



Leaders follow leaders to reunite the colony: relocation dynamics of an Indian queenless ant in its natural habitat

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Several factors cause animals to relocate. In this context, ant species are unique in that they not only have to relocate adults, but they must also move their brood while maintaining colony cohesion. We explored the colony relocation dynamics of the ponerine ant *Diacamma indicum* in its natural habitat. Irrespective of whether ants relocated from their original nest in their natural habitat or from a nestbox in an unfamiliar but natural habitat, colonies experienced fission and multiple fragmentations. However, this fission was transient, and the colonies eventually unified at a single site. The movement of the ants did not exhibit any directional preference, and the gamergate did not enjoy any special attention during the relocation process. Tandem running, a behaviour in which one ant leads a follower ant from one site to another, was used to relocate about 96% of the colony members, and 28% of the colony became tandem leaders on average. The evacuation phase was significantly shorter than the reunification phase, and this may be an adaptive response to a disturbance in the ant's dwelling. Unlike other ants, the leaders were sighted at most of the temporary sites and thus, in principle, had the opportunity to compare the conditions of alternative sites directly. Most leaders discovered the final site by following other leaders; leaders following leaders occurred throughout the relocation process and constituted 30% of the total tandem runs. In the context of these experiments, the colony relocation and reunification dynamics of ants in their natural habitat are discussed.

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Whether they are simple or complex in their design, nests are important to the organisms that occupy them. For many organisms that use nests to rear their immature young, nests provide protection from predators and shelter from adverse changes in the environment. Although organisms expend significant resources in nest construction, sometimes nests need to be evacuated. Environmental disturbance, increased predation and dwindling resources are some of the factors that cause animals to change their nesting site. Social insects such as ants, bees and wasps are examples of species in which nests play a central role, both for rearing their immature young and for storing resources. For these species, nest relocation would be a complex endeavour, as a large number of nestmates and stored resources would need to be transported from one site to another. Ants also need to transport their immature young (egg, larva and pupa) which are particularly vulnerable and represent a significant ratio of the colony's resource investment (Hölldobler & Wilson 1990; Visscher 2007). Despite the costs involved, relocation is necessary for colony reproduction to

occur in some species of social insects. Honeybees, swarm-founding wasps and some species of ants reproduce by colony fission. In this process, the reproductive class and a subset of workers split from the parental colony and disperse to initiate the formation of another colony (Wilson 1971; Banschbach & Herbers 1999; Peeters & Ito 2001; Cheron et al. 2011).

The process of nest relocation has been studied in few species of social insects. Previous research has addressed different aspects of relocation, including the assessment of the quality of new nesting sites, convergence-related decision making for available sites and the flight mechanics of relocating honeybees (Camazine & Visscher 1999; Seeley & Buhrman 1999; Seeley & Buhrman 2001; Seeley 2003; Schaerf et al. 2011). In contrast to honeybees, ants need to transport their brood during the relocation process, making the relocation of ant colonies a more complex process. Ants also lack the dance language that enables honeybees to share information with their nestmates regarding various nesting sites in their environment (Seeley 2010).

Instead of mass movement to a new site, as occurs in honeybees (Visscher 2007), most ants use chemical trails to demarcate the path to a new nest (Hölldobler & Wilson 1990) while others use either carrying and/or tandem running (Hölldobler & Wilson 1990). During tandem running, an ant leads a nestmate to a new location while

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maintaining physical contact (Adlerz 1896). In species of ants that use tandem running, the entire process of relocation is dependent on tandem leaders. Often, tandem leaders recruit other leaders at the beginning of the relocation process to accelerate the rate of nestmate transfer, as has been observed in *Temnothorax rugatulus* (Möglich et al. 1974; Möglich 1978; Hölldobler & Wilson 2009).

The process of nest site relocation has been studied in only a few species of ants. The nest site selection process of *Temnothorax albipennis* shows several similarities to that found in the honeybee *Apis mellifera*; however, there are also significant differences between the two (Franks et al. 2002; Visscher 2007). A study of the relocation process in the ant species *Aphaenogaster senilis* found that colony members reach the new colony site by walking there, rather than being carried or through tandem running. The accumulation of colony members at the new location follows a sigmoid curve (Avargues-Weber & Monin 2009).

Most studies of nest relocation in ant species have been performed in the laboratory under controlled conditions. As a result, there is a lack of information on the behaviour of individual ants when relocating under more natural conditions. Relocation is difficult to observe in a natural setting owing to the unpredictability of natural relocation occurrences and the difficulty of marking and tracking individual ants. Some field studies have focused on nest site characteristics (Thomas 2002) and relocation frequency, which can range from a few days to a decade (Smallwood 1982; Fowler 1986; Tsuji 1988; Briano 1995; Brown 1999; Cerdá et al. 2002; McGlynn et al. 2004). In other field studies, relocation was caused primarily by environmental disturbances (Wheeler 1910; Hölldobler & Wilson 1990; Briano 1995), while nomadic lifestyle, increased competition (Gordon 1992; Brown 1999) and severe predatory pressure was also found to cause relocation (Wheeler 1936; Wilson 1958; Hays et al. 1982; McGlynn et al. 2004; McGlynn 2006; Dahbi et al. 2008; Boulay et al. 2010).

Diacamma indicum, our model system for the current study, is a queenless ponerine ant found in the south and east of India and in Sri Lanka. They form colonies in which the eggs are laid by a mated worker called the gamergate (Wheeler & Chapman 1922). Very little is known about this ant species except that it has a constant species-specific chromosome number and that its genetic variability is lower than that of other sympatric species (Viginier et al. 2004; Karnik et al. 2010). Colony relocation in a closely related species, *Diacamma rugosum*, has been studied under laboratory conditions by Fukumoto & Abe (1983). They found that tandem running was the only means used for relocation and that 5–25% of the colony became tandem leaders.

