

RESEARCH ARTICLE

Caught red-handed: behaviour of brood thieves in an Indian ant

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ABSTRACT

Theft of resources is ubiquitous in the animal kingdom. An evolutionary arms race between thieves and their victims is expected. Although several studies have documented inter- and intraspecific theft of resources in different taxa, studies that delve into the behaviour of thieves and the factors that influence their behaviour have not been undertaken. In the current study on the primitively eusocial ant *Diacamma indicum*, we caught brood thieves red-handed: we observed them in the act of stealing brood and examined their behaviour. Thieves were persistent in their attempts despite facing aggression in the victim colony. Experiencing aggression or failure to steal in the previous attempt negatively impacted a thief's drive to reattempt. To avoid the risks associated with theft, successful thieves exited from victim nests about three times faster than others who were procuring brood from unguarded nests. In a series of experiments examining factors that caused thieves to increase their exit speed, we found that indirect cues of a foreign colony's presence, such as odour or the presence of foreign ants, did not induce these changes in thieves. Thus, we conclude that these ant thieves only respond to the direct threat posed by aggressive foreign ants. In this comprehensive study using behavioural experiments, we reveal the simple rules of engagement between victims and brood thieves.

KEY WORDS: *Diacamma indicum*, Primitively eusocial, Ponerinae, Intraspecific theft

INTRODUCTION

Resources are important for the survival of every animal, and both time and energy is invested in the collection of these resources. One way to reduce this investment is to steal resources from those who have already procured or produced these resources. The most commonly stolen item across the animal kingdom is food, but theft of other resources such as nesting materials and brood have also been documented (Breed et al., 2012). Records of theft are present throughout the animal kingdom, from invertebrates to mammals (Iyengar, 2008). An evolutionary arms race between thieves and their victims would shape the behaviour and morphology of both thieves and victims as they go through cycles of adaptation to enhance their success. Thieves in different taxa employ many interesting strategies, which are found to be fitting to their circumstances and environment. Male satin bowerbirds (*Ptilonorhynchus violaceus*) keep an eye on their neighbouring males, and steal decorations from those that spend less time at their bowers (Wojcieszek et al., 2007). Ravens (*Corvus corax*) keep track

of the location of other ravens' food caches; but while the owners are present they do not approach the caches. Instead, they search at places away from the caches in short bouts to hide their intention of pilfering (Bugnyar and Kotrschal, 2002; Bugnyar and Heinrich, 2005). The ant-eating spider *Zodarion rubidum* employs two different strategies depending on the situation. Thieves initially try to use aggression to monopolize the whole prey captured by a conspecific, but if unsuccessful they resort to becoming inconspicuous and steal part of the prey (Pekár, 2004).

Thievery is widespread in social insects, possibly because thieves can exploit the social mechanisms of the victim colonies (Breed et al., 2012). Thieves in social insects, as in other taxa, also employ various strategies to ensure success. The stingless bee *Lestrimellita limao* raids the nests of other stingless bees and honeybees, resulting in theft of stored honey, pollen, nesting materials and sometimes in the usurpation of the whole nest. Here, raids are initiated by a few scouts, and upon their successful return there is a rapid build-up of attackers at the victim colony to overturn the guards. These attackers also guard the entrance of the victim colony to prevent returning foragers of the victim colony from entering their nest (Sakagami et al., 1993; Grüter et al., 2016). The ant *Ectatomma ruidum* has specialized food thieves, which sneak in and wait inside a conspecific nest to steal food from returning foragers when they enter the nest. The thieves also modify their behaviour to avoid capture while returning to their own nest – taking a roundabout path through dense leaf litter, walking slowly and vigilantly – presumably to avoid encountering conspecifics (McGlynn et al., 2015; Jandt et al., 2015). Ants are a suitable system to study theft as the behaviour is particularly prevalent in these social insects. Observations in this taxa reveals that thieves are equipped to hijack the nestmate recognition system to their advantage. Thieves in many ant species deceive victim colony members by mimicking the colony's gestalt odour or by reducing the quantities of cuticular hydrocarbons they carry (Lenoir et al., 2001; Lhomme and Hines, 2018).

The first three stages of the life cycle of holometabolous insects – eggs, larvae and pupae – together are termed 'brood'. Theft of brood is mostly limited to ants. Brood theft has been observed in three different contexts: for consumption, for increasing workforce in incipient colonies and for rearing slave workers (Buschinger, 2009; Breed et al., 2012). Brood raids for consumption (Gotwald, 1995; LaPolla et al., 2002; Kaspari and O'Donnell, 2003) or for acquiring future slaves (Hölldobler and Wilson, 1990; D'Ettore and Heinze, 2001) are mostly interspecific in nature. Intraspecific theft of brood for increasing workforce to ensure survival is seen in the incipient colonies of some species, and is mostly seen when colonies are founded in a spatially clumped manner (Pollock and Rissing, 1989; Tschinkel, 1992a,b). Brood theft has been previously recorded in about 50 species of ants, all of which reside in the temperate regions and are limited to the subfamilies Myrmicinae and Formicinae (Hölldobler and Wilson, 1990). Recently, however, one additional observation of intraspecific brood theft has been reported in a tropical ant *Diacamma indicum*, belonging to the subfamily Ponerinae (Paul et al., 2016; Paul and Annagiri, 2018).

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D. indicum is a primitively eusocial species recorded in India, Sri Lanka and possibly Japan. The colonies are small, and generally have 12–261 adult females (Viginier et al., 2004; Kaur, 2014). The colonies generally stay in subterranean nests, but also take shelter under bricks and rocks, inside crevices in walls, in tree branches and fallen logs; and are prone to relocate to a new site as a result of physical disturbance to their nest (Viginier et al., 2004; Kolay and Annagiri, 2015). Along with other associated costs, theft of brood was established to be a cost associated with nest relocation in this species. The study also showed that conspecific thieves mostly steal pupae, and their colonies did not consume the stolen pupae, instead allowed them to eclose and increase their workforce (Paul et al., 2016). Victim colonies employed aggression as their primary mode of defence against theft; but thieves bypassed the defence by remaining undetected and by reducing their stay duration in the victim nest, and ensured success by attempting to steal unguarded brood items (Paul and Annagiri, 2018).

