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Construction of nest mound and preference for it during relocation in an Indian ant *Diacamma indicum*

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Abstract
Ants living in subterranean nests face the challenge of nest flooding and require to combat this recurring issue. The tropical Ponerine ant species, *Diacamma indicum*, living in simple nests with a single chamber, was used in the current study to examine mound building in the lab. Upon stimulating rain ($N = 13$ colonies) they built significantly larger nest mounds as compared to controls. Nest mounds proved to be important to colonies that had experienced rain while relocating. Relocating colonies showed significantly higher preference for new nests with mounds (12/13) when choosing between two equidistant, similar quality potential new nests in contrast to control relocations. To the best of our knowledge this study for the first-time documents mound building behaviour in any Ponerine species in laboratory conditions and introduces nest mounds as another architectural feature of interest to relocating colonies.

Keywords
colony relocation, *Diacamma indicum*, nest architecture, nest flooding response.

1. Introduction

Social insects can be considered as one of the best architects in the animal kingdom. They invest considerable time and energy to construct and maintain elaborate nests which protect them from natural adversities (Hansell & Ruxton, 2008). These nests are fashioned out of a variety of material like soil, wax, paper and they can be located in a variety of locations (Hansell, 2005). For organisms dwelling in subterranean nests, flooding is a potential threat that can adversely impact survival of colony members and overall fitness.

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Flooding can not only lead to the drowning and damage of colonies' assets, especially brood items but also cause the collapse of the interior walls. At the community level flooding is known to reduces the species diversity and change species composition within the area (Ballinger et al., 2007; Mertl et al., 2009).

In order to avoid these adverse effects of flooding, ants are known to use many strategies. *Harpegnathos saltator*, an ant species from subfamily Ponerinae is known to wall paper their top most chamber with pupal cases, in addition to having specialised nest architectural features like refusal chambers which aids in drainage of excess water (Peeters et al., 1994). The tropical fire ant *Solenopsis invicta* is known to build rafts i.e., hollow structures made by the arrangement of interlocked workers, which can float in water using the buoyant force of water to help them move to nearby elevated regions to escape flooding (Mlot et al., 2011). *Cataulacus muticus*, another tropical ant living in Malaysian rainforests, frequently experience heavy rainfall and are known to use two methods to reduce the risk of flooding inside their nests: (i) workers block the nest entrance with their workers' heads to prevent water seepage through the entrance and, (ii) they drink up the excess of water that enters the nest and thereafter excrete it out; a behaviour known as "communal peeing" (Maschwitz & Moog, 2000). The tropical Indian ant *Diacamma indicum*, use two methods to combat nest flooding; they occupy nests at higher elevations, and they also modify the nest entrance by building clay mounds which on one hand act as a levee and on the other elevate the nest entrance from the ground level (Kolay & Annagiri, 2015; Bhattacharyya et al., 2021).

By definition nest mounds are "symmetrically shaped pile of excavated soil, perforated with a dense system of interconnected galleries and often thatched with dry plant materials and they are largely known to play an important role in maintaining a suitable microclimate in numerous ant species" (Hölldobler & Hölldobler, 1990; Bristow et al., 1992). Mounds have an array of different functions like thermoregulation, sunlight harvesting, gaseous exchange, maintenance of the balance of Carbon and Nitrogen in soil and preventing nest flooding to mention a few (Korb, 2010). Fungus-growing ant *Acromyrmex lobicornis* build mounds which measures up to 80 cm in height and 100 cm in diameter (Weber, 1982). Mounds aid in harvesting sunlight, i.e. receiving heat from the sun rays, in the tropical fire ant *Solenopsis invicta* (Vogt et al., 2008) and the shade created by the mounds in *Lasius flavus* helps in decreasing the temperature of the inner cavities of the nest, thereby reducing mortality (Véle & Holuša, 2017). Moreover, mounds also help in the maintenance of the temporal pattern of soil CO_2, Methane, and N_2O content (Wu et al., 2013). Mounds of termites belonging to Macrotermitinae serves as agricultural hubs as fungi are grown in the humid interior chambers of the mounds (Darlington, 1994). Building mounds proves to be an effective strategy to combat nest flooding scenario and this phenomenon has been documented in a couple of Ponerine species as well (Peeters et al., 1994; LeBrun et al., 2011; Kolay & Annagiri, 2015). In additions to these functions nest mounds can provide both olfactory and visual cues to help in the navigation of ants (Knaden & Graham, 2016).

