To reunite or not: A study of artificially fragmented *Diacamma indicum* ant colonies

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ABSTRACT

Social insects live together in groups and maintain cohesion to enhance their chances of survival and productivity. Colony cohesion is severely challenged during relocation. We examined the dynamics of colony reunification and the factors affecting nest choice of artificially fragmented colonies of the queenless ant *Diacamma indicum*. None of the twelve undisturbed colonies fragmented or relocated when a good nest was available in their neighbourhood. When colonies were artificially fragmented, they mostly (25/30) reunified into a single nest unlike in randomized time-ordered network models, indicating that reunification is not the result of random recruitment acts. When the reproductive individual was present in a good nest, the colonies reunified at this address. However, when she was present in a suboptimal nest, colonies relocated her to a better quality nest and reunified there, illustrating that quality of the new nest is more important. The work distribution and relocation dynamics of reunification were comparable to intact colonies relocating to a single new nest. This is made possible by enhanced exchange of information among tandem leaders in the form of increased number of tandem runs among them. We conclude that colony cohesion is very important and is maintained after incorporating the risks of relocation and preference for nest quality during decision making.

1. Introduction

Cohesion between individuals of a species to form social groups has been noted in the animal kingdom across a wide range of organisms belonging to different taxa and across a wide range of group sizes (Parrish, 1999; Puckett et al., 2014). Various advantages of maintaining cohesive groups like enhanced protection from predation, increased survival of juveniles, efficient foraging have been found in flocks of birds, shoals of fishes, and swarms of insects (Peeters and Ito, 2001; Wilson, 1990). Further, animals living together in large groups may show new behaviours or functions such as mobbing of predators in some bird flocks (Parrish, 1999) that are not seen when they lead solitary lives. Cohesion operates at a different level in social insect colonies which can range in size from a few individuals to millions of individuals living together as one unit. All members of the colony coordinate their activities and share the work required for survival and raising the next generations (Hölldobler and Wilson, 1990; Wilson, 1990).

While there are many challenges to maintaining colony cohesion among these social insects, it becomes particularly severe when these colonies have to relocate from one nest to another. When colonies of wasps, bees, and ants relocate, they are likely to face the risk of colony fission, leading to decrease in survival among the fragmented subunits and suboptimal fitness for both the reproductives and the workers (Visscher, 2007). Lack of information or coordination in the process of relocation, simultaneous build-up of quorum thresholds at more than one nest option, increased stress levels at the old nest are some of the factors that can cause colonies to fragment. Colony fission has been observed in *Aphaenogaster senilis, Aphaenogaster araneoides* and *Pogonomyrmex badius* in the context of relocation (Galarza et al., 2012; McGlynn et al., 2004; Tschinkel, 2014). Another context in which colony fragmentation occurs is colony reproduction, particularly in those species that reproduce by means of dependent colony founding (DCF), which involves the division of established colonies into smaller autonomous colonies. This occurs in several species of ants, bees, and wasps. The cases in which a group of nestmates accidentally gets separated from the parent colony but has a reproductive individual within it or is capable of requeening forms a new colony in a phenomenon termed as opportunistic dependent colony founding (Cronin et al., 2013). A few studies have considered the outcomes of orphaned workers in the cases where the fragments lack a reproductive individual. In the African army ant *Dorylus molestus*, when the queen is removed from the colony, the orphaned workers fuse with other queen right colonies (Kronauer et al., 2010). In *Aphaenogaster senilis*, it was

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seen that fragmented colonies would merge back with their mother colony within a short time window of less than a week but after this period, the fragments possibly requeen but do not reunify (Ichinose et al., 2009). In *Temnothorax rugatulus*, when monogynous colonies were equally divided between two identical sites, most of the colonies reunified to the site containing the queen (Doering and Pratt, 2016). Irrespective of the reasons for colony fragmentation, it is important to understand whether the fragments reunify, how they reunify and where they reunify.

In the current study, we simulated colony fragmentation in the lab using colonies of *Diacamma indicum*. *D. indicum* is a primitively eusocial ponerine ant species found in the Indian subcontinent. There are no morphologically differentiated queens in this genus; instead, a worker becomes the sole reproductive or gamergate of the colony. Colony size ranges from 12 to 260 individuals and the gamergate can be distinguished from the other workers by the presence of a pair of small appendages called gemmae on her thorax (Peeters and Higashi, 1989). Interestingly, all workers eclose from pupae with these gemmae but the gamergate physically mutilates the callows and maintains her reproductive monopoly (Peeters and Billen, 1991). Thus, if colonies get fragmented but contain pupae they have the potential to become independent. Species belonging to the genus *Diacamma* reproduce obligatorily by fission (Peeters and Ito, 2001). Thus, colony fission is built into the life histories of such species and colonies may be more likely to split when they are ready to reproduce. However, very little is known about the reproductive biology of *D. indicum* and the period during which colonies bud in their natural habitat.