In this study, we examined the relocation mechanism used by *D. indicum* in its natural habitat. We performed two sets of relocation experiments in which we artificially stimulated the colony to vacate their old nest. In the first experiment, colonies (unmarked ants) relocated from native nests in their original habitat. In the second experiment, colonies (of individually marked ants) relocated from artificial nests placed in an unfamiliar, but natural habitat. This allowed us to assess qualitatively the overall process of relocation in the ants' undisturbed natural terrain and subsequently observe the mechanism of relocation at the scale of individual colony members.

METHODS

Eighteen colonies of *D. indicum* were identified in Mohanpur (Nadia district, West Bengal, India, 22°56'N, 88°31'E) between March 2010 and December 2011. We conducted two experiments with these colonies in their natural habitat. In the first experiment, referred to as the dislodging experiment, colonies were made to relocate from their original nests. In the second experiment,

referred to as the displacement experiment, colonies were made to relocate from nestboxes placed in a location different from their collection site. In the dislodging experiment, eight colonies were allowed to relocate in their natural habitat by application of water stress. Qualitative observations were made of the relocation process, and focal observations were made of incidences of tandem running. Water was initially sprayed and later poured into the nest to initiate the relocation. This caused the ants to evacuate the nest with or without brood in their mandibles. While some of these individuals waited at the nest entrance, others dispersed. Soon after finding a suitable shelter, scouts started tandem running colony members into multiple shelters (temporary sites), thus causing colony fission. The number of temporary sites occupied and the different parameters of the temporary sites, such as the nature of the site and the minimum distance and compass direction from the original nest entrance, were noted. Later, ants from these temporary sites merged into one shelter (final site). The final site was defined as the location to which the majority of the colony members converged, and it required ants from temporary sites to relocate to it, although some temporary sites became the final site. The sites from which tandem runs initiated and terminated were recorded. Once the temporary sites were vacated and there were no tandem runs for 30 min, we considered the relocation process to be complete. In cases where the colony did not merge but dispersed to different shelters, we followed ant activities for 6 h and all colonies reunified within this time period. Subsequently, the colonies were observed sporadically over the next 24 h to confirm that no further relocations took place.

In the displacement experiment, 10 colonies were collected and all of the ants were marked with unique combinations of enamel paint colours (Testors, Rockford, IL, U.S.A.) to allow individual identification. In all colonies, the gamergate was identified by the presence of gemma. Colonies consisted of 81.5 ± 34.2 adult females (mean \pm SD, range 36–133) and 64.7 ± 38.4 (range 19–133) different stages of brood. Each marked colony was placed in a plastic box (28.5 \times 21.5 cm and 12 cm high) with a plaster of Paris base and a circular nest chamber (10 cm in diameter) and provided with ad libitum water, honey, ant cake (Hölldobler & Wilson 1994) and termites. One wall of the plastic box had a circular exit (2 cm diameter), which was closed with a cotton plug until the start of the experiment. Colonies were housed in the laboratory for a period of 17 ± 15 (range 1–45) days before the start of the relocation experiment. An area measuring 30.2 \times 19.5 m was identified before the start of the experiment as the release area. The release area was selected based on the following three criteria: relatively sparse undergrowth, low human activity and the presence of *D. indicum* colonies in the surrounding area. On the day of the relocation experiment, the colony was placed on a sand bed in the release area without the glass cover on the nest chamber. Similar to other laboratory-based studies, the standard treatment of removing the top glass cover was applied to stimulate colony relocation (Sendova-Franks & Franks 1995).

The process of colony fission and fusion was tracked by conducting focal observations on tandem running of individually marked ants. The movement of ants from the release site to the temporary sites was classified as evacuation. The evacuation phase starts with the first tandem run out of the release site and ends when the release site is vacated by the ants. The movement of ants from the temporary sites into one final site was categorized as re-establishment. The re-establishment phase starts when tandem running is initiated from the temporary sites to the final site and ends when no tandem running is observed at the final site for at least 30 min. To determine the length of the evacuation phase, it was essential to ensure that the release site was vacated by all of the ants. This was not possible in the dislodging experiment, and we

were unable to discriminate between the evacuation phase and the re-establishment phase in that experiment. Moreover, as ants were unmarked in the dislodging experiment, we could not identify the gamergate or ants involved in tandem running.

In the displacement experiment, ant relocation was recorded by both direct observations and video cameras. For every tandem run, we recorded the identity of the leader and the follower, the initiation and destination sites, whether any brood were being carried and the time the tandem run was initiated and/or terminated. To examine the temporal dynamics of the relocation process, the release time was notated as time zero and the number of ants at the release site was considered to be 100%. We estimated the number of ants occupying a given site by tallying the tandem runs that were terminated or initiated at that site. This method omitted data for ants that walked into or out of the site by themselves. All of the tandem leaders were given ranks based on the number of tandem runs they performed during the entire relocation process. In each colony, the individual that performed the highest number of tandem runs was ranked as one and identified as the maximum tandem leader. Some tandem leaders initiated or terminated their tandem runs at different sites, thus switching their path; these individuals were referred to as switched tandem leaders. Tandem leaders that did not change their initiation and termination sites were referred to as nonswitched tandem leaders. Different parameters, such as the nature of the site and the minimum distance and compass direction from the original nest entrance, were measured in a manner similar to that of the dislodging experiment. Unless otherwise mentioned, the mean values and SDs of these parameters are presented to quantify the results. About 2334 tandem runs were recorded over the course of this study. Of these, 1575 tandem runs were carried out by 231 leaders from 10 different colonies towards 34 different shelters. The dynamics from the displacement experiment were analysed further. StatistixL version 1.8 (www.statistix.com) was used to conduct the Wilcoxon paired-sample test, the Mann–Whitney U test, Rayleigh's test for uniformity and Spearman rank correlation, and the results of these tests were used to quantify the relocation process.