Diacamma indicum, a tropical Ponerine species, which is commonly found in India, lives in very simple nests having a single chamber (Bhattacharyya & Annagiri, 2019). They typically occupy pre-existing cavities in their natural habitat and perform some modification to the interiors if needed (Viginier et al., 2004; Kaur & Sumana, 2014; Kolay & Annagiri, 2015; Bhattacharyya & Annagiri, 2019). In their natural habitat, during monsoons they are known to construct mounds at the entrance of their nest and rebuild these mounds upon damage or destruction (Bhattacharyya et al., 2021). These ants often relocate to new nest locations and their choice of nests is influenced by factors like presence of conspecific nest odour, presence of nestmates at the new nest location (Kaur & Annagiri, 2015). Influence of different physical and architectural features on colony relocation has been studied rigorously in different species of ants (Franks et al., 2002, 2003, 2008). In *Leptothorax albipennis* studies have shown that colonies assess the quality of potential new nests such as the darkness of the nest chamber, the width of the entrance tunnel and the height of the chamber, before they decide to relocate (Dornhaus et al., 2004). In another study on *Leptothorax curvispinosus*, it was shown that colonies' nest choice depend on the potential new nests' entrance size and chamber volume (Pratt & Pierce, 2001).

In this study, we performed experiments which imitate nest flooding situation under laboratory conditions, in order to see if we can trigger mound construction in *D. indicum* colonies. In the next step, we compared these lab built mounds to those built in nature. For understanding the importance of nest mound to colonies with prior experience with rain, we examined if they show any preference for a potential new nest with mound during relocation., Addressing these topics would on one hand convey information about the

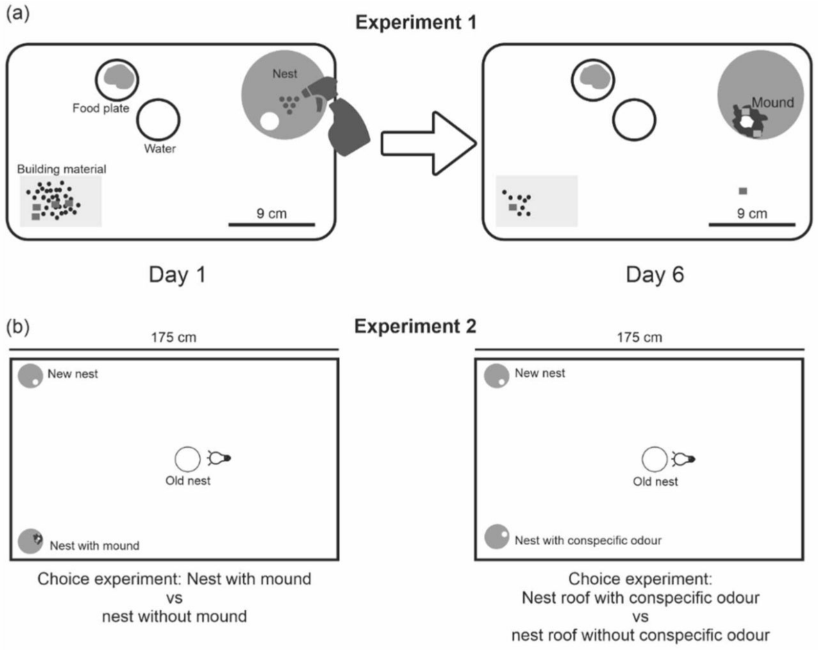

Figure 1. Schematic diagrams explaining the experiments. The upper panel shows the first experiment where colonies had the opportunity to build nest mounds under laboratory conditions. The upper left panel shows the treatment on day 1 and the position of building material plate and the nest along with food and water. The upper right panel shows the changes made on day 6. The grey dark grey area on top of the nest denotes the nest mound and the small pieces of paper incorporated in the mounds are show in light grey. The bottom panel shows two different choice experiments, (i) the left panel shows choices of nests with and without mound and (ii) the right pane shows nests with and without colony specific odour.

colonies' adaptive behaviour towards monsoon and on the other hand highlight the importance of nest mounds in the context of relocation, a facet that has not been explored before in any other species. Thus, using simple experiments (as depicted using the schematic in Figure 1) we addressed some important questions regarding the natural history more specifically the nesting biology of this species.