In the current study, we looked at the behaviour of the thieves at the individual level. We asked whether thieves modified their behaviour to escape capture, and if so, what are the factors that cause this behavioural shift? Brood theft requires the thief to steal a brood item from a guarded nest, and transport it back to its own nest. To assess the behavioural modification in thieves, brood theft was compared with two other contexts that involve an ant carrying a brood item. The first context is the transportation of a colony's own pupae from an old nest site to a new nest site during nest relocation. Brood transport occurs via two methods in *D. indicum*: either the transporters pick up a brood item in their mandibles and carry it to the new nest, or the transporters lead a fellow nestmate holding the brood to the new nest (Kaur and Sumana, 2014). As the former is similar to what the ant thieves do, we used only this category for comparison with brood theft. The second context is procuring brood from an unguarded site and transporting it back to their own nest, a behaviour that we termed 'brood rescue'. Even though the term 'rescue' has anthropomorphic connotations, we use it only as defined above, and use it from the point of view of the brood. Four criteria are used to identify a behaviour performed by an individual as rescue: (1) the victim should be endangered, (2) the rescuer should be under risk by engaging in rescue, (3) the behaviour of the rescuer should be suited to the circumstance of the victim, and (4) the rescue behaviour should not be inherently beneficial to the rescuer, except for the benefit accrued from kin selection or reciprocal altruism (Hollis and Nowbahari, 2013). In our study, the behaviour of the rescuers was directly connected to the vulnerability of the unguarded brood, which were under the risk of dying or being predated; and by being involved in the rescue process the rescuers were under the risk of harm by the potential predator without any direct benefit to themselves. Conspecific brood have not been observed to be discriminated in *D. indicum* colonies, and the brood and the adults eclosing from the brood are integrated into the colony (Paul et al., 2016). Therefore, by bringing the unguarded brood into the colony, the procuring ants are in turn aiding the survival of the brood. Thus, we used the term 'brood rescue' to characterize the behaviour of ants while they picked up unguarded foreign brood and transported it to their own nest.

We then proceeded to investigate the possible factors responsible for modification in the behaviour during theft. Aggressive interactions by the members of the victim colony upon detection of presence of thieves was observed to be the major inhibitor of theft (Paul and Annagiri, 2018). Detection of chemical profile on adult members of the colony by olfactory sensilla is a means of differentiating between self and foreign colonies, whether through

tactile interaction (Meskali et al., 1995) or from a distance, using airborne cues (Brandstaetter et al., 2008). Ant nests have also been reported to contain colony-specific hydrocarbons, as the members of the colony actively mark different parts of the nest with their secretions (Lenoir et al., 2009; Sturgis et al., 2011). Therefore, the colony-specific odour emanating from the victim colony members or the victim nest can be possible factors for modification in behaviour of *D. indicum* brood thieves, along with aggressive interactions. In natural circumstances, all these factors are combined. Using manipulative experiments we decoupled these factors to examine whether any of these factors by itself can cause brood thieves to modify their behaviour. Thus, we tested the impact of three factors on the behaviour of thieves: the presence of foreign odour, the presence of foreign adults and the presence of aggressive interactions from the foreign adults.

MATERIALS AND METHODS

Colony collection, maintenance and experimental setup

Colonies of *Diacamma indicum* were collected from the campus of Indian Institute of Science Education and Research (IISER) Kolkata, situated in Nadia, West Bengal, India (22°56'N, 88°31'E). These colonies were brought back to the lab, and were kept in plastic containers (28.5×21.5×12 cm) with a plaster of Paris base. Petri dishes (9 cm diameter) with a plaster of Paris base were provided inside the containers as artificial nests. The lid of the Petri dishes were covered with red cellophane paper to darken the inside and had a hole (1.6 cm diameter) to act as the entrance. Colonies were provided with *ad libitum* food (Bhatkar and Whitcomb, 1970) and water, and with termites occasionally. All of the colonies were examined under a stereomicroscope (Leica MZ6, Leica Microsystems, Wetzlar, Germany) to ensure the presence of the reproductive female ant, i.e. the gamergate. The adults and brood of the colonies were counted and the adults were individually marked using enamel colours (Testors, Rockford, IL, USA). The colonies were given a minimum of 2 days after collection to acclimate to the new condition before being used for experiments. The experiments were performed in the laboratory arena with a dimension of 1.75×1.45 m. The base of the arena was lined with sand for ease of walking and the walls were coated with petroleum jelly to prevent the ants from escaping. We conducted all our experiments in accordance with the guidelines that are applicable to working with this animal in our country.

Assessment of thieves' behaviour in victim nests

The behaviour of 37 *D. indicum* ants, which acted as brood thieves, was recorded using video cameras (Handycam, Sony, Tokyo, Japan). A total of 18 replicates were performed using 36 colonies consisting of 113.06±38.09 (mean±s.d.) adult females, 29.92±14.90 pupae, 17.86±12.10 larvae and 43.47±21.90 eggs. In the experiment, two colonies were placed at two randomly selected corners of the arena, and at least one colony was made to relocate into an empty nest placed at the centre of the arena (for details, see Paul et al., 2016 and Paul and Annagiri, 2018). Video cameras were placed above both colonies to record the activity of thieves throughout the experiment (see Movie 1 for an example of a successful attempt of brood theft). All visits made by the thieves to the victim colony were taken into consideration in the analysis for understanding their behaviour inside the victim nest. A visit was defined as an event that started when the thief ant entered the nest of the victim and ended when she came out of the nest. All aggression faced by the thief within this period was quantified, and whether the thief achieved success in stealing brood or not was noted. The thieves

met with aggressive interactions of three different categories: ants touching each other with their antennae in quick succession was termed 'antennal boxing', one ant chasing the other was termed 'chase' and one or more ants biting another one and either dragging it or holding it down in one place was termed 'immobilization' (see Paul and Annagiri, 2018 for details of the aggressive interactions). Scores were given to the interactions according to the qualitative assessment of aggressiveness - antennal boxing received a score of 1, chase received a score of 2 and immobilization received a score of 3. The sum of the scores of all the aggressive interactions received during a visit by an individual thief was termed the 'aggression score' and was used to describe the hostility received by the thief. Aggression received across all visits was summed to calculate the total aggression score received by individual thieves.