D. indicum colonies are known to use only tandem running as the means of recruitment during relocation (Kolay and Annagiri, 2015). An individual with information regarding the destination becomes the leader and initiates tandem running with a single follower and leads her to the new nest, maintaining physical contact with her throughout the journey (Adlerz, 1896). In this method of recruitment, only one individual gets recruited at a time and the success of relocation is dependent on a few informed individuals throughout the process (Franklin, 2014). The majority of the tandem runs occur to transport naïve nestmates but some followers are known to become tandem leaders themselves and tandem leaders also become followers (Kaur et al., 2012; Schultheiss et al., 2015). During relocation in the natural habitat, colonies of *D. indicum* fragmented on average into 3.72 subgroups temporarily but reunified into a single nest with about 95% of its colony members (Kaur et al., 2012). Due to the difficulty in studying the structure and quality of the temporary and final shelters in the field as well as the inherent variability present in the natural habitat, we explored different aspects of the reunification dynamics of *D. indicum* by conducting several experiments in the laboratory.

We addressed the following questions in this study. First, we asked if intact colonies in a good nest will fragment or relocate whenever another similar good nest becomes available in their neighbourhood. This would allow us to understand the proclivity of *D. indicum* colonies to relocate even in the absence of any disturbance. In the second step, we enquired if colonies will reunify to a single nest when they were recently fragmented and the fragments were occupying comparable good quality nests. If they show cohesion and reunify, then the questions that become relevant are who brings about this reunification and where do they reunify. We also compared the reunification dynamics to relocations in which intact colonies moved from a single degraded old nest to a new nest in order to understand if colony fragments show differences in relocation dynamics as compared to intact colonies. This would also allow us to examine the impact of higher number of available nest options on the dynamics of nest movement as the colony fragments had multiple potential nests to reunite into while the intact colonies were given only a single new nesting site. We further wanted to examine the effect of a trade-off between the presence of gamergate at a particular site and the quality of the potential new nest site in determining the site of reunification. In order to address these facets, we used three separate

sets of experiment in the laboratory and used behavioural observations to collect data and statistical analysis along with time-ordered network tools to compare and contrast our findings.

2. Methods

2.1. To relocate?

These experiments were conducted using twelve *D.indicum* colonies. The colonies consisted of 94.5 ± 28.3 (mean ± standard deviation) adults, 26.5 ± 12.7 pupae, 8.4 ± 6.4, larvae, and 21.5 ± 5.2 eggs. They were collected from their natural habitat in Mohanpur, Nadia district, West Bengal, India (22°56 N, 88°31 E) between October 2017 and March 2018. These colonies were maintained in the laboratory under standard conditions and given *ad libitum* water, ant cake (Bhatkar and Whitcomb, 1970) and termites occasionally. All ants were marked with a unique colour combination to provide individual identification using non-toxic enamel paints (Testors, Rockford, IL, USA). The experimental arena consisted of rectangular aluminium lined wooden platform of 1.52 m × 1.83 m dimensions with 26 cm high walls of the same material and the base was covered with sand. The new nest was of similar quality as the old nest, both of which consisted of a chamber created by a plastic petri plate (diameter of 9 cm) coated with plaster of Paris. A top plate served as the roof and this roof had a single entrance (diameter 1.65 cm). A red cellophane paper was placed above the roof in order to make the chamber dark since insects are considered to have no visibility in red light. The colony was present inside the old nest while the new nest was empty. The old nest and new nest were placed in two random corners of the experimental arena inside the lab. A video camera (Sony Handycam HDR-CX240) was placed above the new nest to record the activities occurring at this site. The experiment was terminated if the colony did not start tandem running within 3 h of start of the experiment. The discovery time (the time taken by the first ant to enter the new nest), the number of unique individuals who discovered the new nest, the time these individuals spent inside the new nest and any tandem runs were decoded from the video recordings.

