

Patch fidelity in *Camponotus compressus* ants foraging on honeydew secreted by treehoppers

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***Camponotus compressus* ants are thought to be mutualistic with plant sap-sucking insects such as aphids and treehoppers, where the ants provide protection from predation and/or help maintain hygiene, in exchange for sugar-rich honeydew secreted by sap-suckers. We studied the foraging strategies and ant–treehopper interactions among *C. compressus* ants and *Oxyrachis tarandus* tree-hoppers on the plant *Bauhinia tomentosa*. We found that ants show fidelity to specific patches of *B. tomentosa*. We also found a significant correlation between hopper number and the number of visiting ants in foraging patches. Our study raises many questions about the ant–treehopper mutualism, navigation strategies and optimal foraging in *C. compressus*.**

Keywords: *Camponotus compressus*, honeydew, mutualism, patch fidelity, *Oxyrachis tarandus*.

MUTUALISM between ants and sap-sucking insects is a widely studied interaction¹. In this, ants stroke treehoppers with their antennae and make them excrete a sugar-rich liquid, called honeydew, which they then consume (Figure 1). *Camponotus compressus* is a common ant found in South and Southeast Asia, and is known to interact with plant sap-sucking hemiptera like aphids and treehoppers^{2,3}. Although *C. compressus* is common and widespread, its ecology has been poorly studied. While aspects of the *C. compressus*–aphid mutualism have been reported², the *C. compressus*–treehopper interaction has not been studied, except for natural history descriptions³. We conducted a preliminary study exploring the foraging strategies of *C. compressus* and its interaction with *Oxyrachis tarandus*, a species of treehopper.

C. compressus ants are known to follow non-random search patterns while foraging on aphid honeydew in cashew inflorescences², abandoning poor-quality branches and continuing to forage in richer branches. Since group size of treehoppers may determine the amount of honeydew available in a patch, we tested if there is a correlation between group size of tree-hoppers (proxy for patch quality) and number of ants visiting the group.

Furthermore, for efficient foraging, ants need appropriate ways of sampling food patches. Unbiased sampling, i.e. visiting every patch with equal probability, is an efficient way of foraging for ephemeral food resources such as insect prey and seeds. However, in the case of continuously available (as long as treehoppers are present) foods like honeydew, a more appropriate sampling method might be patch fidelity, involving less search time. In this study, we investigated if *C. compressus* ants show fidelity to foraging patches.

The study was conducted in the Indian Institute of Science Education and Research (IISER), Pune campus (18.5203°N, 73.8567°E) during September–October 2013. A colony of *C. compressus* with multiple nest entrances was selected for the study. The study site had three clusters of *Bauhinia tomentosa* plants with at least one nest entrance near the stems of plants in each cluster. Ants that come out of the entrances near the base of a plant foraged on that plant, and we established that all the entrances belonged to the same colony by observing the lack of aggression when four ants from different foraging patches were placed at four different entrances. In contrast, when four ants from this colony were placed among ants of a different colony, also present in the IISER campus (at a distance of approximately 100 m), all of them were attacked and killed.

Treehoppers (*O. tarandus*) were seen in groups, mainly in fresh and terminal branches of *B. tomentosa*. Each group of treehoppers was considered a patch; there were 92 such patches in the study site. All patches were visible and easily accessible for observation. Each patch occupied 5–15 cm of twig and was at least 30 cm from a neighbouring patch. Total number of nymphs in a patch ranged from 15 to 46, while total number of adults ranged from 0 to 5. While nymphs were not seen moving between patches, at least for a week, adults were seen flying from one patch to another. No other ant species was seen on these plants and thus interspecies competition for honeydew was unlikely.