2. Materials and methods

2.1. Colony collection and maintenance

A total of 34 colonies were collected using the nest flooding method (Kaur, 2014) from June 2017 to October 2022 from IISER Kolkata campus and surroundings (Nadia district, West Bengal, 22°56′N, 88°31′E). This area falls under the Gangetic plains and receives an annual rainfall of 150–200 cm, most of which occurs during the months of monsoon (June to September, as recorded from the Haringhata Farm Weather Station, 22°96′N, 88°52′E). After collection, colonies were kept in 30 × 22 × 10 cm plastic boxes with a plaster of Paris base. A plastic Petri plate (diameter 9 cm) was used as artificial nesting site within the box. The bottom plate was coated with plaster of Paris and the top plate acted as the roof of this nest and a hole (diameter 1.7 cm) acted as the nest entrance. The top plate was covered using a red cellophane paper to convert this artificial nest into a dark chamber. The edges of the plastic boxes were coated using petroleum jelly (Vaseline, Hindustan Unilever, Mumbai, India) to prevent ants from escaping the set up. Ad libitum food, termed as ant cake containing honey, agar, egg and multivitamin tablets and water was provided (Bhatkar & Whitcomb, 1970) in two different containers. Colony members were checked individually with the help of feather forceps under the microscope (Nikon SMZ745T, Nikon, Tokyo, Japan) to locate the gamergate (the sole mated female reproductive of the colony) to ensure that we used only colonies that did not have any major reproductive conflict.

2.2. *Mound building*

This experiment was performed to observe the mound-building capabilities of ant colonies under laboratory conditions upon stimulation of rain. Twenty-six natural colonies were brought to the lab and were randomly divided into 2 sets: control and treatment. The treatment set ($N = 13$ colonies) was subjected to water stress whereas the control nests ($N = 13$) did not face the water stress. All the nest boxes were provided with a mixture of dried soil and earthworm-cast mixture and 20 pieces of paper of dimension $0.5 \times 0.5\ \text{cm}^2$, as building material. Earthworm-casts are balls of excreta produced by Earthworms and *D. indicum* workers are seen frequently in natural conditions to use these balls for making mounds (personal observation), which is why we have used it with dry soil in the laboratory set up. All the soil and earthworm casts were collected from the same natural habitat as the ants, in one session and brought to the lab and dried in the hot air oven (Heratherm OGS60, Thermo-Scientific, Waltham, MA, USA) at 60°C temperature for 3 days. All nests got a 4 g aliquot from this mixture and hence was qualitatively similar across replicates. Paper and cardboard pieces have also been seen to be integrated into the mound in the natural habitat and hence they were used in this setup (Figure 2a). Water (50 ml) was sprayed on top the nest roof and its vicinity for 4 consecutive days to imitate rain. On the 5th day no water was sprayed. On the 6th day we recorded the details of the mound. While spraying water a certain amount of air was also blowed from the spray bottle on the treatment colonies. To keep the disturbance caused due to the airflow constant, we blew air from the identical height on top of the control nests from an empty spray bottle. Photographs of all the nests were taken on day 1, just after starting the experiment and on day 6 after the completion of the experiment. The Building index (BI) was used to measure the modifications of the nest at the nest entrance like placement of decorative materials, building of mounds and scores that ranged from 0 to 5 were assigned to each colony. A bare open nest entrance without any modification is scored 0 and a nest entrance which has a consolidated mound and decorative materials receives a score of 5 (for details see Kolay & Annagiri, 2015; Bhattacharyya & Annagiri, 2019; Bhattacharyya et al., 2021). Scores were assigned to nests in a double-blind manner in order to remove a potential source of experimenter's bias. The person assigning scores was given a photograph of the nest entrance only and he/she was neither aware of control and treatment status nor the status of the colony in terms of day 1 and day 6 across replicates.