2.2. To reunify?

Seventeen *D. indicum* colonies were collected from their natural habitat in Mohanpur, Nadia district, West Bengal, India during May and August 2015 and 2017. The colonies consisted of 127.8 ± 31.6 adults, 16.2 ± 10 pupae, 9.9 ± 5.3 larvae, and 10.3 ± 6.2 eggs. Colonies were maintained in nests and all individuals were uniquely marked as described in the previous section. Each colony (both adults and brood) was divided randomly into three fragments in 6:3:1 ratio. This ratio was used in order to simulate the scenario observed in field relocation where colonies initially moved into temporary shelters and the percentage of individuals in these shelters ranged from 5 to 60 (Kaur et al., 2012). The randomization was done by taking 10 chits of paper that had 1, 2 or 3 written on them in the ratio of 6:3:1. Each time an ant and/or brood was picked up from the colony with a feather forceps a chit was also picked. The number in this paper decided where the ant/brood was deposited. Note that the gamergate of the colony was treated like workers and was randomly placed into one of the three fragments. Each colony fragment was kept inside identical plastic boxes (28.5 cm × 21.5 cm × 12 cm) with plaster of Paris base in identical nests as described above. The fragmented colonies were allowed to acclimatize for 90 min in the new shelters. After this period, individuals walking outside the nests were placed inside their respective nests and the nest entrances were blocked with cotton plugs. The three nests containing the fragmented colony (60%, 30% and 10% individuals of their original colony) and an additional empty nest were placed randomly in the four corners of the experimental arena. This was the same arena as detailed in the previous section and all the nests were of comparable quality. The cotton plug was removed after 15 min of

placing the nests in the arena. Focal observation was conducted on transport events. Transport events consisted of brood transport (transport of a brood item by a leader in its mandibles), adult transport (transport of an adult as a follower in a tandem run) and coupled adult-brood transport (transport of an adult who is carrying brood in her mandibles as a follower in a tandem run). We recorded the initiation time, the identity of the leader and the follower, and the initiation and termination sites for each transport event. Before the start of transport, ants entering and exiting different nests were also monitored. The discovery time (the time taken by an individual to discover any nest other than the one from which she comes), latency (the time taken from the discovery of a nest to the start of tandem running to it) and transport time (the time taken from the first tandem transport to the last tandem transport) were recorded. It was possible to get information regarding the discovery time and latency for 12 colonies (88% cases only). The tandem leader that performed the maximum number of tandem runs throughout the relocation was termed as the maximum tandem leader. The experiment was terminated after 45 min of the last transport of adult or brood and the location of the majority of the colony members and brood was noted.

The reunification dynamics was compared with relocation dynamics in which a colony moves out of an unfavourable old nest to a single new nest. Separate relocation experiments were conducted in the same arena and the new nest was similar to the nest previously described. The details of this relocation experiment can be found in Kolay and Annagiri (2017). In these relocation experiments, the old nest was covered by a roof having a semi-circular opening (4.5 cm diameter) and a light was placed above it in order to instigate the ants to relocate. The colonies used in reunification and relocation experiment were of comparable size (reunification experiment: 127.8 ± 31.6, relocation experiment: 143.6 ± 35.7, Wilcoxon rank sum test (unpaired), $W = 66.5$, $N_1 = 17$, $N_2 = 10$, $p = 0.4$).

2.3. Time-ordered network analysis

In order to examine how tandem runs are organised in time, we created time-ordered networks. The nodes represent different nest fragments and each edge represents a tandem run between two nest fragments. The edges are tandem runs conducted from one node to another and hence, are directional and are visualised as arrows connecting between nodes or nest fragments. The edges in a time-ordered network are distributed over the vertical axis which represents time from start of experiment to end of reunification (Blonder et al., 2012). The first step was to construct time ordered networks for observed reunifications in 16 colonies. In the next step, we constructed a null model to assess whether tandem runs are performed randomly across time. During relocation, the rate of tandem runs performed is low in the initial stages and increases as relocation progresses (Kolay and Annagiri, 2017). We wanted to examine whether there is a similar pattern during reunification or whether the number of tandem runs performed across time is random. In these simulations, known as random permuted time models, the data from a given colony's reunification was randomised across time in 100 iterations keeping the number of tandem runs (edges) and initiation as well as destination sites (nodes) conserved to generate a randomised distribution of tandem runs (Holme and Saramäki, 2012).

The distribution of tandem runs in observed and random permuted time model was analysed using Generalized least squares (GLS) test to examine if there was any patterning of tandem runs across time. For this analysis, we have only considered the transports to the nest that contained the gamergate and the cumulative transports (percentage tandem runs) were binned across 5% transport time in each of the 12 replicates (even though in 13 replicates the colonies reunified to the nest containing the gamergate, in one case time keeping was not accurate enough to include in this analysis). For the purpose of comparison, the percentage of tandem runs was taken as the response variable

while transport time and category (observed and random model) were used as predictor variables. A correlation factor for this auto-regression model of order 1 (AR-1) (Adhikari and Agrawal, 2013) and colony ID as grouping factor were also incorporated in the model (see supplementary TextS1 for additional information on the GLS model).

