

RESEARCH ARTICLE

Path minimization in a tandem running Indian ant in the context of colony relocation

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ABSTRACT

The phenomenon of minimizing the path length to a target site in order to increase transport efficiency is described as path optimization, and it has been observed in many mammals, birds and some invertebrates such as honeybees and ants. It has been demonstrated that ants can optimize their foraging path through an emergent process, involving the trail pheromone concentration, without individual ants having to measure and compare distances. In the current study, we investigated whether ants that use only tandem running to recruit their nestmates can minimize their path while relocating their entire colony into a new nest. As colony relocation directly impacts the survival of the whole colony, it would be particularly important to optimize their path to the new nest. Using the ponerine ant *Diacamma indicum*, we conducted relocation experiments, in which ants had to choose between different defined paths, and contrasted our findings with open arena experiments, as they navigated to their new nest. After following 4100 unique transports by 450 different transporters, we found that these ants do minimize their path. Individual leaders, as well as colonies, chose the shorter path significantly more than the longer path, and they showed a significant preference for the shorter arm at multiple decision points on encountering a combination of paths. Thus, we concluded that tandem leaders are capable of path minimization based on the information they themselves collect. Further investigation into the proximate mechanisms by which they achieve this is required.

KEY WORDS: Insect navigation, Ant colony optimization, Non-trail laying ants, *Diacamma indicum*

INTRODUCTION

Animals would be expected to optimize their path when they visit a location multiple times. The phenomenon of path optimization has been documented in the context of foraging in a wide range of animals. Many vertebrates like bats (Lemke, 1984; Racey and Swift, 1985), birds (Davies and Houston, 1981; Gill, 1988), rodents (Reid and Reid, 2005) and primates (Janson, 1998; Watts, 1998; Noser and Byrne, 2010) are known to minimize their path length. Social insects like bumblebees (Heinrich, 1976; Gilbert and Raven, 1980; Ohashi and Thomson, 2009), honeybees and ants are also capable of path optimization in the context of foraging. Ants that use chemical trails to recruit their nestmates, like *Linepithema humile* (previously known as *Iridomyrmex humilis*) are known to use simple means to

minimize their path. Elegant experiments have demonstrated that a combination of being able to differentiate concentration gradients and the longer time required for pheromone concentration to build upon the longer trail was sufficient for the shortest path to emerge as the path of choice, without individual ants themselves having to measure the different distances (Goss et al., 1989). This finding allowed us to appreciate how ants can choose the best solution in a collective manner by following a simple set of rules. It also allows us to wonder if individual ants are capable of deciphering the shortest route with the information that they themselves collect. Furthermore, it would be equally important for non-trail-laying ants to minimize their path. Thus, it is essential to explore if individual ants and colonies of non-trail laying ants can optimize their path.

While pheromone trails are widely used in ants, various species with small colony sizes, rely on other mechanisms such as carrying, tandem running, and in some cases, both (Pratt et al., 2002; Pratt, 2008; Franklin, 2014). Some researchers hypothesize that pheromone trails are easier to maintain for large colonies compared with smaller colonies, as pheromone trails require constant reinforcement. Even though many small-sized colonies do use pheromone trails, they are likely to pay a higher price for maintaining them and alternative mechanisms may have evolved (Beckers et al., 1989; Hölldobler and Wilson, 1990; Beekman et al., 2001; Detrain and Deneubourg, 2008; Planqué et al., 2010; Lanan, 2014; Czaczkes et al., 2015). Carrying is the transport of an individual ant in the mandibles of another ant. Tandem running is the movement of a pair of ants that are in physical contact from one location to another. Typically, the ant that initiates the tandem run and takes the lead is termed a ‘tandem leader’. This individual is familiar with the destination and takes the follower to it (Möglich et al., 1974; Hölldobler and Wilson, 1990). Tandem running is used to recruit for various purposes like foraging, slave raids and colony relocation in different species of ants (Franklin, 2014). In a few species – *Leptothorax acervorum*, *Temnothorax albipennis* and *Diacamma rugosum* – tandem leaders are known to lay individual-specific markings along the path they travel or inside potential new nests (Möglich, 1979; Mallon and Franks, 2000; Maschwitz et al., 2010; Basari et al., 2014).

In the current study, we examined the ability of *Diacamma indicum*, a ponerine ant with small colony size that recruits their nestmates only through tandem running (Kolay and Sumana, 2015), to minimize their path in the context of colony relocation. Choosing a particular path for foraging or relocation will be dependent on the length of different available paths and other factors such as the presence of predators, obstacles and hostile neighbors. Colonies would not only have to minimize their path length but have to decide on the most optimum path considering all other factors. In the current study, we only examined path minimization in terms of length, as all other factors were kept constant. *D. indicum* is found in India, Sri Lanka and possibly in Japan. Their colony size ranges