2.3. *Effect of mound on nest choice during colony relocation*

In this set of experiments, we explored if relocating colonies have any preference for nests with mound as compared to nests without mounds. In order to provide the nest mound, the roof of the previous nest had to be used and this would also have some conspecific odour, thus to control for that we provided alternative nests with conspecific odour but no mound, as detailed below. We used the 13 treatment colonies and the mounds they had constructed, after the previous experiment had been completed. The roof of the nest containing the mound was removed 12 h prior to the relocation experiment and a fresh identical roof without the mound was placed over the colony to maintain the nest integrity, without which the colony will be disturbed and will start to relocate to places within the nest box or will split in different locations. Two identical nests were provided at equal distance of 97 cm from the old nest in a sand arena measuring 175 × 145 cm. One of the target nests contained the mound built by the relocating colony and the other had an identical roof

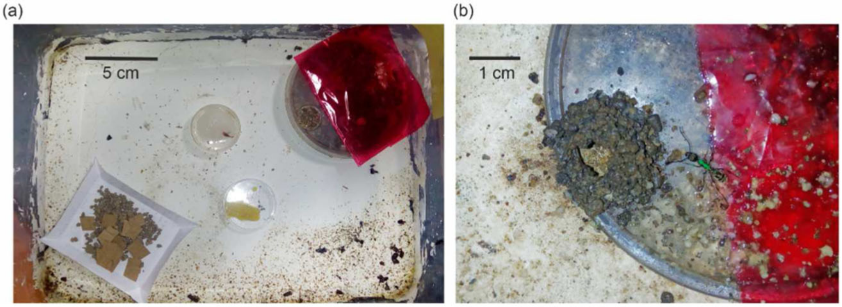

Figure 2. Laboratory setup for rain induced mound building. The photograph on the left panel (a) shows the nest box where *D. indicum* colonies are housed. In one corner, the colony is residing inside the artificial nest. The top plate has a red cellophane cover to make the chamber dark for the colony. The base of whole nest box has plaster of Paris coating. On the opposite corner, there is a mixture of soil balls and cardboard pieces (0.5 cm × 0.5 cm) provided as building material. In the middle of the nest box ad libitum water and food is provided. The photograph (b) shows the mound built by the colony in a span of 6 days. This particular mound had a Building Index (BI) value of 3.5 and an ant marked green is seen in the vicinity of the mound.

without any mound. All other aspects of the two nest options were identical. Relocation was initiated by removing the red cellophane cover from the old nest and the placement of a white light on top of the nest in order to increase light stress on the colonies. After 10 min of this, the roof of the old nest was removed adding more stress to the colony and forcing them to relocate. By taking qualitative observations we tracked ants that were exploring the arena and continued with a replicate only when ants had discovered both the new nests. We noted the final nest choice when more than 90% of the colony members and all the brood were transferred into it.

Our main aim in this set of experiments was to investigate the preference for nest mound. However, in the relocations described above, mounds were present on the colonies' old roof. Thus, chemical cues on the roof are likely to influence the colonies' choice, as it is known that *D. indicum* prefers to occupy previously used nests (Kaur & Annagiri, 2015). In order to address this, we performed additional control experiments. In this set of experiments mounds were scraped off the roof using a paper knife and an identical choice experiment was conducted using 13 colonies. In this set also two options were provided, one nest with old roof but without the mound and another identical nest with a fresh roof which was identical in all other factors. These control relocations were performed with the same colonies mentioned in the previous paragraph (mound choice experiment); however, the second relocation was conducted at least 7 days after the first one. Further, the position of target nests was randomized within the arena every time to avoid any bias from previous experiment. In this set of experiments also we tracked the explorers and noted the choice only after more than 90% of the colony members and all brood were transported to one of the choice nests. A replicate was discarded if both the new nests were not discovered by the colony.

2.4. Choice of good quality nests with odour and good quality nest without odour

This experiment was performed to check colonies' preference towards nests with conspecific colony odour as compared to an identical quality nest without any colony specific odour. A total of 8 colonies were used to perform this experiment in the same arena mentioned in the earlier section to check the preference of relocating colonies to nests with and without their colony odour. The old nest was placed in the middle of the arena and the two choice nests were placed equidistantly in two corners of the arena. The position of the choice nests was randomised before each replicate by tossing a coin. The roof of the old nest which had the colony specific odour in it was removed 30 min prior to the commencement of the experiment very carefully such that the colony is not disturbed and a new roof was installed on top of the colony. In the aforesaid experiment, the roof was removed 12 h prior to the experiment, which might had caused the reduction in the quantity of the nest odourants. In order to address this issue, this additional control experiment was conducted. The roof with the odour was placed on top of a new bottom plate — which was considered as the good quality nest with odour. Another good quality nest with fresh bottom and roof was used as good quality nest without odour. The roof with odour, which did not have any mound, was scraped softly using a paper knife to maintain the same protocol as the experiment mentioned earlier. The colonies were then made to relocate by removing their old nest's roof and putting a white light on top as described previously. Only those relocations were considered for the dataset where both the choice nests were discovered by at least one explorer. Parameters like time taken to discover both the nests, initiation of the relocation and completion of the relocation was noted. At the end of the relocation the choice of the individual colonies were noted and the colonies were observed for another half an hour to determine if that was the final choice.