In the next analysis, we examined if reunification at one nest can occur by random movements of ants among the nests. Although we do not expect ants to move randomly among the nests, we wanted to ensure that reunification cannot occur by chance alone and rather, it is targeted towards one preferred nest. We built a second null model, termed as randomised edge model, in which the number of edges and the temporal patterns were conserved but the destinations were randomised (Holme and Saramäki, 2012). The different fragments of the ant colony acted as nodes and the tandem runs as edges. The site of initiation was maintained as observed but the destination node was randomly chosen with equal probability (1/3). Thousand iterations were conducted across each of the 17 colonies and colonies were considered to be reunified when any node contained 95% or higher of the original colony size.

2.4. Trade-off reunification

Thirteen *D. indicum* colonies were collected from their natural habitat in Mohanpur, Nadia district, West Bengal, India during December 2017 and May 2018. The colonies consisted of 93.5 ± 25 adults, 12 ± 9.3 pupae, 8.6 ± 7.7 larvae, and 20.3 ± 12.2 eggs. Colonies were maintained in the laboratory and all individuals were marked prior to the start of each experiment as explained earlier. The adults and brood in each colony were divided into three fragments, allowed to acclimatize and placed in the arena together with an empty nest at randomly chosen corners as described in the previous set of experiments (2.2 To reunify?). The only difference was that in this set of experiments the red cellophane paper was removed from the nest containing the gamergate, making this nest of mediocre quality as compared to all the other good quality nests. It was termed as mediocre because this nest had only a translucent plastic roof, which allows light to enter into the nest chamber making this nest of inferior quality. In addition, a light source was also placed above this nest containing the gamergate in order to increase the luminosity inside the nest chamber. Note that the gamergate was randomly placed with 60%, 30% or 10% of her nestmates as described in the previous section. All the behavioural observations were conducted as described for the previous experiment (2.2 To reunify?). The discovery time, latency and transport time were recorded as described in previous section. The experiment was terminated after waiting for 45 min following the last transport event.

The effects of different factors - presence of the gamergate, the total number of adults and brood present in a nest at the start of the experiment, the physical position of the nest, the nest from which the highest number of tandem leaders emerged and the nest from which the maximum tandem leader emerged - on the choice of reunification site was examined for the reunification and trade-off experiments using independent Chi-square tests for each factor. Under the null hypothesis, the colonies could reunify at any one of the four nests provided with equal probability. Since multiple Chi-square tests were performed, Holm-Bonferroni correction was used for each series of tests. Statistical analysis as well as graph plotting was done in R software (version 3.4.4) (R Development Core Team, 2008). Non-parametric two-tailed tests such as Wilcoxon rank sum test (unpaired) and Chi-square test were performed and $p < 0.05$ was used as cut off for significance. The time-ordered graphs were constructed using the time-ordered package of R (Blonder, 2015). The progress of tandem runs during the reunification was compared between observed and random model with generalised least squares (GLS) test using the nlme package of R (Pinheiro et al., 2018).

Table 1
This table shows the influence of different factors on the site at which colonies reunify. The factors that were tested, the number of colonies expected to relocate to a nest by chance alone based on the experimental set up (Exp), the number of colonies that actually relocated to a given option (Obs) have been indicated. The degrees of freedom (Df), the chi-square values (χ^2) and the p-values of the chi-square tests that were performed have also been presented. The p-values have been corrected using Holm-Bonferroni correction. NI, N2, N3 and N4 depict the nests with 60%, 30%, 10% and 0% colony members respectively while P1, P2, P3 and P4 depict the fixed positions in the arena where the nests were placed. Significant values have been depicted in bold.* the sample size was lower in this case as two colonies did not have brood on the day they were tested.

Factors	Exp	Obs	Df	χ^2	p-value
Gamergate location	3.75/15	13/15	1	**30.4**	**< 0.01**
Number of adults	3.75/15	5/15 (N1), 6/15(N2), 4/15 (N3), 0/15 (N4)	3	5.5	0.7
Maximum number of brood items	3.75/15	5/13*	1	1.3	1.1
Position in arena	3.75/15	5/15 (P1), 3/15 (P2), 2/15 (P3), 5/15 (P4)	3	1.8	0.6
Number of tandem leaders	3.75/15	5/15	1	0.6	1.4
Maximum tandem leader	3.75/15	5/15	1	0.6	0.9

3. Results

3.1. To relocate?

Out of 12 colonies, 10 colonies discovered the new nest. None of the colonies relocated to the new nest nor did any of the colonies show fission to occupy both the new and the old nests. The discovery time for the new nest was 60.4 ± 32 min. The percentage of individuals who discovered the new nest was 3.3 ± 1.5 and they spent 321 ± 248.4 s inside the new nest.