Assessment of behaviour modification during brood theft

To understand if thieves modify their behaviour to enhance success during brood theft, the behaviour of ants in two different contexts were compared with brood theft: brood transport during nest relocation and brood rescue. The brood transport events used for this analysis were observed during the nest relocations in the laboratory-based brood theft experiments described earlier. The brood rescue events were recorded from experiments in which foreign brood was provided in an empty nest (termed brood plate), and a focal colony was allowed to procure the brood and bring it back to their own nest. A new artificial nest with its top cover removed was used as the brood plate to ensure that no odour cues emanated from the plate itself. The colony was placed at the centre of the laboratory arena, and the brood plate was placed at a randomly selected corner. For recording the brood transport and brood rescue events, a video camera was placed above the source of the brood, i.e. the old nest of the colony in the case of brood transport and the brood plate in the case of brood rescue. Our previous observations regarding brood theft in *D. indicum* established that thieves preferentially stole pupae (for details see Paul et al., 2016 and Paul and Annagiri, 2018); therefore instances where an ant was carrying a pupa was used for the analysis.

The behaviour of the ants were compared in these three contexts – brood transport, brood rescue and brood theft. In each of these contexts the behaviour of an individual ant carrying a pupa out of a nest and taking it back to its own nest was recorded. Two different facets of the behaviour were examined. After an ant enters the brood plate, she must locate a brood item and pick it up in her mandibles before transferring it to the new nest. The period from when the ant picks up the pupae until she starts to walk out of the nest was termed the 'handling phase'. During this phase, transporters seemed to orient the pupae within their mandibles and prepared to walk with the load. The duration of this phase (handling duration) was recorded to compare across the three contexts. From the moment the ant starts to move out of the victim nest or brood plate in the direction of its own nest until it goes out of the nest plate and then the field of view of the camera (a rectangular area of $\sim 17 \times 10$ cm) was termed the 'exit phase'. The speed of the ant in the exit phase (exit speed) was recorded to compare across the three contexts (see Movie 2 for more details). The handling duration and the exit speed of the ants were calculated from the video using MTrackJ, a plugin of the image processing program ImageJ (Meijering et al., 2012). For tracking the ants, the video was loaded into the program and then the position of the ant in each frame was tracked manually.

Causal factors for modification in behaviour

A set of experiments were performed to tease out the factors that cause modification in the behaviour of brood thieves. Three possible

factors would potentially cause thieves to modify their behaviour in the victim nest – presence of foreign colony odour, presence of foreign adults or display of aggressive interactions by the foreign adults. Although we did not observe any thief getting maimed or killed during attempts of theft (Paul and Annagiri, 2018), the thieves potentially could have received physical harm through the aggressive interactions. Therefore, the interactions were considered a 'direct' threat to the thieves. On the other hand, presence of foreign colony odour in the brood plate or odour cues emanating from the adults present in the plate may indicate the possibility of receiving aggressive interactions, but cannot cause physical harm; thus odour was considered an 'indirect' cue. Separate experiments were performed to investigate which of the three possible factors was responsible for the modification in the behaviour of the thieves.

Presence of foreign colony odour

A study on nest selection based on the presence of colony odour showed that *D. indicum* colonies preferentially chose nests that had the odour of their own colony compared with nests with no associated odour (Kaur and Annagiri, 2015). This showed that the artificial nests retain the gestalt odour of the colony inhabiting them in an amount enough for the ants to be able to detect. In order to investigate whether brood thieves modify their behaviour upon detecting the odour of a foreign colony, the following experiment was performed. In this experiment *D. indicum* colonies were allowed to procure foreign brood from brood plates with three different associated odours – one with the odour of a foreign colony (foreign nest with foreign pupae or FN-FP), one with the odour of their own colony (self-nest with foreign pupae or SN-FP) and one without the odour of any colony (novel nest with foreign pupae or NN-FP). We included a fourth treatment as a control where the colony was allowed to rescue their own brood from a brood plate with their own colony odour (self-nest with self-pupae or SN-SP). *D. indicum* has been shown to prefer to relocate into nests containing their own brood (Kaur and Annagiri, 2015); but it is not known whether the ants could detect the odour emanating from the brood items. The fourth treatment was included as a control to compare with the SN-FP treatment to assess whether the thieves can detect the odour of the foreign brood and if it results in modification of their behaviour. These four treatments were performed randomly with a colony on the same day, with a gap of 90 min between each treatment. As previous study showed that on average 20.79% of pupae were stolen from the victim colonies (Paul and Annagiri, 2018), 20% of the pupae of a colony was given on the brood plates. The experiment was performed in the laboratory arena. The focal colony was placed at the centre of the arena and the brood plate was placed at a randomly selected corner. The experiment was started as soon as the brood plate was placed and the colony was allowed to procure the brood without any disturbance. The brood plate was removed from the arena after all the brood was taken away and there were no ants present in the plate, and the colony was rested for 90 min. At 45 min during this rest period, the same number of pupae that was procured by the colony was taken out to remove any impact of increase in number of brood present in the colony. The brood plate with self-odour and odour of a foreign colony was collected 12 h prior by relocating the focal colony and the foreign colony to a new nest. The plates were kept wrapped with cellophane and maintained in a cool place until the start of the experiment to keep the odour level as intact as possible. The self-brood used in the SN-SP treatment were removed 90 min before the start of the experiment. Ten replicates of the experiment were performed during July–November 2015 using 13 colonies. The colonies consisted of

115.85±30.66 (mean±s.d.) adult females, 30.38±17.33 pupae, 17±10.14 larvae and 31.23±18.03 eggs. Video cameras were placed above the brood plate to record the activity of ants. Handling duration and exit speed of the ants while procuring the brood was calculated from the videos to compare across the treatments. A schematic representation of the experimental setup is provided in Fig. 1A,B.