We first tested for correlation between number of treehoppers and number of ants on a patch ('correlation study'). Fifteen patches from terminal non-overlapping branches of *B. tomentosa* were arbitrarily chosen. For our experiments, we have considered only terminal branches. If the patches are not in the terminal branches, they could be on the way to a terminal patch, so sometimes we may encounter ants in transit to their patch. So, to avoid possible over-counting of the ants, we chose only terminal patches. We included only 15 patches in this part of the study to ensure that we obtained a snapshot of ant abundances on foraging patches. Counting on more than 15 patches may have resulted in double counts of ants that might have moved between patches during counting. The number of nymph and adult treehoppers (henceforth referred to as 'nymphs' and 'adults' respectively), and ants present in each patch was recorded. Readings were taken

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Figure 1. *a*, *Camponotus compressus* ant antennating a treehopper nymph. *b*, Honeydew secretion (droplet on the posterior tip of the nymph) by the nymph. *c*, Consumption of honeydew.

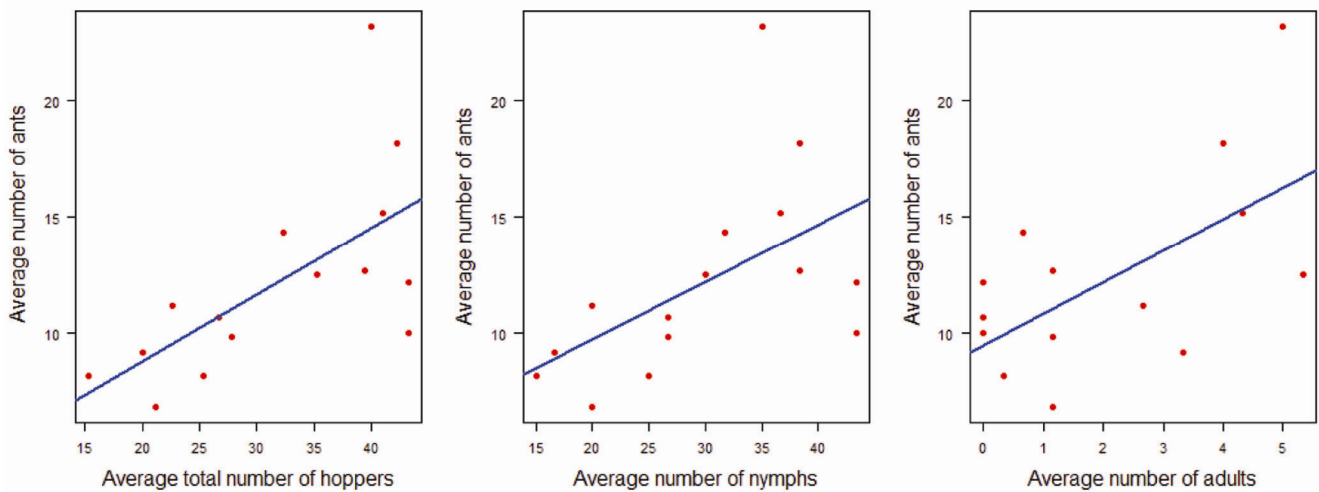


Figure 2. Correlation plots for average number of *C. compressus* ants versus total *Oxyrachis tarandus* tree hoppers ($P = 0.01$, $r = 0.65$), average number of nymphs ($P = 0.04$, $r = 0.54$) and adults ($P = 0.01$, $r = 0.62$) and per patch.

three times a day, each separated by four hours, for two days, to capture temporal variation in the number of ants and treehoppers. The number of nymphs, adults and ants was averaged over all readings for each patch for the correlation analyses.

We found a significant positive correlation between average number of ants and average number of treehoppers per patch, overall (including nymphs and adults; $P = 0.01$, $r = 0.65$), only nymphs ($P = 0.04$, $r = 0.54$) and only adults ($P = 0.01$, $r = 0.62$; Figure 2).

The explanation for this correlation could be that greater hopper numbers reflect increased food resources for the ants. We observed that ants antennate nymph hoppers more often than adults; as mentioned earlier, antennation is likely to be a stimulus for hoppers to secrete honeydew. Nymph treehoppers have long posterior ends from the tips of which honeydew is secreted (Figure 1 *b*). Adults, however, appear to have no such specialized morphology for honeydew secretion. Moreover, adults are winged and mobile, and hence their numbers per patch are more variable temporally. Adults were also observed in much lower numbers than nymphs per patch.