3.2. To reunify?

Most of the colonies (15 out of 17) reunified at a single nest (Chi-square test, N = 17, df = 1, χ^2 = 9.9, p < 0.01). In the remaining two cases, the colonies remained split. On examining where the colonies reunited, we found that presence of the gamergate at one of the nests had significant positive influence as in 13 of the 15 colonies tested, all fragments reunited inside the nest that contained the gamergate (Chi-square test, N = 15, df = 1, χ^2 = 30.4, p < 0.01; Table 1). Apart from the gamergate, many other factors can impact the choice of the final nest. In order to examine this in greater detail, we investigated the influence of five other factors. The total number of adults and brood present in the nest at the start of the experiment and the physical position of the nest did not significantly impact the choice of final nest (Table 1). The nest from which the highest number of tandem leaders emerged or the nest from which the maximum tandem leader emerged also did not become the final choice (Table 1). Thus, we concluded that the presence of the gamergate determines the choice of the final nest during reunification when all the physical parameters of the available nests are comparable.

In the reunification experiments where colonies were in multiple fragments, there was no correlation between transport time and colony size (Spearman's rank correlation coefficient, r_s = -0.1, N = 15, p = 0.7). On comparing the temporal dynamics of relocation with reunification, we found no significant difference in most of the parameters compared. The discovery time (Wilcoxon rank sum test (unpaired), W = 61, N_1 = 12, N_2 = 10, p = 1.0), latency (Wilcoxon rank sum test (unpaired), W = 63.5, N_1 = 12, N_2 = 10, p = 0.8) and transport time (Wilcoxon rank sum test (unpaired), W = 95, N_1 = 15, N_2 = 10, p = 0.3) were all comparable (Fig. 1).

The percentage of leaders involved during transport was comparable between relocation and reunification experiments (Wilcoxon rank sum test (unpaired), W = 82, N_1 = 10, N_2 = 15, p = 0.7; Fig. 2a). However, the percentage of tandem runs in which the followers were themselves tandem leaders and were led to a nest (termed as leader follow leader or LFL tandem runs) was 21.8 ± 11% in reunification experiment and 12.5 ± 3.7% in the relocation experiment, which was significantly lower (Wilcoxon rank sum test (unpaired), W = 129,

N_1 = 10, N_2 = 15, p < 0.01; Fig. 2b). The workload, in terms of tandem runs performed by leaders was analysed across the experiments. It was seen that work distribution was right skewed with skewness of 3.6 (SE = 0.5) and kurtosis of 15 (SE = 1.1) with a long tail in reunification as well as relocation experiment (see supplementary Fig. S2 for more details) (Kolay and Annagiri, 2017).

3.3. Temporal network for reunification

Based on time-ordered network analysis, it was seen that temporal patterning of tandem runs to the nest containing the gamergate as observed in the reunification experiments was only slightly different from random permuted time models even though it was significant (GLS, t-value = -1.93, standard error = 0.01, p = 0.05; Fig. 3). Qualitatively, there was some variability across the colonies and the temporal distribution of observed reunification was lower than random permuted time model in the first half of the reunification process (Fig. 4a and 4b). Randomised edge models revealed that colony reunification is not a random event. Having randomised only the destinations of tandem runs while keeping all other parameters conserved, there was not a single colony that reunified across (17 × 1000) iterations (Fig. 4c). Thus, reunification cannot be expected to occur as a chance event.

3.4. Trade-off experiment

Ten out of thirteen colonies reunified into a single nest and only these 10 were taken into consideration for further analysis. When colony fragments were given a choice between good quality nest and sub-optimal nest containing the gamergate, significantly higher number of colonies (9 out of 10) choose to reunify into the nest of better quality and tandem run the gamergate into the better-quality nest (Table 2). In only one case, the colony reunified at the suboptimal nest containing the gamergate. On examining the influence of other factors like the total number of adults and brood present at the start of the experiment or the physical position of the nest, we found that these did not have a significant impact on the nest choice (Table 2).