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Received 8 May 2019; Accepted 4 October 2019

from 12 to 261 adults, and they are known to relocate upon disturbance to a new nest (Kaur et al., 2012; Sumana and Sona, 2012). Colony relocation is a goal-oriented task, involving the movement of all colony members, both the adults and brood, together with any stored resources from one nest to another. This task has a clear termination point, as it is completed when the old nest has been evacuated and all the colony members reach the new nest. The organization and dynamics of this task are likely to be different from other tasks that are conducted on a regular basis like guarding, nursing and foraging. Ant colonies relocate for various reasons; physical damage to their nest is one of the major reasons (Viginier et al., 2004; Kaur and Sumana, 2014). When colonies relocate, they are particularly vulnerable to abiotic stress factors such as temperature and humidity. Furthermore, they have to deal with biotic stresses caused by predators and even neighboring colonies' thieves (Paul et al., 2016). Thus, a strong selection pressure is expected to operate on colonies to reduce relocation time in order to lessen colony vulnerability. It would also be essential for colonies to maintain colony cohesion and transport all adults and brood to the new nest, as the loss of a reproductive individual or loss of the workforce will have a major negative impact on the survival of the colony. This set of requirements would translate into a need for tandem leaders to optimize their transportation path every time they recruit colony members to their new nest, possibly more so than in the context of foraging. In order to examine whether these ants are capable of minimizing their path length while they relocate to their new nest, we specifically asked four questions. We started by investigating if these ants explore two paths of the same length connecting the old nest to the new one and if they use both for tandem running. Next, we asked whether these ants preferentially use the shorter path, when given one short and one long path. Thirdly, we wanted to know if these ants are capable of choosing the shortest path when a combination of paths is available. Lastly, we wanted to know if the dynamics of the above three constrained path relocations were comparable to relocation in which ants were free to follow paths of their choice in an open arena. All these experiments were carried out inside the laboratory and both colony-level and individual-level comparisons were performed.

MATERIALS AND METHODS

Colony collection and maintenance

Colonies of *Diacamma indicum* Santschi 1920 ($N=47$) were collected using the nest-flooding method (Kaur et al., 2012) from Mohanpur, Nadia, West Bengal, India ($22^{\circ}56'N$, $88^{\circ}31'E$), between November 2015 and May 2017. Our study organism is not an endangered species and the area from where the colonies were collected is not protected. No special permission was required for collecting colonies from this area but our experiments do comply with regulations for animal care in India. The colonies were brought to the laboratory and transferred to a nest box ($28.5\text{ cm}\times 21.5\text{ cm}\times 12\text{ cm}$). Each nest box contained an artificial nest made of a Petri dish lined with plaster of Paris and covered in red cellophane to make the chamber dark. Colonies were provided *ad libitum* food consisting of ant cake (Bhatkar and Whitcomb, 1970), water and termites occasionally. Each colony had a single reproductive individual (gamergate) who was identified by the presence of a pair of gemma. All adult females of the colonies were given individual identities by marking one or more of their body parts (1st and 2nd thoracic segments and gaster) with non-toxic enamel paint (Testors, Rockford, IL, USA) (Kaur et al., 2012). Each colony was used for a single relocation.

Experimental setup

For each relocation, the nest containing the colony (old nest) was placed inside a fresh nest box with a sand floor, which acted as the old nest site. This nest box was connected to a similar nest box, which acted as the new nest site by one or two wooden bridge(s) depending on the experiment being conducted. The latter box contained an empty nest (new nest) which was identical to the old nest. The distances between the two nest boxes were within the range of the distances across which *D. indicum* colonies relocate in their natural habitat. In the natural habitat, colonies traveled a minimum of 61 cm and a maximum of 678 cm to occupy a final shelter (Kaur et al., 2012). The walls of the boxes and the bridges were coated with petroleum jelly (Vaseline, Hindustan Unilever Limited, India) to prevent ants from escaping. The relocation was initiated by removing the roof of the old nest (Petri dish cover) and placing a white light on top of the old nest to motivate the colonies to relocate to the new nest in all cases (Sumana and Sona, 2013). A video recorder (Sony Handycam) was placed at the old nest site as well as at the new nest site to record the behavior of the ants at the exit and entry point to the old and new nests. Before the start of every relocation, the sand base of the experimental setup was replaced with fresh sand.

To examine whether *D. indicum* preferred to take the shorter path as opposed to a longer path during relocation, three different sets of relocation experiments were conducted. In *D. indicum*, the tandem leaders play a crucial role during colony relocation. Tandem leaders transport nestmates one at a time. All adults are brought by means of tandem running and brood is carried mostly by followers of the tandem run and sometimes by the transporters in their mandibles. Unlike trail-laying ants, who follow the trail pheromone to reach their destination, tandem running ants have to navigate to their destination every time they transport nestmates and return to the starting point. This requires tandem leaders to make decisions along the path. By using the setups described below, we investigated whether these leaders explore and use multiple paths, and if so, are they capable of choosing the shorter path.

Equal path relocation

In this experiment, we examined whether *D. indicum* colonies explored and used a single path or both paths when two paths of equal length were available between the old and the new nest during relocation. Here, two nest boxes ($28.5\text{ cm}\times 21.5\text{ cm}\times 12\text{ cm}$) were placed 150 cm apart and two wooden bridges ($150\text{ cm}\times 5\text{ cm}\times 5\text{ cm}$) were used to connect these boxes. As both the paths were identical, they were identified as path 1 and path 2 randomly. The schematic of the setup is presented in Fig. 1A. Colonies ($N=9$), having 100 ± 24.38 adults, 28.22 ± 21.31 pupae, 10.22 ± 9.25 larvae and 26.44 ± 20.51 eggs (means \pm s.d.), were used to conduct this set of experiments.