RESULTS

Relocation Mechanism

In both the dislodging and displacement experiments, most (14/18) of the colonies initially experienced colony fission and later reunified at a final site. **Figure 1** illustrates this process for colony DI-47. In this representative case, colony members moved into three temporary sites within 20 min and 96.3% of the colony members reunited at the final site in 142 min. Tandem runs occurred not only between the release site and the temporary sites, and then to the final site, but also between the different temporary sites (**Fig. 1a**). All colonies in the dislodging experiment experienced colony fission, and only four colonies in the displacement experiment did not experience fission. Three of these four colonies occupied a single temporary site before moving into a final site, and the fourth colony relocated directly to its final site.

Colonies that relocated in their natural habitat and those that did so in an unfamiliar habitat were seen to be comparable in terms of the number of sites they occupied, the distance over which they travelled and the time that they took to relocate (**Table 1**). The number of sites that these colonies occupied ranged from one to eight, and there were no significant differences between the dislodging and displacement experiments (Mann–Whitney U test: $U = 49.5$, $N_1 = 8$, $N_2 = 10$, $P = 0.46$). On average, the distance to the temporary sites and final site from the entrance of the release site was also not significantly different between the dislodging and displacement experiments

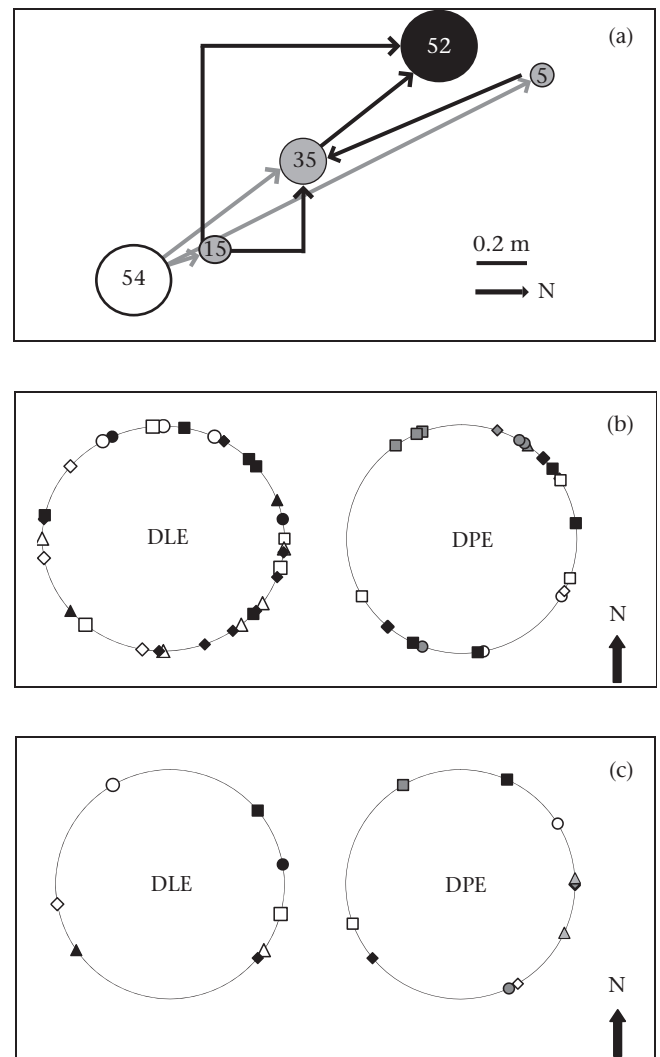


Figure 1. Relocation dynamics and directional preference. (a) Field relocation map for colony DI-47 in the displacement experiment. Circles represent the sites occupied by colony members during the different stages of relocation (white: release site; grey: temporary sites; black: final site). The numbers inside the circles represent the numbers of unique ants that made tandem runs to the site. Arrows (grey: release site to temporary sites; black: temporary sites to temporary sites and final site) represent the tandem runs observed between the sites. (b) Choice of temporary sites in both the dislodging (DLE) and displacement (DPE) experiments (dislodging experiment: Rayleigh's test for uniformity: $z = 1.1$, $N = 32$, $P = 0.3$; displacement experiment: Rayleigh's test for uniformity: $z = 1.7$, $N = 21$, $P = 0.2$). Each symbol represents the direction of the temporary sites occupied by a unique colony, with the release site as the centre of the circle. (c) Choice of final site in the dislodging and displacement experiments (dislodging experiment: Rayleigh's test for uniformity: $z = 0.5$, $N = 8$, $P = 0.2$; displacement experiment: Rayleigh's test for uniformity: $z = 0.9$, $N = 10$, $P = 0.4$). Each symbol represents the direction of the final site occupied by a unique colony, with the release site as the centre of the circle.

(Mann–Whitney U test: temporary sites: $U = 61$, $N_1 = 8$, $N_2 = 10$, $P = 0.07$; final site: $U = 56$, $N_1 = 8$, $N_2 = 10$, $P = 0.2$). Moreover, the time taken for relocation was not significantly different between the dislodging and displacement experiments (Mann–Whitney U test: $U = 47$, $N_1 = 8$, $N_2 = 10$, $P = 0.6$).