4. Discussion

D.indicum colonies were artificially fragmented and kept at different nest sites within a laboratory arena at distances that mimicked what was observed in natural relocations (Kaur et al., 2012) in order to find out if these colonies would reunify into a single nest or remain fragmented. Most of the colonies reunified at the nest in which the colonies' gamergate was present when the quality of the nest she occupied was good. Parameters like the number of nestmates already present at a nest or the number of leaders that emerged from a nest did not influence the choice of a nest for reunification. In previous studies on *D. indicum*, the
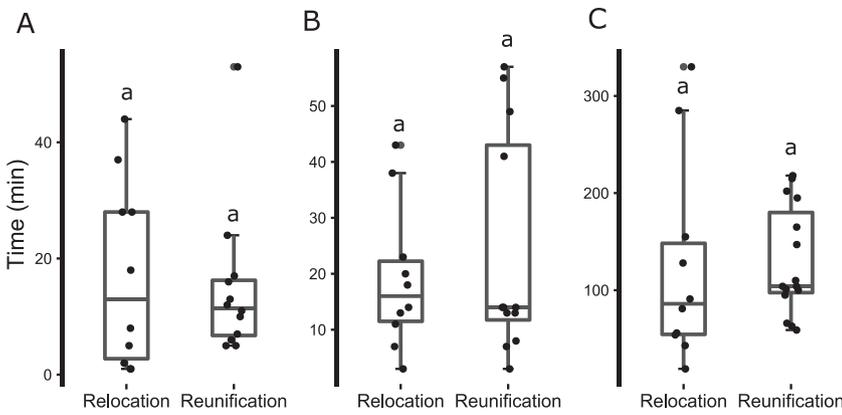
Fig. 1. Temporal dynamics of reunification and relocation. Combined box-and-whisker and dot plot showing discovery time (A), latency (B) and transport time (C) of relocation and reunification experiments have been presented. The boxes represent the interquartile ranges and the line within each box represents the median. The whiskers extend 1.5 times the interquartile range. The dot plot with jitter shows the distribution of the data. Boxes carrying similar letters are statistically comparable.

influence of the gamergate on the choice of new nest has been examined. Disturbed colonies had to choose between good quality nests containing 10% adults and 10% adults with gamergate in one case and between a good quality nest containing 10% adults and an empty good quality nest with only the gamergate (that was tethered) in another case. It was found that the presence of the gamergate did not have a significant impact on the choice of nest of the colonies in either case (Kaur and Annagiri, 2015). However, in the current setup when colonies were artificially fragmented and already housed in good quality nests, we found that the gamergate influenced the nest choice for reunification. The level of stress at the nests possibly plays an important role in this choice. When the stress is relatively high as the old colony is totally exposed, the presence of gamergate does not influence their choice (Kaur and Annagiri, 2015). When the stress levels are low as the colony fragments are within good nests, the presence of gamergate plays an important role and she is not disturbed; instead, all workers are transported to the nest she occupies. This probably ensures minimum exposure of the gamergate to potentially harmful environment and other risks during reunification.

When the nest occupied by the gamergate was of suboptimal quality, the colonies did not reunify at this nest but the gamergate was transported to another nest of better quality and the colonies reunified at this nest. Thus, we conclude that the quality of the nest is more important than the presence of gamergate at a given nest. In *Temnothorax rugatulus,* colonies reunified to the nest site containing the queen, when monogynous colonies were evenly divided between identical nests. Further, when the queen was inside a poor quality nest, *T. rugatulus* colonies reunified at the better-quality nest (Doering and Pratt, 2016). *D. indicum,* a ponerine ant, also showed a similar tendency even though they do not have a queen but a single functional reproductive in their colony termed as the gamergate (Peeters and Higashi, 1989). In both cases, colonies seem to minimize the exposure of the reproductive to potential risks and reunify at the nest in which
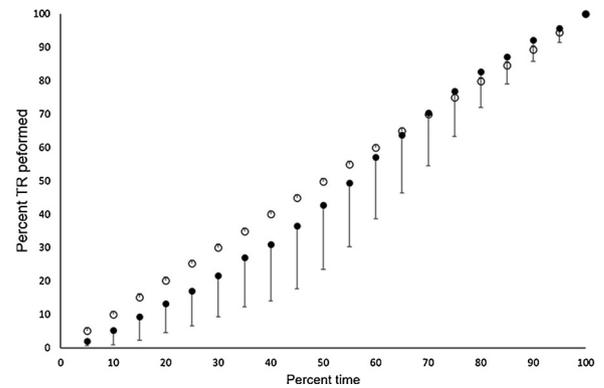

Fig. 3. Temporal distribution of tandem running. Comparison of the progress of tandem runs to the gamergate occupied nest in the observed (filled circle) and random model (empty circle) of reunification has been presented. The x-axis represents percentage of time in 5% bins and the y-axis represents the average cumulative percentage of tandem runs in each bin across the 12 replicates. The upper and lower standard deviations have been shown for random model and observed reunification respectively.

she is present unless she occupies a poor quality nest.