Nymphs, compared with adults, are expected to benefit more from protection of ants, owing to the absence of a hard exoskeleton, and their lower agility. Hence the correlation between nymph and ant numbers is easier to explain compared to that between adult hopper and ant numbers, which requires further study.

To test if ants show fidelity to the patches they forage in ('patch fidelity study'), 7 patches were chosen from the 15 used in the correlation study, such that there were not more than two patches from a cluster of plants. We restricted our sampling to seven patches to minimize the chance of counting the same ant on different patches, as sampling 15 patches took more time during which ants could move from one patch to other. Twice a day (at ca. 9:30 a.m. and 5:30 p.m., covering morning and evening foraging), on days 1 and 2 of the study, all ants found on a particular patch were marked with a unique colour assigned to the patch. Marking was done by spraying fine droplets of 'Fevicryl hobby ideas' acrylic colour on the ants, using a flat painting brush. This caused little disturbance to the ants and was effective in marking many ants at the same time. We decided against marking ants

individually because this procedure was found to stimulate two responses in the ants – many ants were observed returning to the nest immediately and some ants were observed grooming off the paint from marked ants. However, these behaviours were not observed when the ants were sprayed with paint. While marking, even though some droplets also fell on the treehoppers, they were seen in the same patch and no behavioural differences or change in their numbers was evident. Thus, they did not seem to be affected by paint.

On days 3 and 4 of the study no marking was done and no readings were taken, in order to allow the ants and treehoppers to get settled (acclimatized to the paint) after the events of marking. On days 5 and 6 of the experiment, the number of marked ants of each colour and unmarked ants on each patch was noted twice a day. We searched for marked ants in all three clusters of plants entirely, and noted down their numbers and colour as thoroughly as possible.

In the patch fidelity study, the average number of marked ants of a particular colour on days 5 and 6 was much higher in the focal patch (i.e. patch where the ant was marked) compared to the sum of the number of ants of that colour in the remaining patches in the study site (91; 92 patches minus 1 focal patch) averaged over the two days of observation. This was true for all seven focal patches (Table 1; Wilcoxon signed rank sum test, $V = 28$, $P = 0.02$). If ants are foraging randomly in an unbiased manner, we expect the number of marked ants of a particular colour to be much higher summed across all patches, except the patch of that designated colour, compared to the number in the patch of that designated colour. Hence our data clearly indicate fidelity for foraging patches.

Despite marking all ants present on the study patches on days 1 and 2 of the experiment, we found a considerable number of unmarked ants in these patches on days 5 and 6. This could possibly be because the number of marking events (four) was not enough to mark all the ants which visit that patch and/or because we only marked during daytime (these ants are known to be more active at night)⁴. Thus, to determine the role of these factors in explaining the large numbers of unmarked ants, we tested if the proportion of marked ants saturates after sufficient number of marking events ('saturation study').

For saturation study, five patches were selected arbitrarily. Only five patches were selected because we wanted to make sure that all the patches were marked within a short time, to avoid double counting as all ants marked in one patch will have same colour. All ants in these patches were marked during the day (ca. 11 a.m.) and at night (ca. 9 p.m.) every day continuously for eight days. Each patch had two unique colours – one for daytime and one for the night; hence the same ant can have two colours. We chose only two time points because we did not know if spraying the paint many times a day for 8

days could affect the ants, treehoppers and the plant. The number of marked and unmarked ants was recorded approximately around 11 a.m. (before marking) and 3 p.m. (and not before the marking event at 9 p.m.). Night sampling was not possible in our study because the ants moved around rapidly whenever the torch light was shone on them, making it difficult to notice their colour identity.