There was no significant directional preference in the sites occupied by colonies in the dislodging and displacement experiments (**Fig. 1b, c**). In further relocation experiments in which colonies were marked and released at the point of collection, the number of sites occupied, the duration of relocation and site direction were qualitatively similar those in the dislodging and displacement

Table 1
Relocation dynamics of *D. indicum* under field conditions

	No. of sites occupied		Mean distance to temporary sites (m)		Distance to final site (m)		Total relocation time (min)	
	Dislodging experiment	Displacement experiment	Dislodging experiment	Displacement experiment	Dislodging experiment	Displacement experiment	Dislodging experiment	Displacement experiment
	4	5	0.76	2.37	1.1	2.44	353	900
	5	2	0.79	0.61	0.53	6.78	300	757
	3	1	1.05	0.61	1.63	0.61	171	90
	3	3	1	2.69	2.5	2.13	158	440
	5	4	0.77	1.23	1.07	1.93	168	142
	3	3	0.6	2.31	1.38	2.72	1431	432
	2	2	1.87	1.3	1.42	2.16	368	224
	8	4	1.19	1.79	1.56	0.74	131	130
		7		1.79		2.33		537
		3		1.54		1.37		540
Mean	4.13	3.40	1.00	1.62	1.40	2.32	385.00	419.20
SD	1.89	1.71	0.40	0.71	0.57	1.72	432.86	275.14

The number of sites occupied, the distance to temporary sites and the final site from the release site and the total time taken for relocation by colonies in their natural habitat are shown. Dislodging experiment: $N = 8$; displacement experiment: $N = 10$.

experiments (see Appendix). Of the 67 sites that ants from different colonies moved into during relocation, 54 were holes or cracks in the ground, 12 were covered by dense foliage and had a depression in the ground and one was inside the base of a palm leaf. However, the final site was always a pre-existing hole in the ground.

Temporal Dynamics of Relocation

The time taken for the colony to evacuate and reunite was examined in the displacement experiment. The way in which ants moved from the release site to the temporary sites and then to the final site was highly variable and is depicted for colony DI-47 in Fig. 2a–e. The evacuation time (58.1 ± 45 min) was significantly lower than the time for re-establishment (300 ± 191 min; Wilcoxon paired-sample test: $T = 0$, $N = 9$, $P = 0.004$). The relocation rate was measured by the number of tandem runs per min per leader and was significantly higher in the evacuation phase (0.09 ± 0.05) than in the re-establishment phase (0.04 ± 0.05 ; Wilcoxon paired-sample test: $T = 3.5$, $N = 9$, $P = 0.03$). In nine of the 10 displacement experiment relocations, the gamergate reached the final site. However, in one case she was lost while participating in a tandem run. In all cases, the gamergate was led in a tandem run like any other worker and without any retinue. Throughout the process of colony relocation, the gamergate was led in a tandem run to 1.7 ± 0.5 sites. The percentage of colony members that had been led in a tandem run to the final site before the gamergate reached it was 39.4 ± 13.7 . In seven of 10 colonies, the gamergate was led to the final site by a leader whose rank was 3 or lower, and on average, she was led by leaders whose rank was 4.9 ± 5.2 .

Tandem Running

During the displacement experiment relocations, $95.8 \pm 4.1\%$ of the colony members were involved in tandem running either as a leader or as a follower. The colony members that reached the final site by means of tandem running ranged from 69.4% to 96.3% (Fig. 2f). The percentage of ants that used tandem running ($84.8 \pm 9.5\%$) to reach the final site was significantly higher than those that did not use tandem running ($15.2 \pm 9.5\%$; Wilcoxon paired-sample test: $T = 0$, $N = 10$, $P = 0.002$). These statistics demonstrate the crucial role of tandem running in the relocation process.

The number of tandem runs observed during the entire relocation showed a significant positive correlation with colony size (Fig. 3a). It was observed that $28.4 \pm 5.2\%$ of the colony became tandem leaders during the relocation process by leading at least

one tandem run. The number of tandem leaders in the evacuation phase (20 ± 10.1) was significantly higher than that in the re-establishment phase (14.9 ± 9.2 ; Wilcoxon paired-sample test: $T = 4.5$, $N = 9$, $P = 0.03$). The number of tandem leaders involved in the relocation process was significantly positively correlated with colony size (Fig. 3b). However, the number of tandem runs performed by different leaders was skewed. While some leaders performed several tandem runs, others performed just one. In colony DI-47, there were 15 leaders, and the maximum tandem leader (ant identified as X - -) performed 21.5% of the total tandem running during the relocation process (Fig. 3c). In the 10 colonies, maximum tandem leaders performed $18.6 \pm 6.5\%$ of the tandem running during the entire relocation process. Tandem leaders were sighted at multiple sites either as followers or as leaders during the relocation. Considering all sites that were occupied by more than 10% of the colony, tandem leaders visited $75.3 \pm 21.1\%$ of these sites on average, either as a leader or as a follower. Thus, it would appear that tandem leaders are privy to the location of the majority of the available sites.

Leaders Following Leaders

In the displacement experiment relocations, $92 \pm 11.5\%$ of the leaders were followers of the other leaders at least once, and leaders following leaders (LFL) constituted $33.8 \pm 8.8\%$ of the total tandem runs. LFL occurred throughout the relocation process (Fig. 4), implying that this is a prevalent behaviour in this species. The first LFL occurred (25.6 ± 14.7 min) significantly later than the first tandem run of the relocation process (23.7 ± 13.7 min; Wilcoxon paired-sample test: $T = 0$, $N = 10$, $P = 0.02$). However, the time at which the last LFL (386.6 ± 262.7 min) and the last tandem run occurred were comparable (418.7 ± 274.8 min; Wilcoxon paired-sample test: $T = 0$, $N = 10$, $P = 0.06$). This suggests that information regarding possible nesting sites is being disseminated among leaders via the LFL phenomenon.

