

How do workers of the primitively eusocial wasp *Ropalidia marginata* detect the presence of their queens?

Anindita Bhadra^a, Priya L. Iyer^a, A. Sumana^a, Sujata A. Deshpande^a, Saubhik Ghosh^a,
Raghavendra Gadagkar^{a,b,*}

^aCentre for Ecological Sciences, Indian Institute of Science, Bangalore, India

^bEvolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore, India

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Abstract

Queens in primitively eusocial insect societies are morphologically indistinguishable from their workers, and occupy the highest position in the dominance hierarchy. Such queens are believed to use aggression to maintain worker activity and reproductive monopoly in the colony. However, in the primitively eusocial wasp *Ropalidia marginata*, the queen is a strikingly docile individual, who interacts rarely with her workers. If the queen is experimentally removed, one of the workers becomes extremely aggressive within minutes, and eventually becomes the new queen of the colony. We designate her as the potential queen. Experimental evidence suggests that the queen probably uses a non-volatile pheromone to signal her presence to her workers. Here we attempt to identify the mechanism by which the queen transmits information about her presence to the workers. We designate the time taken for the potential queen to realize the absence of the queen as the realization time and model the realization time as a function of the decay time of the queen's signal and the average signal age. We find that the realization time obtained from the model, considering only direct interactions (193.5 min) is too large compared to the experimentally observed value of 30 min. Hence we consider the possibility of signal transfer through relay. Using the Dijkstra's algorithm, we first establish the effectiveness of relay in such a system and then use experimental data to fit the model. We find that the realization time obtained from the model, considering relay (237.1 min) is also too large compared to the experimentally observed value of 30 min. We thus conclude that physical interactions, both direct and indirect (relay), are not sufficient to transfer the queen's signal in *R. marginata*. Finally, we discuss the possibility that the queen applies her pheromone on the nest material from where the workers can perceive it without having to physically interact with the queen.

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1. Introduction

In a typical primitively eusocial wasp, where there is no queen-worker dimorphism, the queen is the most behaviourally dominant member of her colony. In such species, the queen maintains her reproductive monopoly by means of physical aggression toward her workers, and especially toward the beta individual, who succeeds her if she dies

(Fletcher and Ross, 1985; Gadagkar, 1991; Reeve, 1991; West-Eberhard, 1969, 1977).

Ropalidia marginata (Lep.) (Hymenoptera: Vespidae), is classified as a primitively eusocial species due to the absence of queen-worker dimorphism (Gadagkar, 2001). However, *R. marginata* queens are strikingly docile and behaviourally non-dominant individuals. Nevertheless they are entirely successful in maintaining reproductive monopoly in their colonies. Upon the death or removal of the queen, one individual becomes extremely aggressive and goes on to become the next queen if the original queen is not returned. We refer to this individual as the potential queen (PQ). Invariably, the PQ significantly alters her behaviour within minutes of queen removal (Gadagkar,

*Corresponding author. Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India. Tel.: +91 80 23601429; fax: +91 80 23602121.

E-mail address: ragh@ces.iisc.ernet.in (R. Gadagkar).

2001; Kardile and Gadagkar, 2002; Premnath et al., 1996; Sumana and Gadagkar, 2001). This raises the question of how the PQ perceives the presence or absence of the queen.

In *R. marginata*, we can rule out the possibility that the PQ realizes the absence of the queen due to the sudden drop in aggression received by her. This is because the queen hardly shows any aggression toward the PQ, or toward any other worker for that matter. Because workers in highly eusocial species rely on queen pheromones to assess the presence or absence of their behaviourally docile queens, it is an attractive hypothesis that *R. marginata* queens produce pheromones, which serve to inform workers about their presence or absence. Results of experiments involving separation of queens from their workers by wire mesh screens, are consistent with the hypothesis that workers recognize the presence or absence of their queens by means of non-volatile queen pheromone/s (Sumana et al., under review).

Here we consider the possible mechanism by which a non-volatile queen-pheromone is transmitted to the workers in an *R. marginata* colony.

2. The model

We first consider the possibility that the queen of *R. marginata* uses physical interactions to transmit a non-volatile pheromone to the workers. Henceforth we refer to the pheromone as the queen signal. Since the PQ drastically alters her behaviour upon queen removal, it follows that the signal has to be reinforced from time to time. This implies that the signal is not very stable, i.e., it has a short decay time. We define the following three parameters.

Decay time (t_d): the time needed for the queen signal to decay.

Average signal age (t_a): the average age of the queen signal present with the PQ at any instant of time (and therefore at the time of queen removal).