The temporal dynamics of the reunification experiments was similar to that of the relocation experiments although multiple potential nests were present in the first case while only a single new nest was available in the second case. The time taken to discover any one of the colony fragments was similar to the time taken to discover a potential new nest. The time taken for scouts who have discovered a fragment to initiate transport was also comparable and so was the overall transport time from the first transport to the last transport. This was rather unexpected for several reasons. First, the colonies were artificially fragmented and therefore, had no knowledge of the location of the other fragments or whether these were present in the vicinity at all. Second,
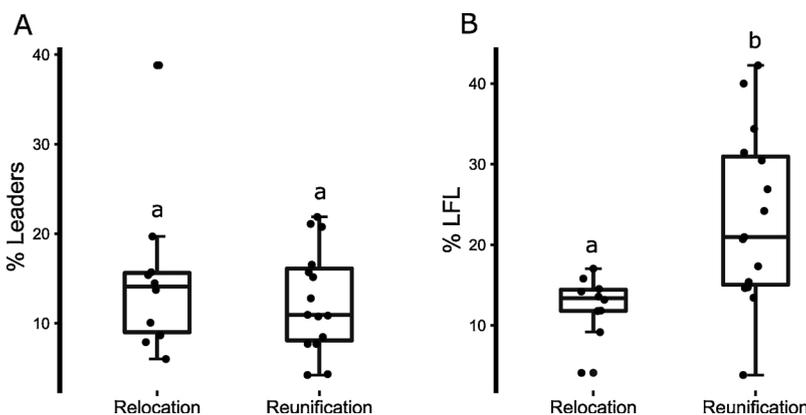

Fig. 2. Communication among leaders. Combined box-and-whisker and dot plot showing the percentage of leaders (A) and the percentage of leader follow leader (LFL) tandem runs (B) in relocation ($N_1 = 10$) and reunification ($N_2 = 15$) experiments have been presented. Each box represents interquartile range and the line within the box represents the median. The whiskers extend 1.5 times the interquartile range. The dot plot with jitter shows the distribution of data. Boxes carrying different letters are significantly different from each other.
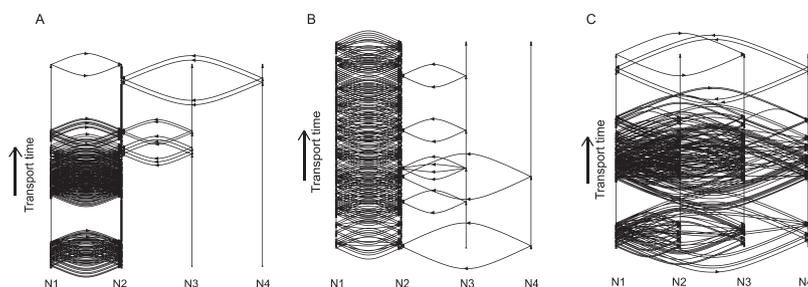
Fig. 4. Time-ordered networks graphs. Time- ordered graphs for observed reunification (A), random permuted time model (B) and randomized edge model (C) have been shown for one colony (DI-502) which reunified to the nest site N2, where gamergate was present in the reunification experiment. N1, N2, N3 and N4 represent four nest sites containing 60%, 30%, 10% and 0% individuals of the original colony. The vertical line represents the time from the start (base of the line) to the end (top of the line) of the transport time of the reunification. The bold line represents the node at which the gamergate was present. The curve lines show individual tandem runs from one particular nest site to other nest site. The arrow heads represent the directions of tandem runs.

Table 2

This table depicts the influence of different factors on the site at which colonies reunify when the gamergate was housed in a suboptimal nest. The factors that were tested, the number of colonies expected to relocate to a nest by chance alone based on the experimental set up (Exp) and the number of colonies that actually relocated to a given option (Obs) have been indicated. The degrees of freedom (Df), the chi-square values (χ^2) and the p-values of the chi-square tests that were performed have also been presented. The p-values have been corrected using Holm-Bonferroni correction. N1, N2, N3 and N4 depict the nests with 60%, 30%, 10% and 0% colony members respectively while P1, P2, P3 and P4 depict the fixed positions in the arena where the nests were placed.