2.5. Calculation of surface area of the mound

The mounds were assumed to be a hollow cylinder with a rough edge. To calculate the surface area covered by the mounds (proxy to the building efforts by the colony) we used the photographs of the nest mounds and in ImageJ software (version 1.51j8), we determined the mound surface area. We utilized the free hand drawing tool to determine the mound outline and used it as a measure of the surface area covered by the mound. The Petri plate diameter (9 cm) was taken as the scale for these calculations.

2.6. Correlation of colony size with amount of building material used

The building materials that were scraped off from the roof of the colonies following 13 experiments were collected into labelled glass Petri dishes individually. These building materials were then dried at 60°C for 3 days in a hot air oven (Heratherm OGS60, Thermo-scientific) We took the weight of this materials three times to the nearest gram (up to three decimal point) and the mean value was considered as an indicator of the total building material used to make the mound. Then the amount of the building materials was correlated with the respective colony size to check if larger colonies used more amount of building material.

2.7. Statistical analysis

Statistical analysis required for the analysis of the data were performed in StatistiXL (Version 1.1) and R (version 3.4.2 (2017-09-28)). Non parametric tests such as Wilcoxon paired sample test and the Mann–Whitney U-test were performed whenever pairwise comparisons were checked. When the data sets were non-paired like Building Index comparison of control versus treatment colonies, we used the Mann–Whitney U-test. When we compared a parameter within the same set of colonies across different conditions, we used Wilcoxon paired sample test, like comparison of BI on day 1 versus day 6 among treatment colonies. Nest choice comparisons were tested using the Goodness-of-fit test, where the categories were good quality nest with mound and good quality nest without the mound for the first set of relocation. We had the null hypothesis that presence or absence of mound would not have any impact on colonies' nest choice, so the expected were 50/50. We followed the protocol in control relocation as well where categories were good quality nest with odour and good quality nest without odour. In all cases, mean ± standard deviation (SD) was presented unless mentioned otherwise. Statistical significance of the data was tested with two tailed $p \leqslant 0.05$ as cut-off for comparisons.

3. Results

3.1. Mound building

Water spraying on the nest's roof and immediate surroundings triggered colonies' mound building behaviour. On day 6 all the treatment colonies made nest mounds and some of the control colonies had mounds as well (Figure 2b). Mounds built inside the laboratory were qualitatively similar in its architecture to the mounds found in nature (Bhattacharyya et al., 2021). These mounds were made of heaps of soil at nest entrance and it incorporated chips of plaster of Paris (collected by the ants by digging the wet nest floor) and pieces of paper. We quantified these nest mounds by comparing the BI of the mounds. The BI values increased significantly in the water treated colonies (Day 1: 0.31 ± 0.25, Day 6: 3.15 ± 1.51) (Wilcoxon paired sample test: $T = 1$, $N = 13$, $p < 0.01$). In control colonies also, the BI values increased significantly (Day 1: 0.27 ± 0.39, Day 6: 1.27 ± 1.24) (Wilcoxon paired sample test: $T = 0$, $N = 13$, $p < 0.01$ (Figure 3a). BI was significantly higher in treatment colonies than in control colonies on day 6 (Treatment: 3.15 ± 1.51, Control: 1.27 ± 1.24) (Mann–Whitney U-test: $U = 138.5$, $\mathrm{df}_1 = 13$, $\mathrm{df}_2 = 13$, $p < 0.01$). The increment in BI from day 1 to 6 was also significantly higher in treatment colonies as compared to control colonies suggesting that even though BI increased in control colonies the amount of building in treatment was significantly higher: (Increment in treatment colonies: 2.85 ± 1.6, Control: 1 ± 1.15) (Mann–Whitney U-test: $U = 129.5$, $\mathrm{df}_1 = 13$, $\mathrm{df}_2 = 13$, $p < 0.01$ (Figure 3b). Surface area covered by mounds also increased significantly in treatment colonies from day1 to 6 (Surface area covered day 1: 0.04 ± 0.07 cm^2, day 6: 8.13 ± 8.87 cm^2) (Wilcoxon paired sample test: $T = 0$, $N = 13$, $p < 0.01$). Amount of building material used by treatment colonies was not correlated with the colony size (Spearman rank correlation test: $r_s = -0.3$, $\mathrm{df} = 13$, $p = 0.32$.