Unequal path relocation

In this experiment, we examined whether *D. indicum* colonies preferentially used the shorter path during relocation to transfer nestmates, when they have one short and one long path connecting the old and new nests. Here, two wooden boxes of $20\text{ cm}\times 20\text{ cm}\times 5\text{ cm}$ dimension were used as nest sites and two wooden bridges of different lengths were used to connect them. One was a straight bridge (short path) with a dimension of $60\text{ cm}\times 5\text{ cm}\times 5\text{ cm}$, and another was a curved bridge (long path) with a dimension of $120\text{ cm}\times 5\text{ cm}\times 5\text{ cm}$. Hence, the longer curved path was double the length of the short straight path. The schematic of the setup is presented in Fig. 1B. Ten colonies, having 94.40 ± 40.99 adults, 18.40 ± 12.82 pupae, 11.90 ± 5.58 larvae and 20.90 ± 9.04 eggs, were used to conduct this set of experiments.

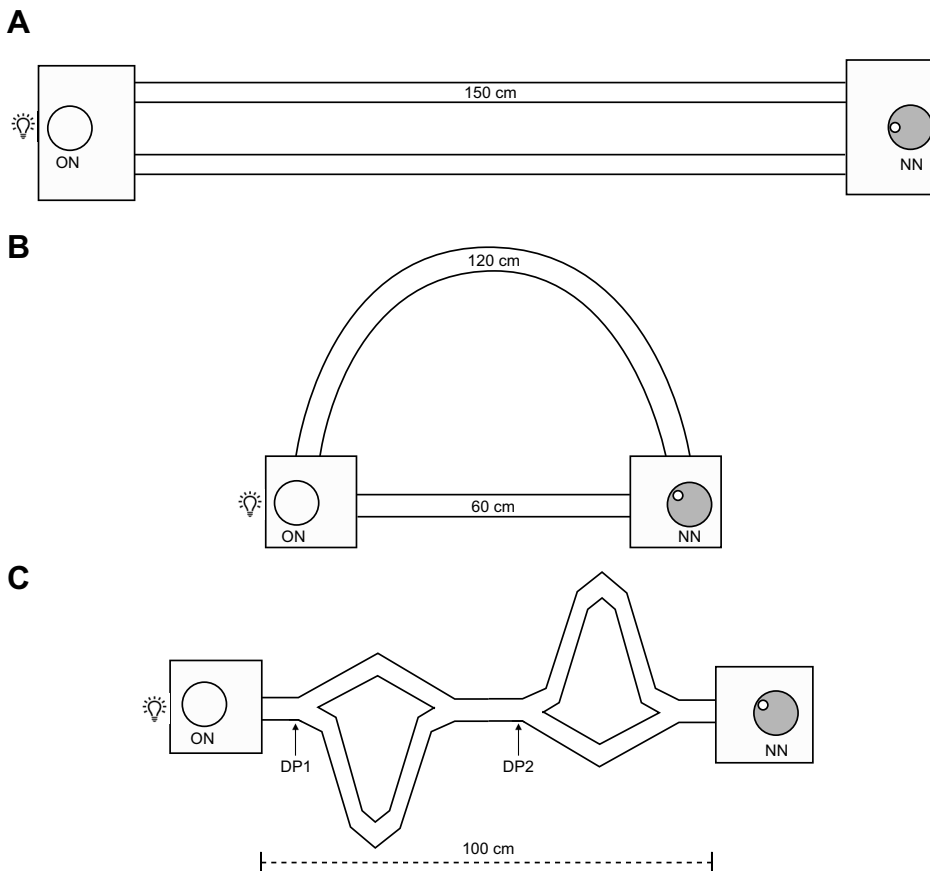


Fig. 1. Schematic representation of the experimental setups used in the laboratory. In all cases, the old nest (ON; which lacked a roof and had a light source placed above it in all cases) was connected to the new nest (NN) by a wooden bridge. (A) Two paths of equal length were used in equal path relocation. (B) Two paths of different lengths were used for unequal path relocation. (C) A combination of paths of different lengths was used in combined path relocation. The decision points at which ants had to choose the direction of the short or long path along their outwards journey (ON to NN) are labeled as DP1 and DP2.

Combined path relocation

In this set of experiments, we assessed whether *D. indicum* used the shortest path among a combination of four different paths. More specifically we enquired if *D. indicum* is capable of choosing shorter path at two different decision points along its way to the new nest. The design of the bridge connecting the old to the new nest was adopted from Goss et al. (1989) and this design ensures that no external bias or any inherent left or right preference of the ants can lead to the selection of short path at both decision points. While previous experiments examined if ants can choose one path over the other when only two paths were available, this experiment explored if these ants can indeed decipher the shortest route from a combination of four available routes. The old and new nest sites (20 cm×20 cm×5 cm) were placed 100 cm apart from each other and were connected by a bridge, consisting of two modules as detailed in Goss et al. (1989). This bridge has one entry and one exit point along with four possible paths of three different lengths. The length of the shortest path was 110 cm, the medium path was 144.9 cm and the longest path was 178.8 cm. The bridge was designed in such a manner that the ants had to face a short followed by a long path (SL path) in one direction and the alternative combination of long followed by a short path (LS path) in the other direction. Thus, if they had a left bias, they would be expected to use SL path, and right bias would lead them to use LS path when the old and new nests were placed as shown in the schematic (Fig. 1C). Along these paths, there were two decision points where tandem leaders or transporters had to choose a left or right turn that leads to either a short or long path. Ants that chose the direction that led them on the short path or the long path in both the decision points were denoted as taking the SS and LL respectively. Nine colonies

having 99.6 ± 27.8 adults, 15.50 ± 11.21 pupae, 9.50 ± 5.25 larvae and 21.62 ± 15.92 eggs, were used for this set of experiment. Video recorders were placed such that we could record events occurring both at the old and new nest sites, as well as the two decision points.