The proportions of marked ants, which include both day-marked and night-marked ants and a few ants with both the marks, and unmarked ants were plotted against marking event number. The best-fitting curve for the observed trend in the proportion of marked ants with the marking event number was chosen according to Akaike Information Criterion⁵. Models used for fitting the curve were: $y = (a*x)/(b + x)$; $y = m*x + c$; $y = (1/x*a) + (b*x)$; $y = \exp(a + b*x)$ and $y = a - \exp(-b*x)$, where y is the proportion of marked ants, x the marking event number, and a , b , c and m are constants. All the statistical analyses were done in R studio.

Our saturation study showed that the proportion of marked ants in a patch saturates to an average of 85% in all the patches, suggesting that most of the ants seen on a patch are revisiting it. Among all the models that we tried to fit the data to, the best fit occurred with $y = a - e^{(-b*x)}$, which corresponds to a saturating function for the proportion of marked ants (see Figure 3 for a representative plot). From the shape of the curve (linear, exponential, parabolic or saturating), we can see if number of marking events is a reason for the unmarked ants found in the fidelity study. Each curve indicates a different kind of relationship between the variables and thus different biological explanation. For example, a linear curve could indicate that the number of ants that forage increases with time, while previously marked ants stay in the patch. Since the best fit is a saturating function (i.e. as the number of marking events increases, the proportion of marked ants increases and then saturates), we can conclude that the reason for finding many unmarked ants in the fidelity experiment is not enough marking events to mark all the ants which visit that patch. This also shows how many marking events are required to cover most of the ants that forage in a patch. It would be interesting to see how these results change the interpretation of the fidelity experiment (perhaps magnitude of fidelity changes with time).

Furthermore, we found that marking ants at night was crucial to achieve the saturation of the proportion of marked ants as removing them from the data does not achieve clear saturation (data not shown). We also found that even after marking the ants in a patch about 16 times, there are always a few unmarked ants (Figure 3). These few unmarked ants could be (i) Active explorers sampling for new food sources at any particular point of time. (ii) Ants whose previous patch may have lost its value and thus they forage in a different patch. One way to test this is to mark the ants and change the number of treehoppers

Table 1. Patch fidelity experiment. Number of marked and unmarked ants in the seven patches observed twice a day for two days; $N = 92$ patches of treehoppers on *Bauhinia tomentosa* plants. Number of marked ants of a particular colour was much higher in the patch designated with that colour (i.e. where ant was marked) compared with the sum of ants of the same colour in all other patches

Patch no.	Day 5						Day 6					
	0930 h			1730 h			0930 h			1730 h		
	Number of marked ants in the focal patch	Sum of the same colour marked ants in all other patches	Number of unmarked ants in the focal patch	Number of marked ants in the focal patch	Sum of the same colour marked ants in all other patches	Number of unmarked ants in the focal patch	Number of marked ants in the focal patch	Sum of the same colour marked ants in all other patches	Number of unmarked ants in the focal patch	Number of marked ants in the focal patch	Sum of the same colour marked ants in all other patches	Number of unmarked ants in the focal patch
1	6	1	2	1	1	2	3	1	2	1	1	3
2	5	2	5	6	1	3	6	1	2	4	1	1
3	4	1	4	5	1	4	4	1	6	3	0	8
4	4	0	2	4	0	1	5	0	5	3	0	1
5	6	3	8	2	1	6	3	4	7	5	1	5
6	7	3	20	6	3	20	5	6	25	6	2	20
7	3	2	4	5	0	1	5	1	4	1	2	5