Average realization time (t_r): the average time taken for PQ, after queen removal, to realize her absence.

The PQ should realize the loss of the queen at the instant of time when the queen signal present with her decays. Hence these three parameters are related as:

$$t_r = t_d - t_a \quad (1)$$

Since the average age of the pheromone with the PQ at any instant of time cannot exceed the decay time of the pheromone,

$$t_a \leq t_d \quad (2)$$

We estimate the realization time both empirically by observation of the behaviour of the PQ upon queen removal as well as theoretically by experimentally estimating t_d and t_a and using the above equations. If the queen transmits her pheromone by physical interactions, the

realization times estimated directly and indirectly should match.

3. Experimental procedures

Fifty post-emergence colonies of *R. marginata* were collected from various nesting sites in Bangalore (13°00'N, 77°32'E), Mysore (12°25'N and 76°50'E) and Mudumalai (11°34'N, 76°38'E), and transplanted to the Vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore (Gadagkar, 2001). All individuals in each colony were uniquely colour coded with small spots of quick drying paints.

Dominance behaviours in *R. marginata* consist of aggressive biting, being offered liquid, chase, crash, hold another individual in mouth, nibble, peck, and sit on another individual. Their obvious counterparts, like being bitten, offer liquid, being chased, etc. are designated as subordinate interactions. The sum of all the dominant behaviours was used to compute the frequency of dominant behaviour (Gadagkar, 2001). The sum of all dominance-subordinate interactions, antennate and being antennated, allogroom and being allogroomed, solicit and being solicited, snatch and lose food, liquid and building material, was designated as total interactions.

Each session of behavioural observations lasted for five minutes, and was followed by a one-minute break. Sampling methods consisted of instantaneous scans and all occurrences sessions (AOS), which were randomly intermingled. We recorded behavioural states of all individuals in the scans, and every occurrence of a set of selected behaviours in the AOS (Gadagkar, 2001). Each colony was observed for 5–8 h in the presence of the queen (day 1) and for another 5–8 h in the absence of the queen on the following day (day 2).

4. The realization time (t_r)

As mentioned above, one individual (referred to as PQ) becomes very aggressive as soon as the queen is removed from a colony of *R. marginata*. We conclude from this altered behaviour of the PQ that she has perceived the absence of the queen. It is difficult to determine precisely when the PQ perceives the absence of the queen because this would depend on demonstrating a significant difference in the rate of dominance behaviour shown by the PQ. Such demonstration would require observational data with sufficient sample sizes. With our sampling strategy, we require at least 30 min of observational data to calculate the rate of dominance behaviour. Hence we compare the rate of dominance behaviour shown by the PQ in the first 30 min after queen removal with her rate of dominance behaviour in the presence of the queen. If the rate after queen removal is significantly greater than the rate in the presence of the queen, we can conclude that the PQ has perceived the absence of the queen within 30 min of queen removal. Using data from 50 colonies, we calculate that the

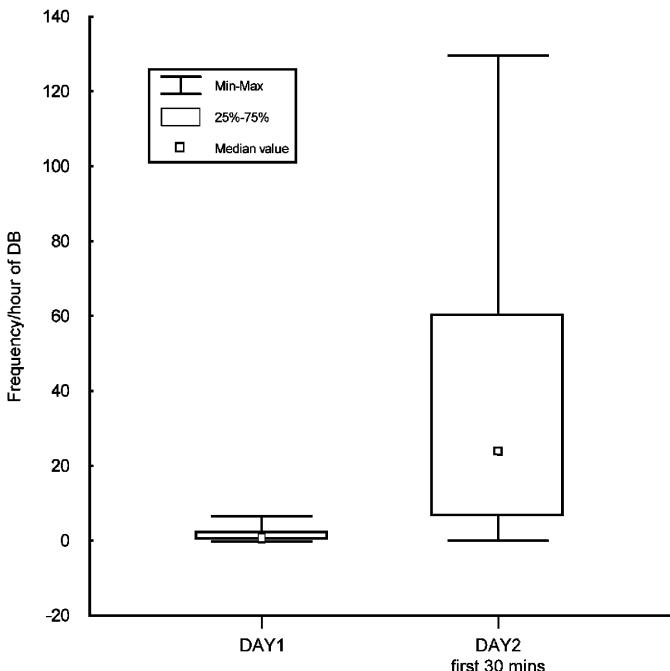


Fig. 1. Frequency per hour of dominance behaviour shown by the PQ within 30 min of queen removal, as compared to her own levels of dominance in the queenright colony. (Wilcoxon matched pairs signed ranks test; $N = 50$, $T = 38$, $Z = 5.49$, $p = 0.000$).