Open arena relocation

In nature, *D. indicum* colonies relocate across open fields where long defined paths are not expected. Instead, ants would be free to choose any path that they want in an open arena. Thus, in order to get a perspective of our defined path relocation in comparison to what is typically seen in nature, we conducted another two sets of relocations with different colonies. These relocations were performed from an old nest to a new nest across similar distances as the defined path relocations and in a similar manner as described above and thus were considered as control experiments. The idea was to check if the overall relocation time and transportation time across the defined path and open arena relocation were comparable. These lab arenas consisted of wooden boxes lined with aluminium, and the arena floor was covered with sand. This sand base was shuffled thoroughly before the start of any experiment. To prevent the ants from escaping, the walls of the arena were coated with petroleum jelly (Vaseline, Hindustan Unilever Limited, India). Relocation dynamics are influenced by several parameters, of which distance over which colonies relocate would be an important parameter (Sumana and Sona, 2012). For control experiments, the distance from old to new nest and the colony size were kept comparable to the defined path setups (Table S1). The open arena 1 experiment (OA1) was performed with ten colonies in a 145 cm×176 cm×30 cm arena, by placing the old and the new nests at diagonally opposite corners of the

arena. Thus, the colonies relocated over a distance of 180 cm in this experiment. Colonies ($N=10$), having 124 ± 53.67 adults, 37.1 ± 30.13 pupae, 28.9 ± 16.32 larvae, and 40 ± 26.14 eggs, were used for OA1. The open arena 2 experiment (OA2) was performed with nine colonies in a $91.4\text{ cm}\times 91.4\text{ cm}\times 30\text{ cm}$ arena, by placing the old and the new nests at diagonally opposite corners. Thus, colonies relocated over a distance of 90 cm in this experiment. Colonies ($N=9$), having 91.67 ± 38.72 adults, 17.44 ± 21.80 pupae, 8.44 ± 9.33 larvae and 36.78 ± 29.45 eggs, were used for open arena 2. OA1 was used to compare with equal path relocations (the distances between two nests were 180 cm and 160 cm respectively). OA2 was used to compare relocation dynamics with unequal path and combined path relocation. The distance between old and new nests was 90 cm in OA2 while the shortest distance between old and new nest was 80 cm in unequal path relocation and 110 cm in combined path relocation.

Behavioral observations and statistical analysis

Behavioral observations were conducted by using an audio recorder and multiple video recordings. Data was decoded from these recordings into datasheets and then entered in Microsoft Excel 2013 (Windows 10) for analysis. Information regarding the time at which the colony was placed inside the arena, the time at which the first tandem run reached the new nest (defined as start of transport) and the time at which the last tandem run reached the new nest (defined as end of transport) were recorded for every relocation in both open arenas and defined path experiments. Based on these inputs, the total transportation time (time between the start to the end of transport) and total relocation time (defined as the differences in time between when the colony was placed in the arena to the final tandem run into the new nest) were calculated. Following the last tandem run, we waited for 30 min to ensure that there were no additional transport events and that relocation was complete.

We collected data at the level of individual ants for different parameters from both the audio and video recordings. Ants that came out of the disturbed old nest, moved around the arena and found the new nest were labeled as explorers. We noted the individual identity of explorers on different paths in each of the defined path experiments. In the case of combined path relocation, we considered the first bifurcation in the setup as first decision point as the ants would have to start by choosing either the short or the long arm at the junction as they headed to the new nest. All explorers' and leaders' decisions regarding their choice was considered at this decision point. We considered the start of

transportation as the end of the exploratory phase in any relocation. Once transportation was initiated, the identity of the transporters, initiation site and initiation time were recorded. The identity of the followers and any brood (eggs, larvae or pupae) that she carried was also noted. Once transporters started walking on any one of the paths, we recorded the choice made by the particular transporter for the particular transport for every outward or forward journey from the old to the new nest. Thus, we had information on every transport and transporters in terms of their path. The percentage of colony members that became explorers and transporters and the percentage of the transporters who traveled along each of the available paths was calculated separately for each experiment. Statistical tests were performed using StatistixXL (version 2.0) and R (version 3.2.3). Two-tailed non-parametric tests were used for all the analysis and $P<0.05$ was considered as the cut-off value for statistical significance. Unless mentioned otherwise, mean \pm s.d. values are presented.

RESULTS

Equal path relocation

In the first experiment, all nine colonies relocated successfully to the new nest and colony cohesion was maintained as all colony members moved into the new nest. Both the paths were explored before relocation started. On average, $13.76\pm 5.53\%$ of the colony members became explorers and $15.63\pm 4.31\%$ of the colony became tandem leaders. Of the explorers, $69.83\pm 11.93\%$ explored only a single path, which was significantly greater than those who explored both paths (Wilcoxon paired-sample test, $T=0.001$, $N=9$, $P<0.01$; Fig. 2A). Of the leaders, $45.48\pm 20.79\%$ used only a single path to lead tandem runs during relocation, which was statistically comparable to leaders who used both paths (Wilcoxon paired-sample test, $T=17.0$, $N=9$, $P=0.59$; Fig. 2B). At colony level, there was no significant difference in the use of two paths, 42.56 ± 29.36 and $57.44\pm 29.36\%$ tandem runs occurred along path 1 and 2, respectively (Wilcoxon paired-sample test, $T=16.0$, $N=9$, $P=0.49$). Individual leaders had a significantly higher preference for the path through which they initiated their tandem runs compared with the alternative path (Table S2).