Factors	Exp	Obs	Df	χ^2	p-value
Gamergate location	2.5/10	1/10	1	1.2	0.6
Number of adults	2.5/10	5/10 (N1), 2/10 (N2), 2/10 (N3), 1/10(N4)	3	3.6	0.3
Maximum number of brood	2.5/10	5/10	1	3.3	0.3
Position in arena	2.5/10	4/10 (P1), 2/10 (P2), 4/10 (P3), 0/10 (P4)	3	4.4	0.9
Physical Quality	7.5/10	9/10	1	1.2	0.8

the colonies were inside good quality nests and thus, were expected to be less eager to explore and decide on transportation. Further, the total relocation time was also expected to be longer as assessing multiple probable nests and moving from three separate unknown nests into one was expected to be more complex than moving from a single old nest into a single target nest. The manner in which tandem runs were distributed across transport time was only slightly different from random especially in the first half of the process as indicated by time-ordered network analysis. Even though individual fragments had to be discovered from different locations and brought to one destination, they were conducted in a random manner in terms of time and no clear pattern was apparent qualitatively as well (Supplementary Fig. 1). All these results put together show that relocation dynamics is relatively conserved.

The percentage of the colony that became leaders was also conserved across the reunification and relocation experiments. A few leaders performed most of the tandem runs and most of the leaders performed only a few tandem runs in the reunification experiments (Supplementary Fig. 2) similar to what was seen in the relocation experiments. This kind of uneven work distribution had been previously observed among leaders during relocation process in *D.indicum*, both in the natural habitat and in the laboratory (Kaur et al., 2012; Kolay and Annagiri, 2015; Sumana and Sona, 2012) and in other social insects in the context of different tasks (Pinter-Wollman et al., 2012; Sendova-Franks et al., 2010).

Leaders interact not only with followers but also among themselves via tandem running. Many tandem runs are performed among tandem leaders themselves presumably to lead each other to potential new nest sites. This plays an important role in transfer of information regarding the location of potential nests when multiple options are available. Thus, the leader follow leader (LFL) tandem runs are important for maintaining colony cohesion and preventing fragmentation (Kaur et al., 2012). Reunification is expected to be more complex as colonies had to

decide their final nest sites from four available options in the reunification experiments, whereas only one final nest was available in the relocation experiments. The percentage of LFL tandem runs out of the total tandem runs for reunification was significantly higher than in the relocation experiments. In a previous study, it has been shown that *D. indicum* colonies split into as many as eight temporary fragments before reunifying to a single site during relocation in the natural habitat of these ants. The number of LFL tandem runs for this field experiment (33.8 ± 8.9) was qualitatively higher than both the reunification and relocation experiments (Kaur et al., 2012). Thus, the number of LFL tandem runs required may be correlated to the complexity involved in terms of the number of potential nesting sites available. The present study showcases the usefulness of LFL interactions for reunification but additional laboratory experiments involving direct manipulation of LFL tandem runs during relocation will allow us to explore this aspect further.

When there was no fragmentation and their nest site was not disturbed, *D.indicum* colonies did not relocate to a similar quality new nest. The cost of relocation is presumably non-zero and it is avoided as long as the old nest is habitable. Thus, they do not fall into the category of nomadic species (McGlynn, 2012). The discovery time of the new nest was qualitatively higher when the nest was undisturbed as compared to the relocation and reunification experiments. When established nests are not under stress, there seems to be no urgency for individuals to search for a new home and usually, only a few foragers who come out searching for food or for monitoring their environment encounter the potential new nest, probably by chance. However, when colonies are disturbed and fragmented artificially, they still reunify into a single new nest. Across 17,000 iterations of the randomised simulation models, not a single case of reunification was noted when the destination was randomised. This reiterates clearly that reunification is a targeted and coordinated process that is robust and cannot be brought about by random movement of ants among the nests. Therefore, the null hypothesis of random movement was rejected. Colony reproduction is expected to occur by colony fission in this species as there are no winged queens that disperse to found a separate colony (Peeters and Ito, 2001). Thus, colonies would be expected to split more frequently during the reproductive period. Although the experiments were performed at different times of the year, fragmented colonies almost always reunified showcasing the importance of colony cohesion. Even when a good nest was available in the vicinity and colonies were aware of it, they did not show colony fission in a single case. Considering these results together, we speculate that the circumstances that lead to colony fission are entirely different from those that cause colony relocation.

In conclusion, undisturbed *D. Indicum* colonies occupying good quality nests do not relocate whenever they find a new nest of similar quality. Even though randomized simulations indicated that colony fragments in which the individuals move between nests randomly will not reunify, colonies that were artificially fragmented were observed to reunify in most cases. Colonies reunified at the site containing their gamergate when she was inside a good quality nest and the distribution of tandem runs to the nest containing this individual was slightly different from random model. However, when the nest site occupied by

the gamergate was of suboptimal quality, the colony reunified in a different nest of better quality. Thus, the quality of the new nest is more important in this species than the risks associated with relocating the gamergate. The work distribution and dynamics of reunification were comparable to relocation dynamics and the challenges of fragmentation seem to be overcome by enhanced communication among tandem leaders.

Competing interests

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.beproc.2018.10.017.

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