Switched Tandem Leaders

In the relocations in the displacement experiment, the percentage of tandem leaders that switched ($49.2 \pm 14.1\%$) was not significantly different than that of nonswitched leaders ($50.8 \pm 14.1\%$; Wilcoxon paired-sample test: $T = 22$, $N = 9$, $P = 1$). However, switched tandem leaders contributed more to the relocation process, as measured by (1) overall contribution and (2) final site contribution. The overall percentage of tandem runs performed by switched tandem leaders

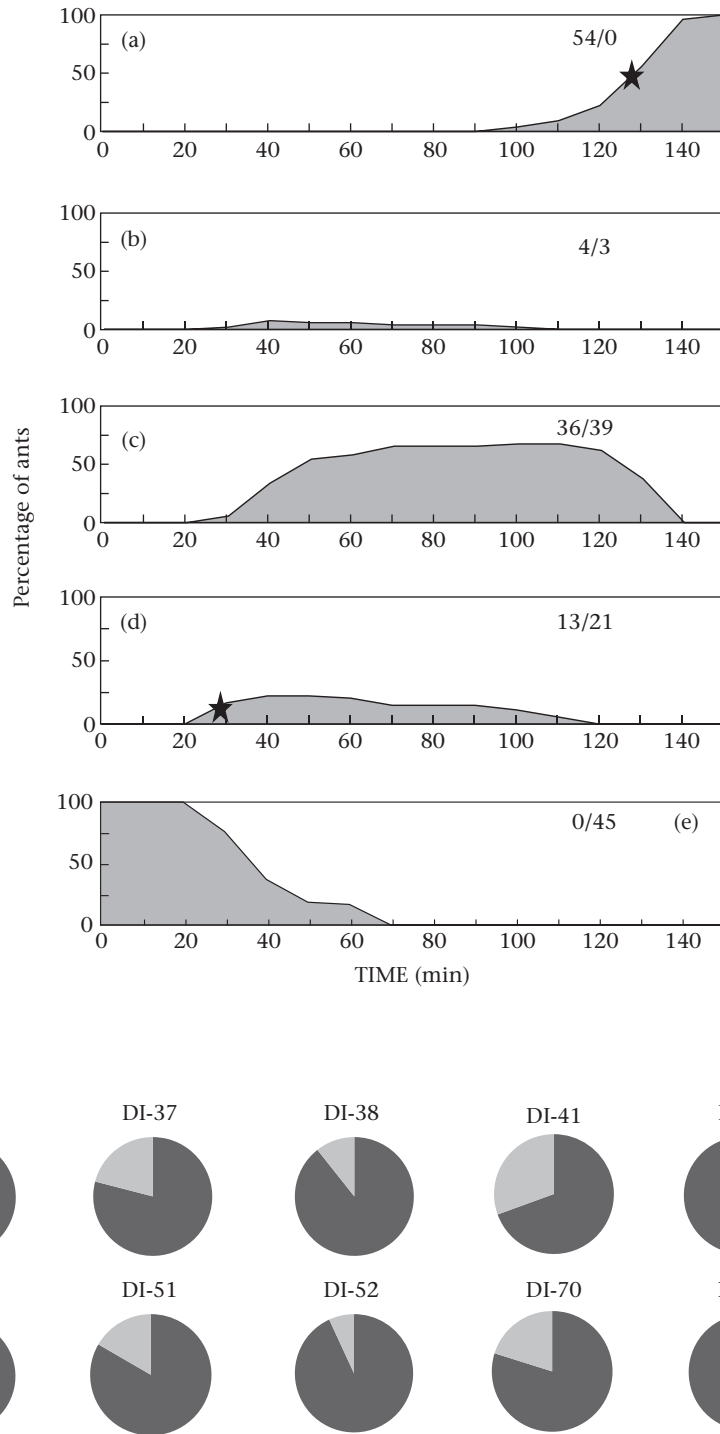


Figure 2. Temporal dynamics and the extent of tandem running. (a–e) Temporal dynamics of tandem running in colony DI-47. The net percentage of ants present at (a) the final site, (b–d) three different temporary sites and (e) the release site, based on tallying tandem runs, is plotted against time. Time was initialized (at zero) at the start of the relocation experiment. The star represents the terminal location of the relocated gamergate, which was first relocated to a temporary site and later to the final site. The number of tandem runs terminated and initiated at any given site is given on the right-hand side. (f) The percentage of each colony that reached the final site through tandem running (black region) and those that were lost or reached the final site through independent exploration (grey region).

(77.4 ± 14%) was significantly higher than that of nonswitched tandem leaders (22.6 ± 14%; Wilcoxon paired-sample test: $T = 0$, $N = 9$, $P = 0.004$). The percentage of tandem runs performed at the final site by switched tandem leaders was also significantly higher than that of nonswitched tandem leaders (Fig. 5a). In most cases, the location of the final site was discovered by following another leader and not by independent exploration (Fig. 5b).