Presence of foreign adults

An experiment was performed to investigate whether the mere presence of adults was sufficient to cause modification in behaviour of brood thieves. *D. indicum* colonies were allowed to procure foreign brood from three different brood plates – one with their nestmates (self-adults or SA), one with adults from the foreign colony (foreign adults or FA) and one without any adults (adults absent or AA). All the adults were separated from the brood by a mesh partition. A nylon mesh of approximately 1 mm grid size was used for this separation. While occasional antennation between the focal ants and the ants across the mesh partition was observed, aggressive interaction was not possible. The brood plates used were new, thus no colony odour was present on the plates. The plates contained 20% of the total pupae of a colony placed in the open half of the plate, while the other half contained 20% of the total adults of a colony inside the mesh enclosure. The experiment was done in a similar fashion to the previous experiment. Brood plate, which in this case is the plate with the imprisoned adults, was placed at a random corner while the undisturbed colony was placed at the centre of the arena. The AA and FA treatments for a colony were done in a random order on the same day with a 90 min rest in between, with the procured pupae taken out from the colony after 45 min in the rest period. Ten replicates were performed during May–November 2016 with 12 colonies consisting of 124.75±33.85 (mean±s.d.) adult females, 39.67±20.02 pupae, 26.33±11.74 larvae and 41.42±27.57 eggs. The SA treatment was performed separately during October–November 2016 with a different set of 10 colonies consisting of 100.8±40.27 adult females, 34.8±14.23 pupae, 17.2±9.89 larvae and 28.6±22.58 eggs. The activity of ants in the brood plate was recorded using a video camera placed above the plate. Handling duration and exit speed of the ants while procuring the brood was calculated from the videos to compare across the treatments. A schematic representation of the experimental setup is provided in Fig. 1A,C.

Statistical analysis

Data were analysed using non-parametric tests and generalized linear mixed-effects models (GLMM). Non-parametric tests were performed using *statix* version 1.11. GLMMs were performed using the ‘lme4’ package (Bates et al., 2015) in R version 3.4.3 (<https://www.r-project.org/>). The ‘fitdistrplus’ package (Delignette-Muller and Dutang, 2015) in R was used to assess the error distribution of the response variable for performing GLMMs. Mean±s.d. values are reported throughout unless mentioned otherwise. Two-tailed values of $P<0.05$ were used as the cut-off to reject the null hypothesis.

RESULTS

Assessment of thieves' behaviour in victim nests

Thieves made multiple visits to the victim nest and attempted to steal pupae. Among the 37 ants that were investigated, only 4 made a single visit and, on average, individuals made 5.81±4.61 visits (mean±s.d.) with a highest count of 18 visits by a single ant (Fig. 2). Visits of thieves resulted in aggressive interactions with the members of the

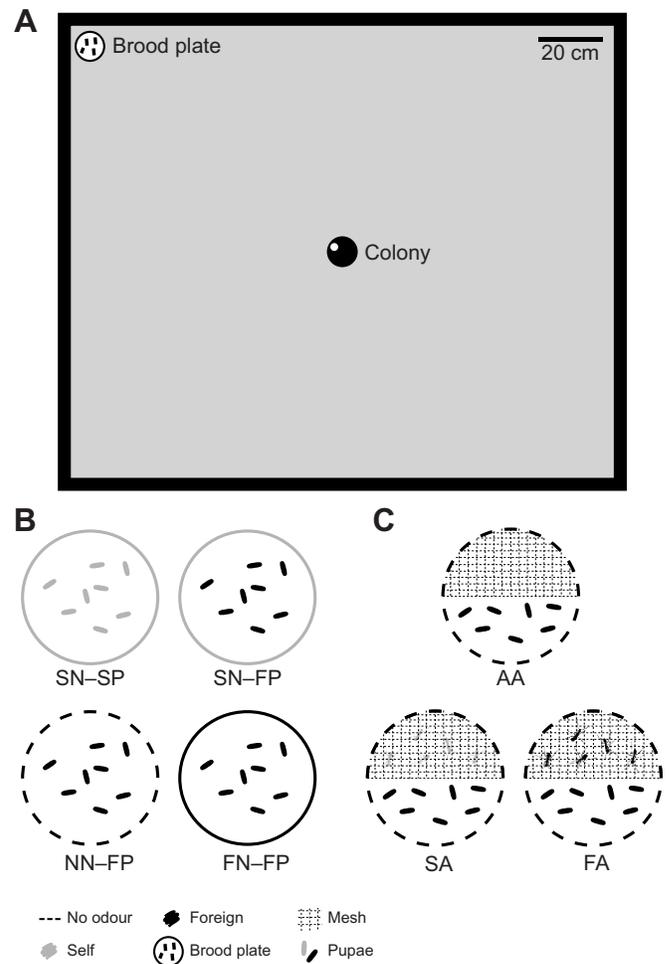


Fig. 1. Schematic for the setup of experiments to assess ant behaviour. The laboratory arena used for performing the experiments is depicted in A. (B) The experimental setup used for the ‘presence of foreign colony odour’ experiment. (C) The experimental setup used for the ‘presence of foreign adults’ experiment. In B and C, objects with the self-odour of the focal colony are represented in grey and objects with the odour of a foreign colony are represented in black. Objects which have no colony odour associated are represented using dashed lines. Brood plates are represented by circles, pupae are represented using filled rod shapes and wire mesh used to encage adult ants as dotted grid. SN, self-nest; NN, new nest; FN, foreign nest; SP, self-pupae; FP, foreign pupae; AA, adults absent; SA, self-adults; FA, foreign adults.

victim colony. A positive correlation was observed between the number of visits made by an individual thief and the total aggression score received by the thief (Spearman rank correlation: $r_s=0.70$, d.f.=37, $P<0.001$) (Fig. 3A). The latency for thieves to return to the victim’s nest for the next visit was significantly higher when they faced immobilization during the previous visit (7.19±10.61 min) (mean±s.d.) compared with when they did not face immobilization in the previous attempt (3.95±4.68 min) (GLMM: $P=0.031$, see Table S1A) (Fig. 3B). Thieves also showed a significantly lower latency for returning to the victim nest when they were successful in stealing a pupa in their previous visit (2.15±1.51 min) compared with when they were unsuccessful in stealing a pupa in their previous visit (7.10±9.78 min) (GLMM: $P=0.003$, see Table S1B) (Fig. 3C). However, success or failure in previous visits did not impact the success of thievery in the subsequent visits (Wald–Wolfowitz runs test: $P>0.1$ in all cases).