PQ shows dominance behaviour at the rate of 1.50 ± 1.69 per h in the presence of the queen and at the rate of 33.56 ± 34.36 per h in the first 30 min after queen removal (Fig. 1). These rates are highly significantly different from each other (Wilcoxon matched pairs signed ranks test; $N = 50$, $T = 38$, $Z = 5.49$, $p = 4.05 \times 10^{-8}$). Thus we conclude that the PQ takes no more than 30 min (probably much less) to realize the absence of the queen and we therefore set the realization time (t_r) to be a maximum of 30 min.

5. Pheromone transfer by direct interactions?

In this section we consider the possibility that the queen transfers her pheromone to the PQ by means of direct physical interaction with her. In 26 out of 50 colonies the PQ did not interact with the queen of her colony even once in the 5 h of observation on day 1. This means that in these colonies the PQ's did not receive the queen pheromone for at least 5 h. Nevertheless they did not behave as if the queen was absent. We conclude therefore that the queen pheromone cannot have a decay time of less than 300 min. Hence we set the decay time for the queen signal to be a minimum of 300 min.

Pooling data from the 26 colonies in which PQ did not interact with her queen even once in 5 h (rate of interaction set to zero) and the remaining 24 colonies in which PQ had one or more interactions with her queen, we calculate the average rate of interaction of PQ's with their queens to be 0.28 ± 0.39 per h (Fig. 2), which corresponds on an average of one interaction in every 213 min. Because we might have

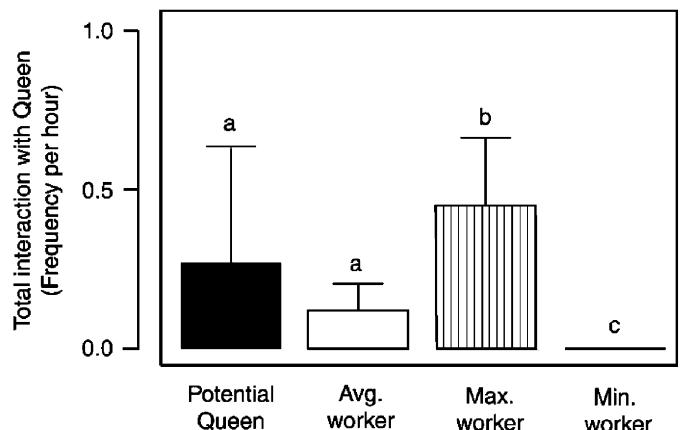


Fig. 2. Mean and standard deviation of the frequency per hour of total interaction with the queen, by potential queen (filled bars), average worker (empty bars) (defined as the average value for all workers, other than the potential queen), Max worker (vertical bars) (defined as the wasp which shows the highest rate) and Min worker (horizontal bars) (defined as the wasp which shows the lowest rate) in the queen removal experiment ($n = 50$ colonies). Comparisons are by Wilcoxon matched-pairs signed-ranks test; bars with different letters are significantly different ($p < 0.05$) from each other.

removed the queen anywhere from just after an interaction with the PQ to just before the next interaction, we estimate that the average age of the pheromone with the PQ at any instant of time (t_a) is equal to $213/2 = 106.5$ min. From the decay time of at least 300 min estimated above and the average age of the pheromone with the PQ estimated here of 106.5 min, Eq. (1) yields a minimum realization time (t_r) of $300 - 106.5 = 193.5$ min. However we have experimentally determined the realization time to be a maximum of 30 min. It is therefore clear that the queen could not possibly be using direct physical interactions to transmit her pheromone to the PQ.

6. Pheromone transfer by relay?

Information transfer through networks has been studied for some years in social insect colonies, especially in ants (Adler and Gordon, 1992; Gordon, 1996, 2003; Gordon et al., 1993; Gordon and Mehdiabadi, 1999; Pacala et al., 1996). The focus of these studies has primarily been interactions between workers in the context of task allocation and regulation of worker activity in the colony. Such models, designed to study information transfer, are based on the rates of direct physical interactions between the workers. However, in our system, the direct interactions do not seem to be responsible for information transfer pertaining to the queen. So we build a model to study the mechanism of indirect transmission—or the transmission of the pheromone through a chain of physical contacts between the members of the colony. We used the Dijkstra's algorithm (Dijkstra, 1959) to determine the fastest possible path of interaction between the queen and PQ and estimate the average age of queen signal with the PQ, given that the signal can also be transmitted indirectly.