Unequal path relocation

When colonies had to relocate across two unequal paths, we found that they completed the task in all cases and colony cohesion was maintained. On average, $14.87\pm 8.97\%$ of the colony members became explorers and $8.75\pm 2.25\%$ became tandem leaders. Both

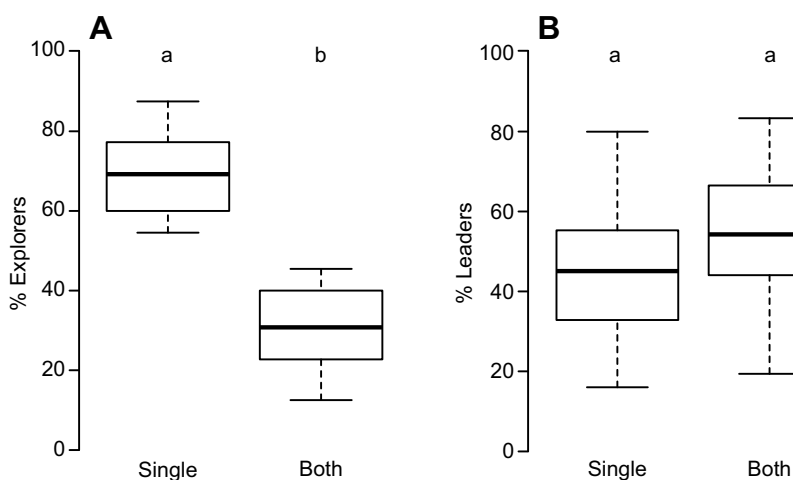


Fig. 2. Path choice of *Diacamma indicum* ants when two paths of equal length are provided. Box plots show percentage of (A) explorers who explored the different paths and (B) leaders who used those paths, across all colonies ($N=9$). The bold black line inside the box denotes the median value, and the box represents the interquartile range, the whiskers represent the data points which are within 1.5 times the interquartile range. Comparisons were done using Wilcoxon paired-sample test, and significant differences ($P<0.05$) across the categories are represented using different letters.

paths were explored by the explorers before the start of relocation and the explorers who explored only the short path ($46.25 \pm 23.62\%$) and both the paths ($41.53 \pm 23.77\%$) are statistically comparable but the percentage of explorers who explored only the longer path ($12.21 \pm 7.99\%$) is significantly lower (Friedman test, chi-square=14.26, d.f.=2, $P < 0.001$; *post hoc* Wilcoxon paired-sample test with Bonferroni's correction, short vs both, $P = 0.36$; short vs long, $P < 0.001$; both vs long, $P < 0.01$; Fig. 3A). The percentage of leaders who transported only on the short path (60.44 ± 30.99) or both the paths (37.40 ± 29.34) was significantly higher than leaders who used only the long path (2.16 ± 5.04), whereas the percentage of leaders who used only the short path or both the paths were statistically comparable (Friedman test, chi-square=12.39, d.f.=2, $P = 0.002$; *post hoc* Wilcoxon paired-sample test with Bonferroni's correction, short vs both, $P = 0.08$; short vs long, $P < 0.001$; both vs long, $P < 0.01$; Fig. 3B). At the colony level, $87.96 \pm 12.03\%$ of tandem runs followed the short path during relocation, which was significantly higher than those who followed the long path (Wilcoxon paired-sample test, $T = 0.0$, $N = 10$, $P < 0.01$; Fig. 3C). Even at the level of the individual leader, they showed the same preference (Table S2). This indicates that even though both the paths were explored before transports were initiated, leaders preferred the shorter path to relocate their colony.