DISCUSSION

Previous studies have recognized that emigration is a relatively frequent event in the life of many social insects and has significant implications for their survival and reproduction. However, this phenomenon has received little attention in the context of ants, and we do not know of any studies focusing on relocation in natural

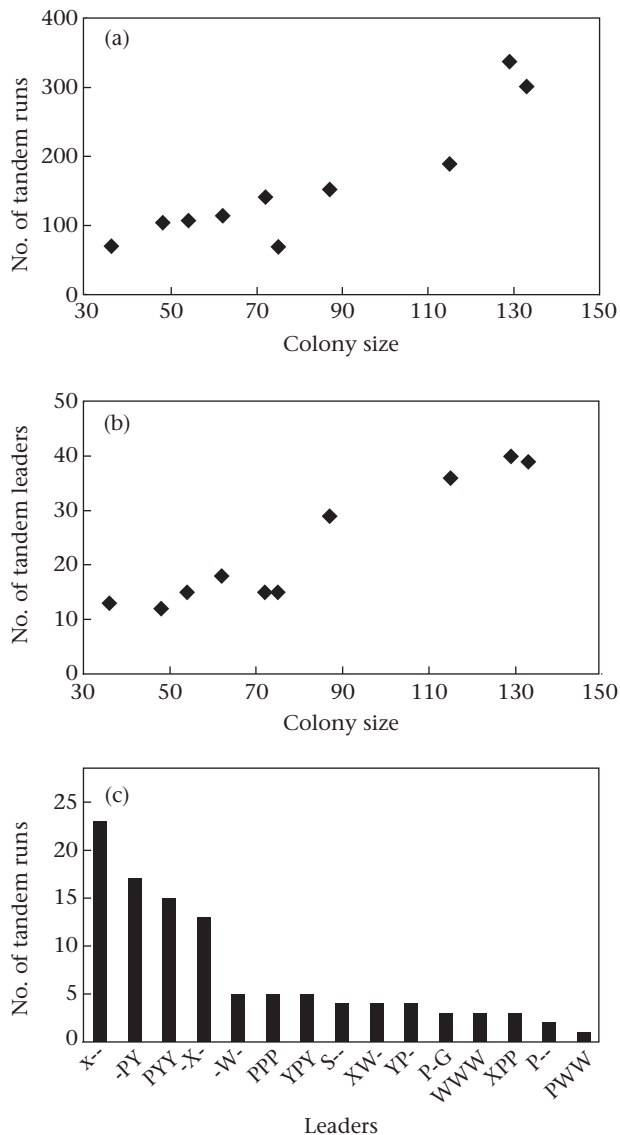


Figure 3. Tandem running: influence of colony size and its distribution among tandem leaders. (a) The number of tandem runs in relation to colony size (Spearman rank correlation: $r_s = 0.81$, $N = 10$, $P = 0.007$). (b) The number of tandem leaders in relation to colony size (Spearman rank correlation, $r_s = 0.91$, $N = 10$, $P = 0.001$). (c) The number of tandem runs performed by all of the leaders in colony DI-47 during field relocation, arranged in descending order.

habitats at the scale of individual colony members. In the current study, we examined the mechanism by which *D. indicum*, a ponerine ant, relocated in its natural habitat in two different contexts. In the first scenario, colonies emigrated out of their native nest in their natural habitat, and in the second scenario, colonies with individually marked members relocated from an artificial nest in natural but unfamiliar territory.

Our observations revealed a new dimension in the relocation process, namely, the maintenance of colony cohesion. When colonies faced an emergency in which their nests became uninhabitable, they moved into multiple temporary sites, which caused colony fission. However, this colony fission was transient; eventually, the colonies reunited into one single site. Colony fission is disadvantageous for many reasons but less so for species such as *D. indicum* that reproduce by budding. Despite this, all of the colonies in both experiments managed to reunify. Colony fission and other relocation parameters such as the duration of relocation, the distance to different sites and

the number of temporary sites did not change in the two treatments. This suggests that familiarity with the original habitat did not influence the process and that this process had an underlying commonality. Perhaps this common dynamic originated from intrinsic species-specific factors, even possibly in combination with extrinsic factors such as nest availability and habitat type. This postulation was supported in a third scenario in which colonies with marked individuals relocated at their original nesting site (see Appendix).

Unlike other species of ants that use trails, carrying and/or tandem running, *D. indicum* only used tandem running to relocate adults. Brood, males and termites were carried from one site to another. While it is known that several species of ponerine ants use tandem running for relocation (Hölldobler & Wilson 1990), this experiment reconfirms the robustness of that observation based on studies in these ants' natural habitat.

The percentage of the colony that became tandem leaders during relocation varied with the phase of the relocation process. In the evacuation phase, there were significantly more tandem leaders than in the re-establishment phase. Because of this, it is possible that colonies relocated to their temporary sites significantly faster, even though the distance and the number of tandem runs were comparable. We believe that faster movement during the evacuation phase is an adaptive response, as finding any kind of shelter is important when the brood and adults of the colony are exposed and vulnerable. Three different and nonmutually exclusive factors can explain the additional time required during the re-establishment phase. Ants may require more time to identify a suitable final site. Establishing a consensus on the best possible site among all of the available sites may also require additional time. Colony reunification from multiple sites into a single site may also be more complicated and time consuming than colony fission. However, the time invested to reach this consensus indicates the importance of the site selection for the final nesting site. In this sense, the final site could be considered as the winning site. However, we found that tandem leaders that discovered this site independently and led tandem runs to this site also became followers of other leaders. Leaders working for the winning site were also motivated to explore other sites by becoming followers of other leaders, irrespective of the winning or losing status of these sites. It is noteworthy that, in these relocations, the winning site was not apparent at the beginning of the process but evolved dynamically through the discovery of different nesting options by different leaders.