We then used data from the 50 colonies mentioned above to examine if indirect transmission can account for the emergence of the PQ within half an hour of queen removal.

7. Dijkstra's algorithm

By the medium of physical inter-individual interactions, the queen's pheromone could reach the PQ either directly from the queen or indirectly through other workers who have interacted with the queen. We are interested in the shortest of all such possible paths, considering both direct and indirect routes. Applying Dijkstra's algorithm to experimental data on rates of interaction between every pair of individuals in each colony, we calculate such a shortest path and for convenience, we refer to this as the relay path.

We assume that the direct interaction rate for a pair of wasps is fixed. We also assume that the queen continuously generates fresh signal (i.e., the signal does not age with the queen), and that the time taken for the actual physical contact resulting in signal transfer between any two wasps is negligible. Let the queen and wasp A interact with each other directly every x minutes (Fig. 3). Then at any given instant of time, the previous interaction between queen and A would have taken place on an average, $x/2$ min earlier, and hence the average age of the pheromone with A is $x/2$ min. This is defined to be the average direct transmission time between queen and A .

Suppose also that wasps A and B interact every y min. Then as above, at any given instant of time, the previous interaction between A and B would have taken place on an average, $y/2$ min earlier. Assuming that A 's interactions with B and with the queen are stochastically independent events, the signal transmitted by A is on an average, $x/2$ min old when B interacts with A . Hence the average age of the signal with B is $x/2 + y/2$ min. We call this the average indirect transmission time between queen and B through A . Note that the indirect transmission time adds up for every link in a chain of interactions involved in transmitting the signal.

We define the relay transmission time between the queen and a wasp as the minimum of the average direct transmission time between them and the average indirect transmission times corresponding to all possible chains of contacts between the queen and that wasp. Hence the

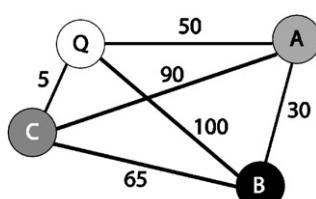


Fig. 3. A model to explain relay mechanism. The weights on the edges are the direct transmission times.

relay transmission time gives the average minimum age of the queen signal with that wasp. We use a graph-theoretic approach to calculate this quantity. Consider an undirected weighted graph G associated with each colony of wasps in which every node corresponds to an individual wasp. Let the weight of an edge connecting two nodes be the average direct transmission time between the corresponding pair of individuals. We claim that the relay transmission time between the queen (corresponding to node Q in G) and wasp A (corresponding to node A in G) is exactly equal to the weight of the minimum weighted path from Q to A in graph G . For example, the minimum weighted path between Q and B in the graph shown in Fig. 3 is $Q-C-B$, i.e., Q and C interact every 10 min and hence have a direct transmission time of 5 min. Similarly, the direct transmission time between C and B is 65 min. Hence the relay transmission time between Q and B is given by the weight of the path, which is 70 min. Notice that the direct transmission time between Q and A is 50 min, which is less than the shortest indirect transmission time (via C), which is 95 min. In such cases we set the relay transmission time to be equal to the direct transmission time.

The shortest path between any pair of nodes in an undirected graph with all edges having positive weights can be determined by the Dijkstra's algorithm. This is a greedy algorithm since it determines the shortest path by selecting, at each step, the node having the shortest distance from among all the remaining nodes. The algorithm is implemented by maintaining a subset S of the set of all the nodes N . S represents the set of visited nodes at each stage, and initially consists of only the source node Q . The weight of the edge connecting any node W to a node V is defined to be the distance of W from V , $\text{dist}(W, V)$. If there is no such edge, the value of $\text{dist}(W, V)$ is set to infinity. At each step of the algorithm, the node V with minimum distance from Q is added to the set S , and the distance of any node W not in S is updated to be the minimum of these two quantities—the weight of the already known path to it and the weight of the path through node V . Thus, $\text{dist}(Q, W)$ is now set to:

$$\min \{\text{dist}(Q, W), \text{dist}(Q, V) + \text{dist}(V, W)\}.$$

For any vertex V in S , $\text{dist}(Q, V)$ then equals the weight of the shortest path from Q to V . The algorithm terminates when $S = N$.