Combined path relocation

All nine colonies relocated successfully when they were given a combination of paths of different lengths between the old and new nests. Colony cohesion was maintained in all cases. On average $12.86 \pm 3.71\%$ of the colony explored the paths from the old nest to the new nest and $11.33 \pm 2.80\%$ became tandem leaders. All four possible combinations of paths were explored by the explorers before the transportation started. On considering the first decision point where the ants had to decide whether to take the short or long path we found that the percentage of explorers who explored only the short path ($45.38 \pm 9.61\%$), both the paths ($31.75 \pm 13.32\%$) and only the long path (22.86 ± 13.29) were statistically comparable [Friedman test, chi-square=14.26, d.f.=2, $P = 0.03$; *post hoc* Wilcoxon paired-sample test with Bonferroni's correction, short vs both, $P = 0.06$; short vs long, $P = 0.02$; both vs long, $P = 0.41$ (following Bonferroni's correction for a significant difference, P should be lower than 0.016)]. The percentages of leaders who used only the short path ($52.46 \pm 35.58\%$), both the paths ($36.27 \pm 29.39\%$) and only the long path ($11.23 \pm 12.31\%$) were statistically comparable (Friedman test, chi-square=14.26, d.f.=2, $P = 0.16$). At the colony level of the outward journey, $56.92 \pm 28.28\%$ of the tandem runs occurred on the SS path, $27.34 \pm 27.4\%$ on LS path, $12.86 \pm 16.4\%$ on SL path and $2.88 \pm 3.56\%$ on the LL path. The percentage of tandem runs on the SS path was significantly higher than on the SL and LL paths, whereas on the LS path, this figure was statistically comparable with runs on both the SS and LL paths (Friedman test, chi-square=16.57, d.f.=3, $P < 0.001$; *post hoc* Wilcoxon paired-sample test with Bonferroni's correction, SS vs LS, $P = 0.23$; SS vs SL, $P = 0.01$; SS vs LL, $P < 0.01$; LS vs SL, $P = 0.23$; LS vs LL, $P = 0.03$; and SL vs LL, $P = 0.08$). For analysis at the level of individual leaders, only those leaders who had done at least two transports from the old to the new nest successfully were considered. Of the 77 such tandem leaders, 15 used the shortest path, 2 leaders used either of the intermediate paths exclusively and 60 leaders used all the four available paths for transportation. None of these leaders used the longest path only for transporting the colony. They showed a significant preference for the shortest path

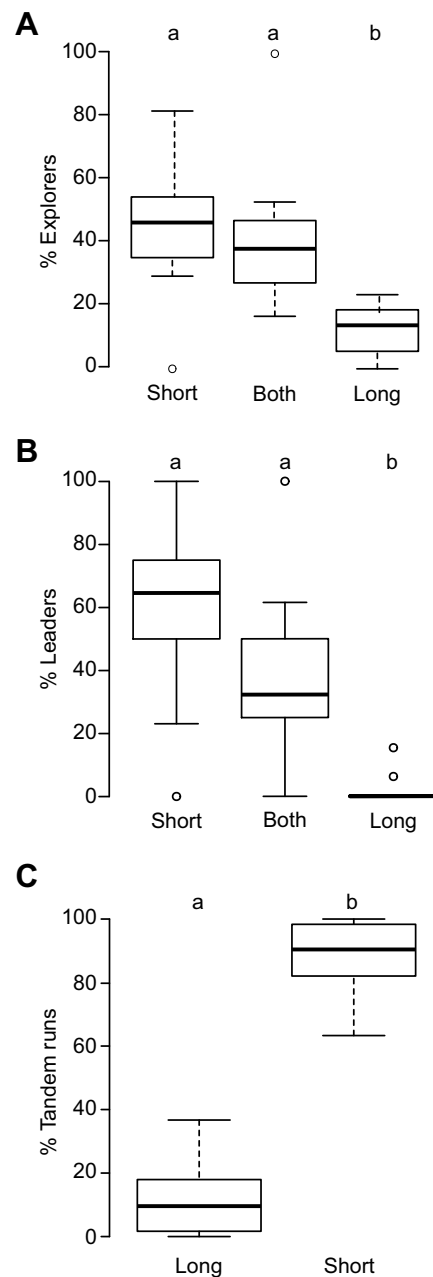


Fig. 3. Path choice by *D. indicum* when two paths of different lengths are provided. Comparison of percentage of explorers (A) who explored different paths, leaders (B) who used different paths and overall path choice for the colony (C) across all colonies ($N = 10$). The bold black line inside the box denotes the median value and the box represents the interquartile range, the whiskers represent the data points which are within 1.5 times the interquartile range and the circles denote the outliers. Comparisons for percentage of explorers and leaders were made using Friedman's test and *post hoc* Wilcoxon paired sample test with Bonferroni's correction. Comparison of the path choice for the colony was conducted using Wilcoxon paired sample test. Significant differences ($P < 0.05$) across the categories are represented using different letters.

over the intermediate and longest paths along their outward journey. Among these 77 tandem leaders $55.53 \pm 35.64\%$ of their tandem runs occurred on the SS path, $28.34 \pm 33.73\%$ on the LS path, $11.73 \pm 20.25\%$ on the SL path, and $4.39 \pm 10.82\%$ on the LL path (Friedman test, chi-square=81.571, d.f.=3, $P < 0.0001$; *post hoc* Wilcoxon paired-sample test with Bonferroni's correction, SS vs LS, $P < 0.001$;

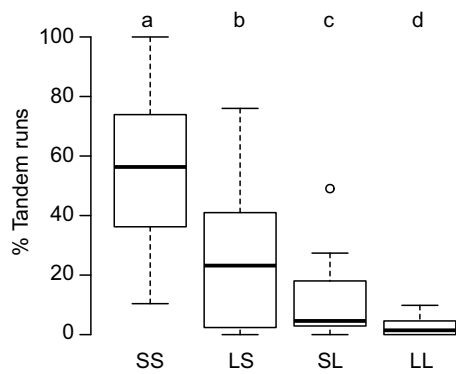


Fig. 4. Path choice by *D. indicum* when four combinations of paths of different lengths are provided. The path choice of individual leaders ($N=77$) is shown for the forward tandem run. The bold black line inside the box denotes the median value and the box represents the interquartile range, the whiskers represent the data points which are within 1.5 times the interquartile range and the circle denotes the outlier. Comparisons of explorers and leaders were done using Friedman's test and *post hoc* Wilcoxon paired sample test with Bonferroni's correction. Significant differences ($P<0.05$) across the categories are represented using different letters. SS, tandem leaders taking the short path at both decision points; LS, long path followed by short path; SL, short path followed by long path; LL, long path at both decision points.