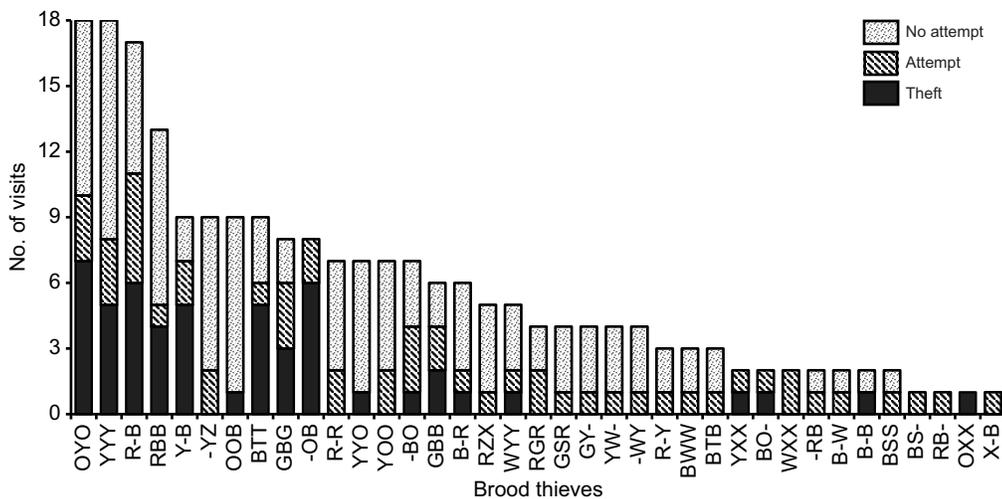


Fig. 2. Visits made by ant thieves to victim nest. The visits made by the 37 thief ants to the victim nest is depicted using stacked bar plots. The names of the thieves are shown on the x-axis. The whole bar for an individual thief depicts the total number of visits made by that particular thief. The dotted portion of a bar depicts those visits in which the thief did not make any attempts of brood theft (no attempt), the striped portion depicts cases in which the thief made attempts but was unsuccessful (attempt) and the black portion depicts cases in which the thief was successful in stealing a pupa (theft).

Modification in behaviour during theft

The behaviour of thieves in the victim colony was compared using two parameters – handling duration and exit speed – and was contrasted with the behaviour of ants while transporting pupae during nest relocation and rescuing of pupae from unguarded nests. For this analysis, 56 instances of brood transport, 99 instances of brood rescue and 42 instances of brood theft were compared. Handling duration for brood transport and brood theft was 1.88 ± 1.05 s (mean \pm s.d.) and 1.19 ± 0.64 s, respectively, and values were comparable (GLMM: $P=0.15$, see Table S3) (Fig. 4A). The handling duration during brood rescue was 3.29 ± 3.53 s and this was significantly higher compared with that for transport and theft (GLMM: rescue vs transport: $P=0.013$; rescue vs theft: $P=0.001$, see Table S2A) (Fig. 4A). Exit speed for brood theft was 10.44 ± 5.32 cm s⁻¹ and was significantly higher than exit speeds for brood transport (5.76 ± 2.25 cm s⁻¹) and brood rescue (3.27 ± 1.36 cm s⁻¹). Exit speeds across the three categories were significantly different (GLMM: $P<0.001$ for all comparisons, see Table S2B) (Fig. 4B). The highest exit speed of a *D. indicum* ant observed was 23.74 cm s⁻¹ and was observed during brood theft.

Causal factors for modification in behaviour

Presence of foreign colony odour

The behaviour of the thieves in the four treatments was compared using handling duration and exit speed during procurement of the pupae from the brood plate. For this analysis, there were 54, 56, 57 and 55 instances of brood procurement in SN–SP, SN–FP, NN–FP and FN–FP treatments, respectively. The handling duration was 2.36 ± 2.84 s (mean \pm s.d.) for SN–SP, 2.53 ± 3.76 s for SN–FP, 2.12 ± 2.87 s for NN–FP and 2.43 ± 2.20 s for FN–FP, which were comparable (GLMM: $P>0.4$ for all comparisons, see Table S3A and Fig. 5A). The exit speed for SN–SP was 3.41 ± 1.95 cm s⁻¹, which was significantly lower than for SN–FP (3.69 ± 2.09 cm s⁻¹) and NN–FP (4.64 ± 2.81 cm s⁻¹) (GLMM: $P<0.05$ for both, see Table S3B) (Fig. 5B), but was comparable to FN–FP (3.61 ± 1.78 cm s⁻¹) (GLMM: $P=0.064$, see Table S3B) (Fig. 5B). The exit speed for SN–FP, NN–FP and FN–FP treatments were comparable (GLMM: $P>0.4$ for all comparisons, see Table S3B) (Fig. 5B). Thus, handling duration was comparable in the four treatments, and the exit speed was significantly different among some treatments but not others. Even in the two cases where it was significantly different, the difference was marginal, and was not enough to explain the large increase of speed observed in the case of thieves stealing from victim nests.