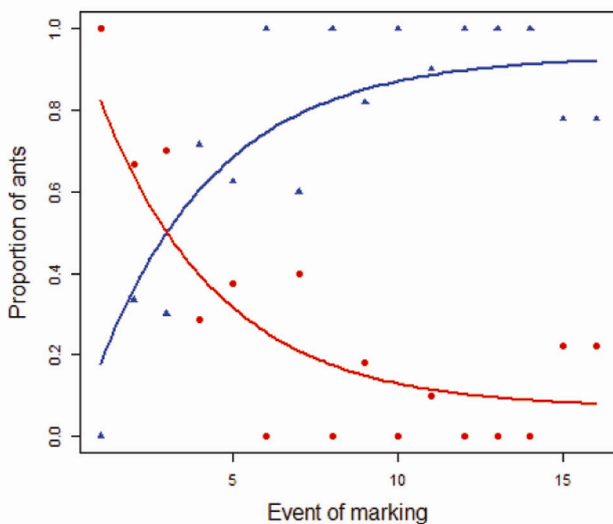


Figure 3. A representative plot with proportion of marked ants (including ants marked once during daytime and once at night-time; blue triangles) and unmarked (red circles) ants in five patches (plots for other four patches are similar and thus not shown here) for 16 events of marking spread over 8 days. Proportion of marked ants saturates as the number of marking events increases, indicating that high number of unmarked ants observed in fidelity experiment is due to limited number of marking events. Best fit model for this, according to Akaike Information Criterion is $y = a - e^{(-b \cdot x)}$.

and observe where the marked ants forage. (iii) Ants which may have forgotten their patch of fidelity and are recruited to the current patch. It would be interesting to further explore how long an ant can remember and revisit a patch.

Unbiased sampling of patches by ants can lead to colony-level redundancy in foraging, i.e. more ants than re-

quired visit each patch. By adopting patch fidelity, ants can reach food faster than by sampling each and every patch. Fidelity is useful especially when food is not completely depleted in a short time. However, patch fidelity also has disadvantages. Given that hopper numbers in a patch may fluctuate over time, ants need to be flexible enough to change their preferences accordingly. One strategy might be that ants reduce fidelity to a patch as its quality falls. Further investigation about how ants assess and respond to change in the patch quality is required.

C. compressus ants are recruited to food sources by a phenomenon known as group recruitment, where a ‘leader’ takes a small group of ants to a newly discovered food source. As *C. compressus* is not known to use chemical trails⁶, returning to the same patch everyday may involve memory and suitable navigation strategies (for example, using landmarks or other cues). Past experiences of an ant may also affect its foraging decisions. These questions remain to be answered.

Fidelity for foraging sites has been reported in multiple species of ants, in different forms, including in relation to group⁷, direction⁸, path⁹, seed patch¹⁰ and leaf¹¹. One of the advantages of our study was the small size and simple architecture of the plants on which ants were foraging. This helped in getting an accurate count of all marked ants in all patches. Our study also had a few limitations: (1) All readings were taken during daytime, whereas these ants are known to forage more at night. (2) Given the constraints of our field study, we did not have a way to observe ants inside the nest in ground and hence lacked basic data such as size of ant colony (we had not collected the data for proportion of marked ants resampled which could have been used to estimate the colony size).

(3) Since this study was done as a semester project, there were time constraints for the student (R.P.N.), which partly influenced the sampling design we chose (e.g. in saturation study, we restricted our sampling to 5 out of 7 patches). Despite these constraints, our results about fidelity seem to be evident.

Our study shows that there is a positive correlation between the number of *C. compressus* ants on a foraging patch and hopper density, and that ants are likely to return to the same foraging patch repeatedly. These preliminary results raise many questions for future studies. While we have assumed a mutualistic relationship between ants and hoppers, and it is evident that ants ingest honeydew secreted by hoppers, the benefits that hoppers obtain from ants is yet to be demonstrated in this system. Furthermore, the costs and benefits of mutualistic interactions are typically complex and condition-dependent¹². These commonly occurring and easily studied species may offer an accessible system to explore general questions about species interactions.

Our results also raise questions about patch fidelity, such as whether there is more fidelity to higher quality patches, and how ants are able to regulate their likelihood of visiting a patch. *C. compressus* ants are observed to consume insects and honeydew (continuously available food), a source of carbohydrates for which they show fidelity. Is there specialization among the workers for foraging for honeydew (carbohydrate source) and insects (protein source), such that the optimal strategy for honeydew foragers may be patch fidelity, whereas the optimal strategy for insect foragers may be unbiased sampling? These and other questions may be fruitfully explored with the *C. compressus*–treehopper system commonly found in India.

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