SS vs SL, $P<0.0001$; SS vs LL, $P<0.0001$; LS vs SL, $P=0.002$, LS vs LL, $P<0.0001$; and SL vs LL, $P=0.006$; Fig. 4).

Impact of path on relocation dynamics

The relocation experiments in which discrete paths were provided was compared with relocation experiments where ants were free to choose any path within an open arena. This set of comparisons allowed us to examine if the overall relocation time and transportation time were impacted as a result of the constraints imposed by the defined paths. Total relocation time (Fig. 5A) was significantly higher in all three defined path setups compared with their respective controls. Total relocation time for equal path and OA1 relocation was 110.28 ± 225.61 min and 82 ± 14.89 min, respectively (Mann–Whitney test; equal vs open arena 1, $U=77.0$, $N_1, N_2=9, 10$, $P=0.01$). Total relocation time for unequal path, combined path, and OA2 relocation was 131.70 ± 43.22 min, 132.56 ± 56.02 min and 76.32 ± 19.20 min, respectively (Mann–Whitney test; unequal vs OA2, $U=73.0$, $N_1, N_2=10, 9$, $P=0.02$; combined vs OA2, $U=68.0$, $N_1, N_2=9, 9$, $P=0.01$). The transportation time (Fig. 5B) was comparable between defined path setups and the controls. Average transportation time for equal path and OA1 relocation was 51.98 ± 15.62 min and 48.1 ± 16.35 min, respectively (Mann–Whitney test; equal vs OA1, $U=54.0$, $N_1, N_2=9, 10$, $P=0.49$). Average transportation time for unequal path, combined path and OA2 relocation was 59.7 ± 19.43 min, 67.44 ± 25.87 min and 45.44 ± 11.72 min, respectively (Mann–Whitney test; unequal vs OA2, $U=64.0$, $N_1, N_2=10, 9$, $P=0.13$; combined vs OA2, $U=63.0$, $N_1, N_2=9, 9$, $P=0.05$).

DISCUSSION

Colony relocation is an essential and relatively common task among ants (Hölldobler and Wilson, 1990). Relocation is different from foraging in three ways. Firstly, relocation is a task that has a clear termination and hence is termed 'goal-oriented', unlike foraging, which is conducted in a continuous manner. Relocation involves the movement of the whole colony, all adults and immature young,

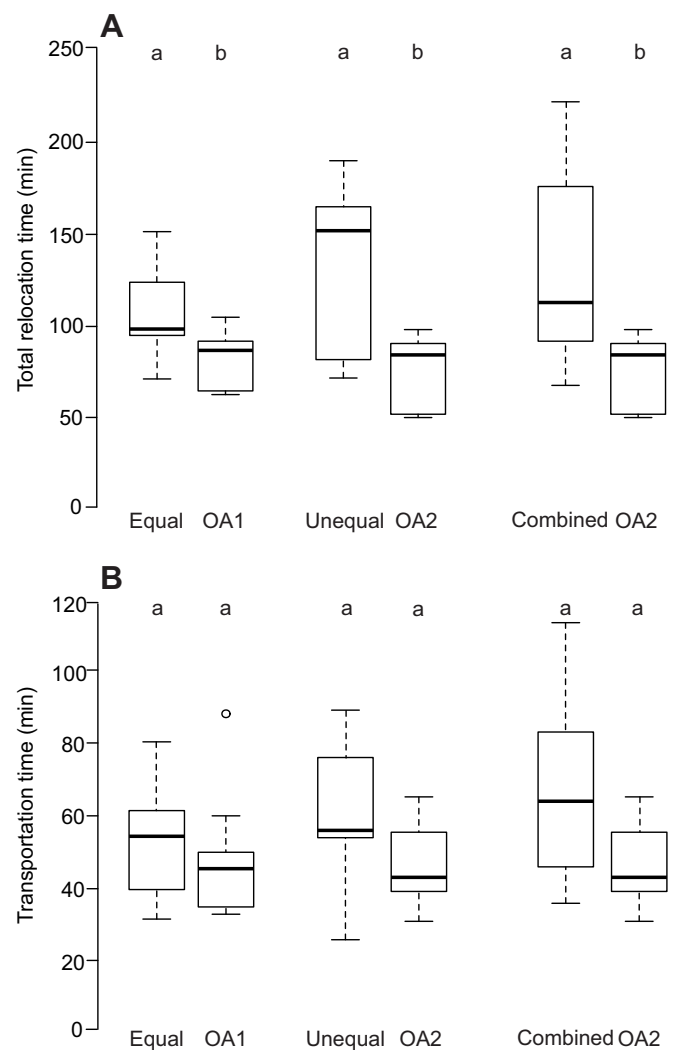


Fig. 5. Total relocation time and transportation time of *D. indicum* in open arena relocations compared with the three different test setups. (A) Total relocation time and (B) transportation time in two open arenas (OA1 and OA2) compared with the three experimental setups. The bold black line inside the box denotes the median value and the box represents the interquartile range, the whiskers represent the data points which are within 1.5 times the interquartile range and the circle denotes the outlier. The controls and the tests were compared using the Mann–Whitney test. Significant differences ($P<0.05$) across the categories are represented using different letters. OA1 ($N=10$) was compared with equal path ($N=9$) experiment; OA2 ($N=9$) was compared with unequal path ($N=10$) and combined path ($N=9$) experiment.