Presence of foreign adults

The behaviour of the thieves in the three treatments was also compared using handling duration and exit speed during procurement of the pupae from the brood plate. For this analysis, we quantified and analysed 96 instances of brood procurement in both the AA and FA treatments, and 86 instances in the SA treatment. Handling duration for AA was 2.70 ± 2.79 s (mean \pm s.d.), which was significantly higher than FA (2.02 ± 2.13 s) (GLMM: $P=0.028$, see Table S3C and Fig. 6A), but was comparable to SA (2.75 ± 3.11 s) (GLMM: $P=0.926$, see Table S3C and Fig. 6A). Handling duration for FA and SA was comparable (GLMM: $P=0.072$, see Table S3C and Fig. 6A). Exit speed for FA was 4.20 ± 2.20 cm s⁻¹, which was significantly higher than for AA (3.57 ± 1.53 cm s⁻¹) and SA (3.18 ± 1.42 cm s⁻¹) (GLMM: FA vs AA: $P<0.001$, FA vs SA: $P=0.013$, see Table S3D) (Fig. 6B). Exit speed for AA and SA were comparable (GLMM: AA vs SA: $P=0.785$, see Table S3D) (Fig. 6B). Handling duration was low and exit speed was high in case of the FA treatment, which suggests that the mere presence of foreign adults significantly impacted the behaviour of ants during procurement of brood from brood plate, but the magnitude of this impact is relatively small.

Presence of aggressive interactions

Thieves faced aggressive interactions from victim colony members during attempts of brood theft, but during the FA treatment where foreign adults were present in the brood plate across a mesh partition, there were no risks of facing aggression. Although we did not observe displays of aggression during the replicates of the FA treatment, occasional non-aggressive interaction between the foreign adults and the ants procuring brood were observed. Antennae could be extended through the mesh, and occasional antennation was observed between the ants on opposite side of the mesh. Therefore, some direct interactions were present, but unlike brood theft attempts, the ants procuring brood in FA treatment did not face any aggressive interactions. In order to assess the impact of aggressive interactions on the modification of behaviour of brood thieves handling duration and exit speed during these two contexts were compared. Handling duration during brood theft (1.19 ± 0.64 s) was significantly lower compared with FA treatment (2.02 ± 2.13 s) (GLMM: $P=0.035$, see Table S3E and Fig. 6A), and exit speed during brood theft (10.44 ± 5.32 cm s⁻¹) was also significantly higher compared with that observed in FA treatment (4.20 ± 2.20 cm s⁻¹) (GLMM: $P<0.001$, see Table S3F) (Fig. 6B). Therefore the ants modified their behaviour significantly during brood theft when there was a

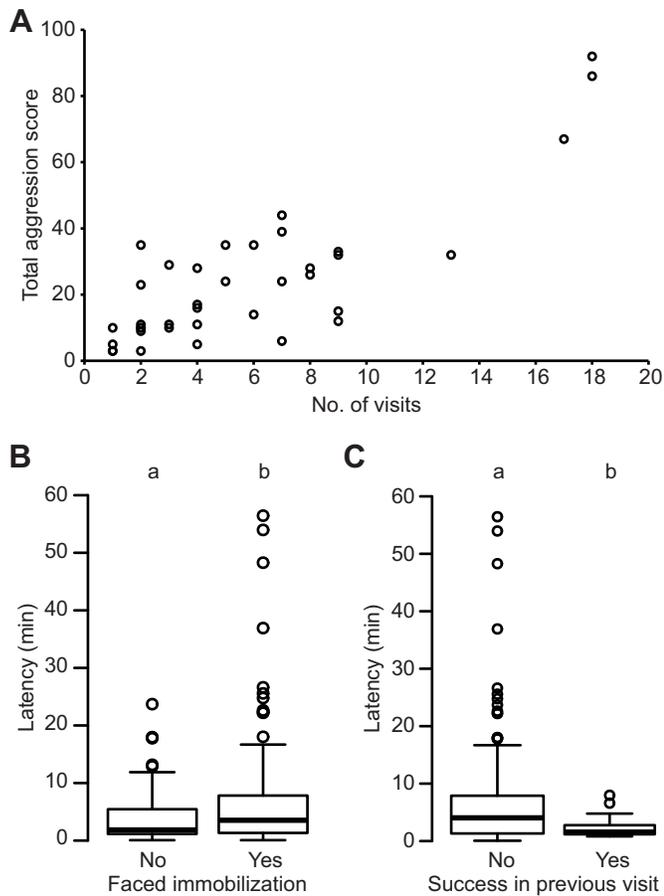


Fig. 3. Visits by thieves in the victim nest and the factors impacting the visits. (A) Correlation between the number of visits made by thieves to the victim nest and the aggression they faced (Spearman rank correlation, $n=37$, $P<0.05$). (B) The latency to return to the victim nest of the thieves who faced immobilization in the previous visit is compared with the thieves who did not face immobilization in the previous visit. (C) The latency to return to the victim nest of the thieves who were successful in stealing a pupa in their previous visit is compared with the thieves who were unsuccessful in their previous visit. The different letters above the boxes depict significant differences among the categories (GLMM, $n=33$, cut-off $P<0.05$).

potential for facing aggression or when the thieves actually received aggression from the members of the victim colony.

DISCUSSION

Even though theft is ubiquitous across the animal kingdom, catching thieves red-handed, i.e. observing them in the act of theft is difficult. This is probably why studies attempting to understand how thieves behave during the process of theft is rare. In this study, we investigated the behaviour of brood thieves from multiple points of view. We tried to understand how thieves behave while performing the task of brood theft in the hostile environment of a foreign colony. Thereafter, we investigated the behavioural modifications employed by the thieves in order to achieve success and the factors responsible for causing this shift in behaviour.