3.2. Choice of good quality nests with odour and good quality nest without odour

All the 8 colonies relocated successfully and none of them remained split after the completion of the experiment period. Colonies did not show any

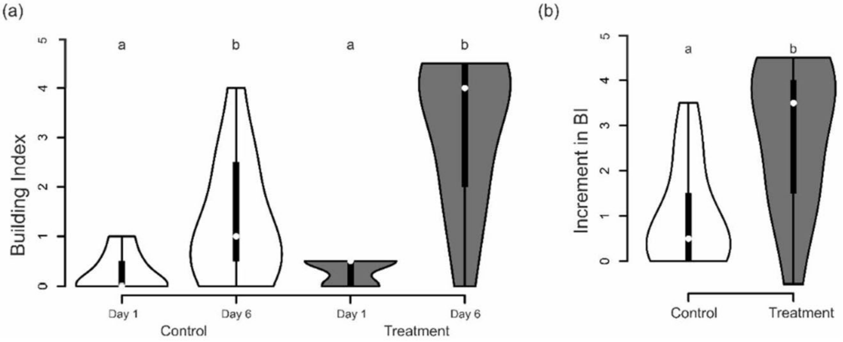

Figure 3. Comparison of Building Index (BI) on day 1 and day 6 and increment in BI in control and treatment colonies. This plot (a) compares the Building Index values of nest mounds constructed by *Diacamma indicum* colonies inside laboratory conditions within the two categories — control (no water treatment- represented in white) and treatment (water sprayed for consecutive 4 days, represented in grey), on day 1 vs. day 6. The plot (b) shows the increment in Building Index (BI) in both control and treatment sets from day 1 to day 6. These violin plots display the median as a white dot and box on either side are the first and third quartiles with the lines representing the range of data. The density distribution of the data is represented as a white (in control) and grey (in treatment) colonies. Significantly different bars carry different letters (Wilcoxon paired sample test: $p < 0.05$).

clear preference towards nests with or without odour (Good quality nest with odour vs. good quality nest without odour: 5/3, Chi square test: χ^2 value = 0.5, df = 1, $p = 0.48$). So, the results show that colonies' nest choice did not depend on odour of nest roof.

3.3. *Effect of mounds on nest choice during colony relocation*

All the 13 colonies relocated successfully and none of them were split at the end of the relocation process. Colonies showed clear preference for nests with the roof that had their own mound over good quality nests without mounds (Old roof with mound vs. new roof: 12/13, Chi square test: χ^2 value = 9.308, df = 1, $p < 0.01$). When mound was absent and only self-roof was provided (control), colonies did not show any clear preference (Old roof without mound vs. new roof: 8/13, Chi square test: χ^2 value = 0.692, df = 1, $p = 0.41$). The results show that relocating colonies with previous exposure to rain preferentially relocated into nests with mounds when they have a choice of equal quality, equidistant nest.

4. Discussion

In nature, *D. indicum* colonies were found to have nest mounds during monsoon and post-monsoon and these mounds were actively repaired upon destruction (Bhattacharyya et al., 2021). This strongly suggests mounds are important for colonies and they are not only built on facing water stress in the vicinity of their nest entrance but that they are actively maintained. These findings inspired us to examine if we can get colonies to construct mounds in the controlled conditions inside the lab.

The current study showed that mound building can be initiated in laboratory conditions for the first time in any Ponerine species. The experiments were designed to mimic the natural scenario of monsoons to anchor the water treatment to what these ants are likely to face in their natural habitat. If this rain stimulation can induce changes to the nest architecture than it can become a stepping stone to explore different topics like the mechanisms of building mounds, the presence of specialised mound builder, the time and resources invested into mound building and the importance of these mounds across contexts like navigation towards the colony, maintaining the colony gestalt. Questions like what percentage of the colony members get involved in this job, does the mound height correlate with the amount of water stress experienced at the nest entrance need to be addressed in controlled lab environment.