The workload associated with relocation was not shared equally among tandem leaders. Although 28.4% of the colony became tandem leaders, most of these leaders (84.8%) performed less than 10% of the tandem running during the relocation. A single ant, the maximum tandem leader, was a switched tandem leader in nine of the 10 colony relocations (one colony did not occupy any temporary sites but moved directly into the final site, and thus there was no opportunity for switching), performed 25% of the tandem runs to the final site and was sighted at almost all of the nesting sites occupied by the colony. Further studies are needed to determine whether the maximum tandem leader was the first individual to discover the final site and whether this same individual becomes the maximum tandem leader in repeated relocation events. We observed that one gamergate was lost during a tandem run to the final site, and 22 attacks by other ants or spiders led to injury or death of *D. indicum* workers during these relocations. On average, 85% of the colony arrived at the final site through tandem running. Others possibly found the site by independent exploration or were lost. These observations indicate some of the costs incurred by the colony during relocation. These can only be meaningfully studied in the natural habitat of the ants, and this could be a focus of future investigations.

One relocation dynamic of note was that the gamergate did not receive any significantly different treatment compared to other workers. We observed that the gamergate was not surrounded by

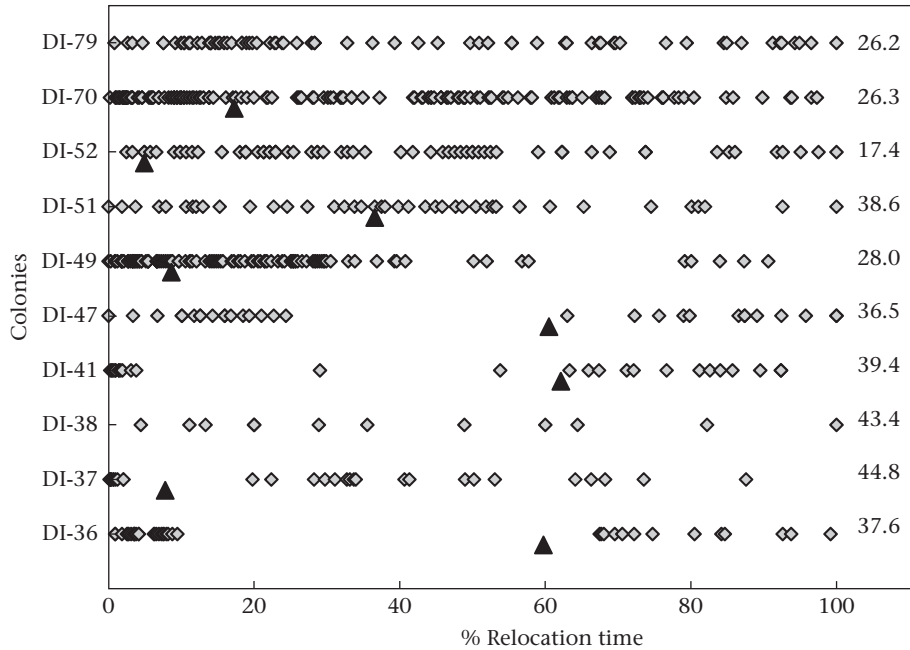


Figure 4. Temporal dynamics of leaders following leaders (LFL). The instances of LFL across 10 colonies are tracked over the relocation time (%). Grey diamonds (◊) represent LFL events, and black triangles (▲) represent the start of the re-establishment phase. The numbers on the right-hand side represent the percentage of LFL events during relocation.

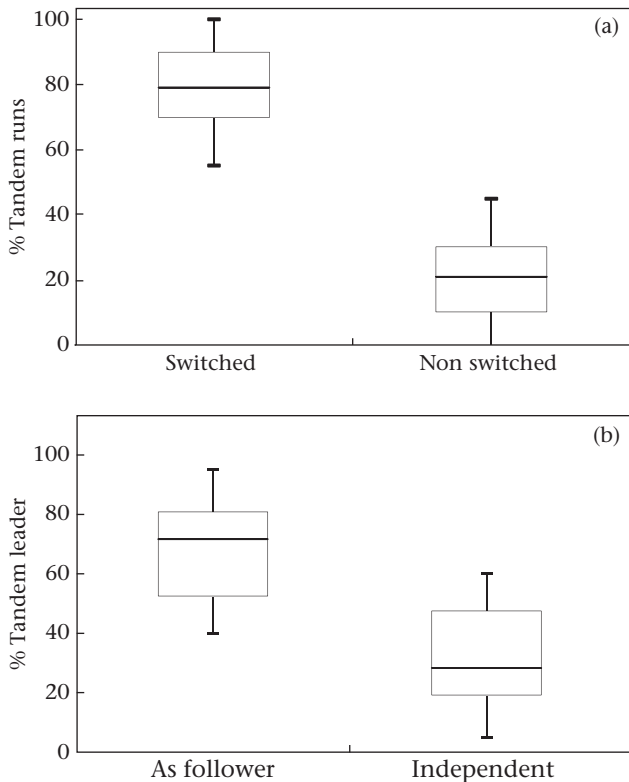


Figure 5. Switched tandem leaders and final site discovery. (a) The percentage of tandem runs performed by switched and nonswitched tandem leaders (Wilcoxon paired-sample test: $T = 0.001$, $N = 9$, $P = 0.004$). (b) The percentage of final site tandem leaders that discovered the final site by following another leader or by independent exploration (Wilcoxon paired-sample test: $T = 3$, $N = 10$, $P = 0.01$). For both (a) and (b), the median is represented by the line within the box, the box represents the upper and lower quartiles and the whiskers represent the smallest/largest values.

a retinue during the tandem run. Neither did the mere presence of the gamergate at a site ensure the reunification of the fissured colony at that site. She was often observed to be led in a tandem run to the temporary sites and then to the final site. Based on this, we speculate that the investment required to replace the gamergate in the *Diacamma* genus is relatively lower than that of ant species that have a queen.