Thieves were persistent in their attempts to steal pupae, and the majority of the thieves visited the victim nest multiple times and attempted to steal pupae. The number of visits by thieves were significantly positively correlated with the total aggression score, which suggests that the amount of aggression received continued to increase with each additional visit. Therefore, thieves making more

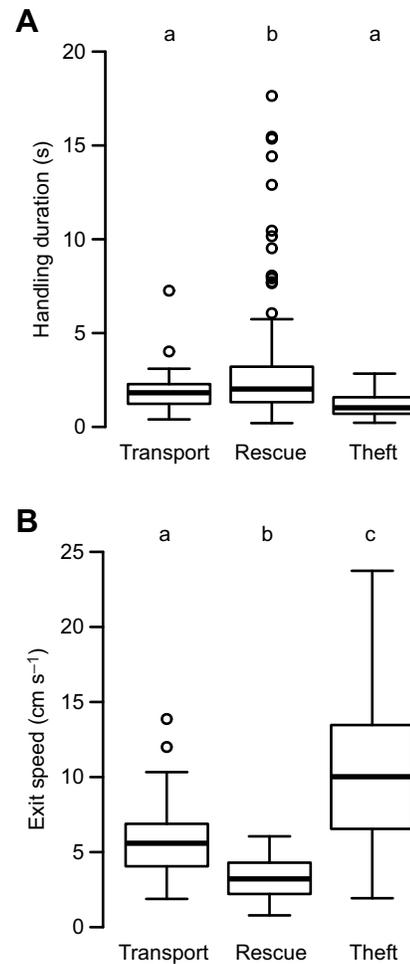


Fig. 4. Handling duration and exit speed of ants during brood transport, brood rescue and brood theft. (A) Comparison of the handling duration of ants during brood transport in the process of nest relocation (transport, $n=56$), brood rescue (rescue, $n=99$) and brood theft (theft, $n=42$). (B) Comparison of the exit speed of ants during transport, rescue and theft. The different letters above the boxes depict significant differences within the categories (GLMM, cut-off $P<0.05$).

attempts were equally prone to receive aggression as thieves that made fewer attempts to steal. Although none of the thieves were observed to be killed or maimed by the members of the victim colony (see Paul and Annagiri, 2018), the aggression faced did impact the drive towards revisiting the victim nest. When a thief was detected and immobilized during a visit, the time taken for the thief to revisit the nest was significantly longer. When the thieves were able to steal successfully during a visit, the latency for revisiting the victim nest was significantly reduced. Absence of lethal levels of aggression against thieves may influence the behaviour of thieves and the success of theft, and is also suggested in the case of food thieves of the ant *E. ruidum* (McGlynn et al., 2015).

The behaviour of ants during brood theft deviated to a large extent when compared with brood rescue and brood transport during nest relocation. During brood rescue, the focal colony was undisturbed and was not involved in any emergency task. The brood in the brood plate was also unguarded. Therefore, the rescuers from the focal colony did not face any particular risk or any urgency. This was reflected in the behaviour of the ants during brood rescue as handling duration was highest among the three categories and exit speed was lowest. During nest relocation ant colonies face an

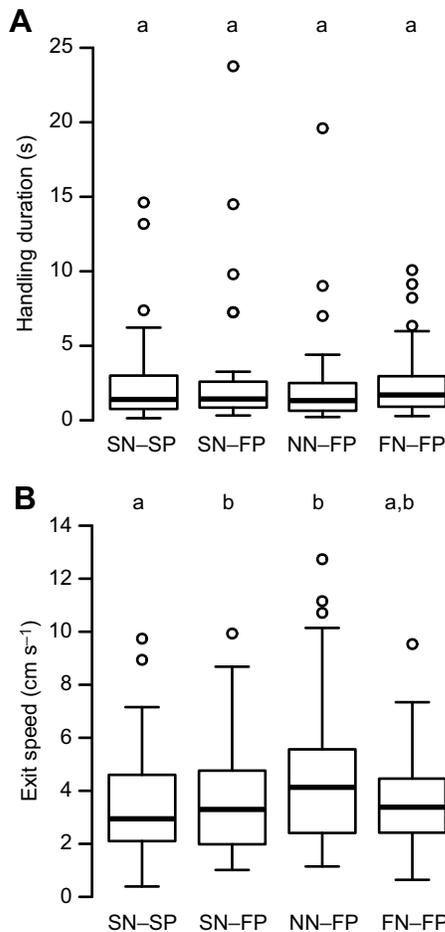


Fig. 5. Impact of the presence of foreign colony odour in the brood source on the behaviour of ants procuring brood. Comparison of (A) handling duration and (B) exit speed of ants during treatments SN-SP ($n=54$), SN-FP ($n=56$), NN-FP ($n=57$) and FN-FP ($n=55$) to assess the impact of foreign colony odour. The different letters above the boxes depict significant difference within the boxes (GLMM, cutoff $P<0.05$). A detailed description of the different treatments used in the experiment is provided in the Materials and Methods section. SN, self-nest; NN, new nest; FN, foreign nest; SP, self-pupae; FP, foreign pupae.

emergency as their colonies are housed under suboptimal conditions and their survival depends upon the colony relocating to a secure new nest site. Under these circumstances the handling duration is low and the exit speed is high compared with levels in brood rescue. The risk for the thieves is relatively higher during brood theft, where the thieves are inside a foreign nest and may have to face aggression from the members of the foreign colony. In this situation, thieves showed handling duration as low as that seen for brood transport during relocation. The exit speed for brood theft was significantly higher, it was on average 1.8-times higher than the exit speed for brood transport during relocation, and 3.2-times higher than the exit speed for brood rescue. The exit speed is the speed of the ants while moving away from the nest with pupae. The risk for the thieves is highest while the thief is inside or near the victim nest as the density of non-nestmates will be high in this region. Whether the thieves maintain this speed all the way back to their nest was not examined in the current study, but we expect this not to be the case as the density of foreign ants would be lower away from the victim nest. Increase in the speed of thieves while in the home range of the victim colony was also reported in *E. ruidum* ants (McGlynn et al.,