Water-induced modification to nests has been studied in great detail in different species like *Paraponera clavata* that nest more in dry regions than in wet surroundings. The slope of the nesting site also changes according to the humidity conditions (Elahi, 2005). In *Ectatomma ruidum* the nest depth and type of nesting (monodomic or polydomic) varies in different habitat conditions particularly the soil properties. The deeper the nest chambers, the lower is their volume and building is not dependent upon presence of plants or roots, whereas we saw *D. indicum* mounds getting supports from those structures in nature (Santamaría et al., 2022). While these studies were conducted in the natural habitat of the ants and gives information about the abiotic factors that affect them, they have the limitation of being influenced by a plethora of factors and not just water. Thus, in the current study we conducted experiments in controlled lab conditions to systematically examine the changes to the nests under the influence of water. Further by using choice experiments, we were able to examine the importance of nest mounds to relocating colonies, a novel architectural feature, for the first time in this species. This makes our study novel and important from the perspective of the species' natural history. Even without facing water treatment colonies built mounds at their nest entrance but this was at a smaller scale as compared to colonies that faced water stress. This is probably because mounds have several other functions which are not connected to monsoon or rain, like gathering heat from sunlight, working as a porous surface suitable for gaseous exchange and thermoregulation to mention a few. Our findings from a previous study performed in nature also showed that mounds were present even when there was no rain and were actively maintained (Bhattacharyya et al., 2021).

No correlation was found between colony size and amount of building material used by the colony for building mounds. This lack of correlation can be explained by the fact that the mound is an outer structure and is not used by the colonies to live in or as storage space. However, from previous studies we know that even the nest volume of these species was not correlated with the colony size (Bhattacharyya & Annagiri, 2019); which indicates, that this species might not be very strict about their mound volumes. Building and maintaining large mounds will involve a large investment in terms of time and energy. A couple of other things that could contribute to this lack of correlation are that the time period for building nest mounds was limited to 6 days and the colony received a low degree of nest inundation. Another nest architectural feature which might have impacted the mound building is the nest entrance size, which was uniform across these experiments. Further experiments performed through longer time periods and varied degree of water treatment and nest entrance size can help in fully comprehending this aspect of mound building like the study on *Acromyrmex fracticornis* where turret building was observed in great detail (Cosarinsky et al., 2020), or in case of *Brachyponera senaarensis* colonies, which generally occupy dry areas can inhabit wet tropical habitats if the areas are well drained and the soil is sandy (Lachaud & Déjean, 1994).

Prior exposure to nest inundation impacted nest choice by this species. Nests with mounds were preferred as compared to good quality equidistant nests by the relocating colonies which had faced nest inundation. So, nest mounds add to the list of nests-associated factors which have influence on nest choice by *D. indicum* colonies (for details see Kaur & Annagiri, 2015). This is the only architectural feature that impacts the nest choice of relocating colonies other than chamber volume (personal observation). While it is unlikely that relocating colonies will find alternate nests that are non-inhabited with fully built mounds in the natural habitat, as these mounds require constant effort for its maintenance (Bhattacharyya et al., 2021), preliminary observations in the natural habitat show that they encounter nests with rudimentary mounds in the form of clay modifications around the nest entrance, further if colonies reoccupy their abandoned nests they are also likely to find some modifications at their nest entrance. Only field surveys performed in different seasons to address the frequency at which colonies undertake relocation and also the propensity at which they find nests with mounds when they relocate can answer this particular question.

Control experiment excluded the effect of two nest-associated factors i.e., nest mound and nest odour showing that the mound was responsible for the choice. Nest odour on the other hand was not primarily responsible as almost equal number of colonies relocated to the two available options, i.e, nests with and without colony specific odours. A potential limiting factor could be that the intensity of the nest odour was reduced upon removal of the nest mound as the next set of experiments were conducted after a 12 h gap, though it is known to persist at least 12 h in other species (Cammaerts & Cammaerts, 1998). To overcome this limitation, we performed additional experiments, where colonies' choice of nests with and without colony specific odour was checked. In this case, the roof was removed 30 min before the commencement of the experiment and without the presence of any mound, thus, there would have been minimal depletion of odourants from the roof of the nest. Even in this experiment colonies did not show any clear preference towards either of the nests. So, to conclude we can say that nest odour on the roof of the new nest has not been a contributing factor, and that presence of mounds influenced colonies' nest choice.

This study is important as it not only strengthens the fact that mound building is a direct response to water stress but also points out that, exposure to water stress makes colonies choose nests with pre-existing mounds if available. Put together these two results showcase how this tropical Ponerine species combats the challenges of nest flooding that has the potential to negatively impact their fitness.

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