of interests We have no conflicts of interest to declare.

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