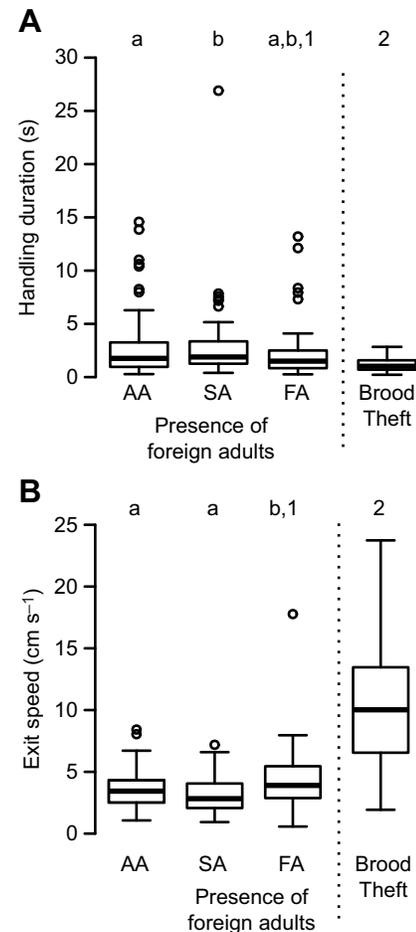


Fig. 6. Impact of the presence of foreign adults in the brood source on the behaviour of the ants procuring brood. Comparison of (A) handling duration and (B) exit speed of ants during the treatments AA ($n=96$), FA ($n=96$), SA ($n=86$) to assess the impact of foreign adults is depicted left of the dotted lines. The different letters above the boxes depict significant difference within the boxes (GLMM, $P<0.05$). The box plots on the right of the dotted line in A and B depict handling duration and exit speed of ants during brood theft ($n=42$), respectively. The different numbers placed above the boxes representing FA and brood theft represent a significant difference within these two categories (GLMM: cut-off $P<0.05$). A detailed description of the different treatments used in the experiment is provided in the Materials and Methods section. AA, adults absent; SA, self-adults; FA, foreign adults.

2015). The average length of the body of an adult female *D. indicum* ant is about 1 cm. Therefore, the average initial speed of a thief was around 10 body lengths per second; and the highest initial speed achieved was around 24 body lengths per second. This high initial speed along with the low handling duration during brood theft suggests that the thieves modified their behaviour to a great extent to avoid facing aggression from non-nestmates and to steal successfully.

The mere presence of a foreign colony odour while procuring brood from a nest did not seem to cause any behavioural change in the ants. Experiments performed on nest selection on the basis of associated odour demonstrated that *D. indicum* colonies are able to detect the colony-specific odour present on the artificial nests (Kaur and Annagiri, 2015). Thus the focal colonies were presented with brood placed on nests with different associated odours. The handling speeds were similar across the four treatments, and the exit speed was marginally higher in the treatments where the nest

had the odour of the focal colony or did not have any colony odour. This suggests that the mismatch of odour between the nest and the pupae may have caused slight modification in the behaviour of the thieves. This speculation assumes that the pupae had a detectable amount of the odour of the source colony on their surface, which requires further experimentation to confirm. Looking at the overall comparisons, it can be concluded that the presence of the odour of a foreign colony alone was not able to cause modification in the behaviour of the ants.

Presence of foreign adults on the brood plate, but inside a mesh enclosure, prevented the foreign adults from interacting aggressively with the focal ants. This allowed us to investigate whether the presence of foreign adults was enough to cause behavioural modification. Looking at the overall comparisons, the handling duration of the ants procuring pupae was not different in the treatments compared with control. The exit speed of the ants procuring pupae was comparable in control and in the treatment with self-adults, but exit speed in the treatment where foreign adults were present was significantly higher than in the other cases. This suggested that ants modify their behaviour while procuring pupae in the presence of foreign adults. To tease out whether the potential for aggressive interactions is required for ants to modify their behaviour or the presence of foreign adults is enough, behaviour of ants during brood theft was compared with the behaviour during procurement of brood in the presence of foreign adults. In the first case the ants procuring brood had the potential of facing aggression, while in the latter case they did not. The handling duration when ants had the potential of facing aggression was significantly lower. The exit speed of ants with the potential of facing aggression was significantly higher, and it was on average 2.5-times higher than the exit speed of ants who did not have the risk of facing aggression. This suggests that although the presence of foreign adults during brood procurement impacts the behaviour of ants, the modification is moderate compared with the extent of modification observed while the ants were at risk of facing aggression from the foreign adults.

In this study, we performed a detailed exploration of the behaviour of brood thieves in *D. indicum*. The experimentation revealed that thieves are able to modify their behaviour on facing a direct threat of aggression, and ensured the success of theft by means of the modification. Thieves were persistent in their attempts at theft and were observed to return to the victim colony repeatedly, even though they faced aggression. This may be indicative of the balance tipping towards benefit in the trade-off of brood theft, as the risk for the thieving colony is low in terms of the number of ants involved in theft but the gain in terms of future workforce is high (Paul and Annagiri, 2018; Paul et al., 2016). The drive of individual thieves to steal was explored in this study, but the tendency of colonies to steal and procure brood has to be investigated using experiments in the future. It was established that thieves of *D. indicum* steal pupae, which become a part of the thieving colony and do not get consumed (Paul et al., 2016); thus it is unlikely that nutrient status of a colony in the short term exerts an influence on the drive to steal. There was also no correlation found between the number of thieves in colonies or number of attempts of theft performed by the thief colonies with the size of their colonies, i.e. number of adults and brood present (Paul and Annagiri, 2018); but further manipulative experiments involving the colony size as a variable are required to corroborate these findings. Detection of the thieves in the victim colonies led to aggressive interaction towards thieves and failure of attempts of theft. Semiochemicals are important in detecting the presence of intruders (Howard and

Blomquist, 2005; Sturgis and Gordon, 2012; Sano et al., 2018), but may not be sufficient or required for behavioural modification in the brood thieves of *D. indicum*. The presence of foreign gestalt odour failed to cause modification in thieves' behaviour. Presence of foreign adults without the potential of aggression caused a moderate shift in thieves' behaviour. The modification seemed to be triggered to a considerable extent only by the potential of facing aggression, in response to which the thieves quickly picked up brood items and egressed as fast as possible to avoid capture. While indirect cues of foreign colonies were not sufficient to elicit change in the thieves' behaviour, it clearly indicates the ability of ants to react according to external circumstances upon facing the direct threat of aggression. Exploration of the behaviour of social insects at the level of individuals is essential to appreciate the complexity of their societies and to understand the rules of engagement between partners across intra- and interspecific interactions.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.193755.supplemental>

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