unlike foraging, in which only a subset of the colony members participate. Relocation directly impacts colony survival, as it exposes the colony to several risks such as fragmentation of the colony, vulnerability to predators, parasites and thieves and even increases the dangers posed by abiotic factors like temperature, humidity and rain (Hölldobler and Wilson, 1990; Tschinkel, 2014; Paul et al., 2016). Thus, any mechanism that optimizes the manner in which relocation is organized and conducted would be under strong selection pressure. In *D. indicum*, tandem leaders play a central role in the process as tandem running is the only recruitment method and these tandem leaders have to navigate the terrain multiple times to transport colony members one at a time to the new nest, they have the opportunity to optimize their path. In the current

set of experiments, we asked whether tandem leaders choose the shortest path between their old and new nest.

To the best of our knowledge, path optimization has not been investigated in the context of colony relocation nor has it been investigated in ants that navigate in their environment without the assistance of pheromone trail. When the trail laying ant *Lasius niger* was provided two paths of equal length between their nest and food, they were initially using both paths, but with time, they used only one of the paths and ignored the other. It was understood that this occurred because of magnification of initial small differences in the pheromone concentration (Dussutour et al., 2005). This is in contrast to what occurred in the current set of experiments with equal paths connecting the nests. We found that tandem leaders used both paths to transport throughout the relocation, indicating that trail pheromones do not function in a similar manner even if they are present. When *D. indicum* was given one short and another long path between the nests, colonies and individual leaders choose the shortest path. Furthermore, we found a bias in the exploration, as the number of explorers on the long path was significantly lower than on the short path, suggesting that there could have been some visual or thigmotactic cues that prevented ants from choosing the long path when an alternative short path was available.

Instead of a binary choice at a single point when these ants were presented with the opportunity to choose from four different paths, we found that at the colony level, tandem runs occurred on the shortest and a combination of short and long paths, while the longest path was avoided. Experienced tandem leaders, on the other hand, showed a significant preference for the shortest path. This clearly indicates that non-trail laying ants are capable of minimizing their path even when a combination of paths was available. Even though emergent properties of a pheromone trail allows ants to use the shortest path (Goss et al., 1989) and solve dynamic tower of Hanoi to take the shortest path (Reid et al., 2011), the current experiments showcase the ability of individual ants to minimize their path by themselves. Thus, we propose that individual ants are capable of deciphering the shortest path based on information they collect directly, without the aid of a pheromone trail or emergent properties of these pheromones. Information gathered by path integration, visual landmarks, thigmotactic cues or path integration could be involved in this process of path minimization.

Typically, tandem leaders would not be conducting relocation over defined paths as provided in these experiments. Experiments in the natural habitat showed that tandem leaders are unlikely to use specific trails towards the new nest and the path efficiency was $83.95 \pm 12.41\%$ on average (Anoop, 2019). Thus, we wanted to compare the relocation dynamics of these defined path experiments within the natural habitat as well as in the open arena within the lab. Specifically, we enquired whether the total relocation time and transportation time was significantly altered in defined path experiments. In a previous natural habitat relocation experiment, we found that these ants took on average 419.2 ± 275.4 min to relocate, $28.4 \pm 5.2\%$ of leaders participated and the average distance they covered to reach the new nest was 232 ± 172 cm (Kaur et al., 2012). Qualitatively, we found that the total relocation time and the percentage of colony members that became tandem leaders was higher in natural habitat relocation compared with defined path and open arena relocation. In the natural habitat relocation experiments, colonies occupied multiple temporary shelters, traveled through heterogeneous terrain and faced potential predators, making the natural habitat particularly complex and are hence not the most

suitable data set for comparison with the current experiment. Upon comparing open arena relocations that had a similar homogeneous substrate and temperature range as the defined path relocations, we found that colonies took longer to enter and explore the defined path and thus total relocation time was significantly longer, even though the distance across which they relocated was similar. This could be due to an initial reluctance of the ants to enter the bridges. However, once transports were initiated, colonies took comparable lengths of time to complete the relocation, indicating that transportation time is conserved across conditions and that this goal-oriented process is designed to be completed quickly.

Even though *D. indicum* lives in small colonies and employs tandem running only in the context of relocation, the cost associated with this process is high enough to drive these tandem leaders to evolve the means to minimize their path using inputs from the environment they explore. The mechanism by which tandem leaders achieve path minimization is a whole new avenue for research. Performing different manipulative experiments involving visual and thigmotactic cues, together with testing path integration skills of these ants, would be the first step towards understanding these mechanisms.

Acknowledgements

We thank Basudev Ghosh for his assistance in the collection and maintenance of *D. indicum* colonies. We also thank the two reviewers for their input, which helped improve the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.A.; Methodology: S.M., M.K.P., S.A.; Software: S.M.; Formal analysis: S.M.; Resources: S.A.; Data curation: S.M., M.K.P.; Writing - original draft: S.M.; Writing - review & editing: S.A.; Visualization: S.M., S.A.; Supervision: S.A.; Project administration: S.A.; Funding acquisition: S.A.

Funding

The funding for this study came from the Indian Institute of Science Education and Research Kolkata.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.206490.supplemental>

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