In honeybees and ant species in which the nest relocation process has been studied in detail, scouts are known to reach a unanimous decision for the new nest site before initiating the transport of colony members to that site (Visscher 2007). Studies conducted in the laboratory on *T. albipennis* determined that this species uses a decentralized system involving recruitment latency and quorum sensing to choose the best available relocation site without having individual scouts explore alternative sites (Mallon et al. 2001; Britton et al. 2002; Franks et al. 2002; Pratt et al. 2002). In the present study, we were unable to examine the nest quality or track all of the ants that walked into or out of different sites throughout the relocation process. We were thus unable to examine whether *D. indicum* also employs a similar mechanism to *T. albipennis*. Unlike *Temnothorax* (Robinson et al. 2009), tandem leaders in *D. indicum* have, in principle, the opportunity to compare different sites directly, as these tandem leaders were sighted at the majority of the sites in our experiments; leaders involved in tandem running to the final site were sighted at 67.6% of sites occupied by the colony.

In previous research, colony reunification dynamics have not been addressed because the first phase of the relocation process, colony fission, was not well understood (see Lindauer 1955; Franks et al. 2003; Seeley & Visscher 2003; Raghavan 2003, personal communication; Pratt 2005). Nevertheless, colonies have been artificially fragmented and reunified under laboratory conditions in other contexts (Boulay et al. 2007; Ichinose et al. 2009). In the current study, colonies managed to reunify despite being fissured into as many as eight fragments. This reunification can only be accomplished through the efforts of tandem leaders. Two types of leaders could be distinguished. The first comprised leaders that

showed a high degree of fidelity to their initiation and termination sites and led tandem runs only to these sites. The second type of leader showed poor fidelity to their initiation and termination sites. The latter type switched their initiation and (or) destination sites and were thus switched tandem leaders. If all leaders showed high fidelity, new leaders would be needed for each pair of sites, which would make the reunification process less efficient. However, switched tandem leaders are more flexible and can make informed decisions based on parameters such as nest quality, number of nestmates already present and the distance to be travelled. Even callows are capable of becoming followers. Thus, the ability to follow is probably an innate behaviour that persists throughout the ants' lifetime, and this ability could provide a proximate reason for why leaders can also become followers. The ultimate function of leaders following other leaders could be two-fold: the need to reunite lost (or separated) colony members and the need to explore and compare alternative nesting sites, which enables the colony to make an informed decision regarding their nest choice. We conclude that the cost of investing time in this decision-making process is outweighed by the benefit of an informed choice by switched tandem leaders, and these leaders eventually facilitate the reunification of the colony at a final nesting site.

However, it is unclear how leaders acquire information on the location of the alternative sites. Both, high-fidelity and switched tandem leaders would need this information to reunify the colony. There are three methods that could potentially allow leaders to discover the location of other colony members: random search, odour trail and tandem running (Hölldobler & Wilson 1990). Leaders probably find the first shelter by random searching and thus can be expected to locate alternative sites by the same method. Odour trails may also be applied to mark the path to different sites. The third method could allow leaders to use tandem-running behaviour to locate their nestmates. Tandem running is understood to be the means by which new leaders are recruited or colony members are led to a new site. In this study, we found that leaders used tandem running to take other leaders from different locations to a site of interest, thus providing the follower with information on alternative sites and facilitating reunification. Upon entering an alternative site, leaders may examine it and choose to switch their initiation or destination site. Switched leaders have been found in the relocation process of *T. albipennis* (Franks et al. 2008). In *T. albipennis*, tandem running is used only during the initial phase of the relocation, as the colony then shifts to carrying individuals. Only during this initial phase do leaders recruit other leaders and transport specialists (Möglich 1978; Hölldobler & Wilson 2009). However, in *D. indicum*, a third of all tandem run were LFL-type, and they were observed to occur throughout the relocation process.

When explored under field conditions, the process of colony relocation in ants is both more complex and more interesting than current findings indicate; it is also a fundamental requirement for the survival and propagation of ant species. In this context, it would be particularly interesting to analyse the costs and benefits of nest relocation, the methods by which colony cohesion is maintained and the decision-making processes employed by these superorganisms. The research outlined in this paper represents a step towards achieving these larger goals.

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Appendix

Four colonies with individually marked members were allowed to relocate in their natural habitat at the point of collection. To keep the area surrounding the nest intact, we collected these colonies by sprinkling water at the nest entrance and housed them in the laboratory for a maximum of 2 days, similar to the displacement experiment. On the day of the relocation, the nestbox was placed on a sand bed, and its entrance was placed in the same direction as that of the original nest entrance. We observed the subsequent relocation dynamics by following tandem runs (as discussed in the displacement experiment) and collected information such as the number, distance and direction related to different sites. In addition, the total relocation time was also recorded (Table A1). All of the four colonies experienced fission and occupied 4.25 ± 2.1 (mean \pm SD) sites. The distance to the final site was 1 ± 1.2 m from the original nest entrance, and the time taken for relocation to occur was 209 ± 136 min. Qualitatively these results are comparable to what was observed in both the dislodging experiment and displacement experiment. Quantitative analysis of the directional preference of the temporary sites (Rayleigh's test of uniformity: $z = 1.7$, $N = 17$, $P = 0.18$) also showed similarity with the results from the dislodging and displacement experiments. Thus, we can conclude that the relocation dynamics observed in this experiment were similar to those observed in the dislodging and displacement experiments, including values for fission and reunification and even in those colonies that were housed in the laboratory and subsequently reintroduced at their original nesting site.

Table A1
Number, distance and direction of final relocation sites

Colonies	No. of sites	Distance to final site (m)	Direction of final site (degree)	Total relocation time (min)
DI 126	6	2.7	30	283
DI 127	3	0.6	120	98
DI 128	2	0.2	135	91
DI 129	6	0.4	180	362