We implement the algorithm (using a program written in MATLAB 6.0.0.8) by calculating the relay transmission time matrix R from the direct transmission time matrix D . The (i, j) th entry of D is the average direct transmission time between wasps i and j . If no direct interaction takes place, this value is set to infinity. The (i, j) th entry of R is the relay transmission time between i and j , and is calculated from the Dijkstra's algorithm. Both these square matrices are hence symmetric, positive valued and with zeros on the diagonal.

8. The advantage of relay over direct interaction?

Before we considered whether interaction by relay is adequate to explain the rapidity of the emergence of the PQ after queen removal, we studied some properties of the relay mechanism. We did this by examining whether relay transmission offers an advantage over direct transmission, and, whether and how this advantage varies with colony size and interaction rates. The parameters we considered were, (i) the mean time taken to transmit information between any two individuals directly (which is the mean of the upper triangular part U of the direct transmission matrix D), (ii) the variability in this time, expressed as coefficient of variation (CV, which is the ratio of the standard deviation of U to its mean), and (iii) the size of the colony (expressed as the number of individuals in it). The questions above were then answered by studying the relay transmission times for random D matrices generated with the prescribed parameters, the entries of D being

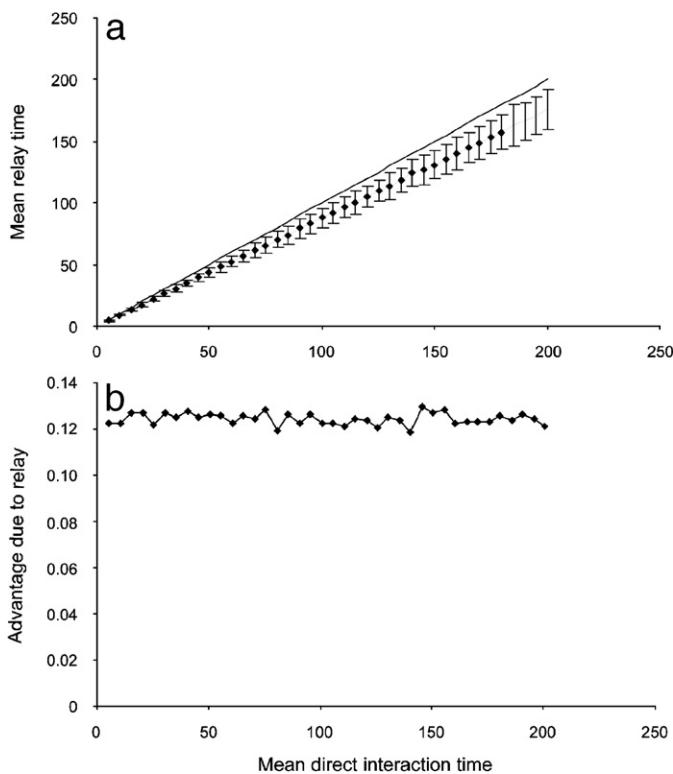


Fig. 4. (a) Variation in mean relay transmission time with mean of the direct transmission time. Mean relay time in this graph is the average of means of R matrices corresponding to 100 randomly generated D matrices with fixed parameters (CV = 0.4, colony size = 40). The standard deviation of the 100 mean values is used to plot the 95% normal confidence interval around the average mean obtained, and to hence check if the relay transmission time is significantly different from the direct transmission time. (b) Proportionate advantage due to relay when the mean of direct transmission time varies. The advantage of relay over direct transmission is given by the mean of the difference between D and R matrices as a ratio of the mean direct transmission time ($D-R/D$). It can be seen that the advantage due to relay remains nearly constant at 12.5% (p -value of 0.403 for a test of significance of difference of the slope of the regression line from 0).

randomly generated from a normal distribution with the specified mean and CV.

The mean relay transmission time increased with but was always significantly less than the mean direct transmission time (Fig. 4(a)). However the proportional advantage of relay over direct transmission ($(D-R)/D$) did not vary with increase in mean direct transmission time (Fig. 4(b)). For very low values of CV, there was no significant advantage of relay over direct transmission but when the CV of the direct transmission time increased above about 0.3, the mean relay transmission time became significantly lower than the mean direct transmission time (Fig. 5(a)). In this case the proportional advantage of relay over direct transmission steadily increased with increase in CV of the direct transmission time (Fig. 5(b)). For all values of colony size upward of 20 individuals, relay was always faster than direct transmission (Fig. 6(a)) and the proportional advantage of relay over direct transmission steadily increased with colony size (Fig. 6(b)).

In summary, the improvement in the transmission time due to relay was 12.5% when colony size was kept constant at 40, CV at 0.4 and the mean of D was varied from 5 to

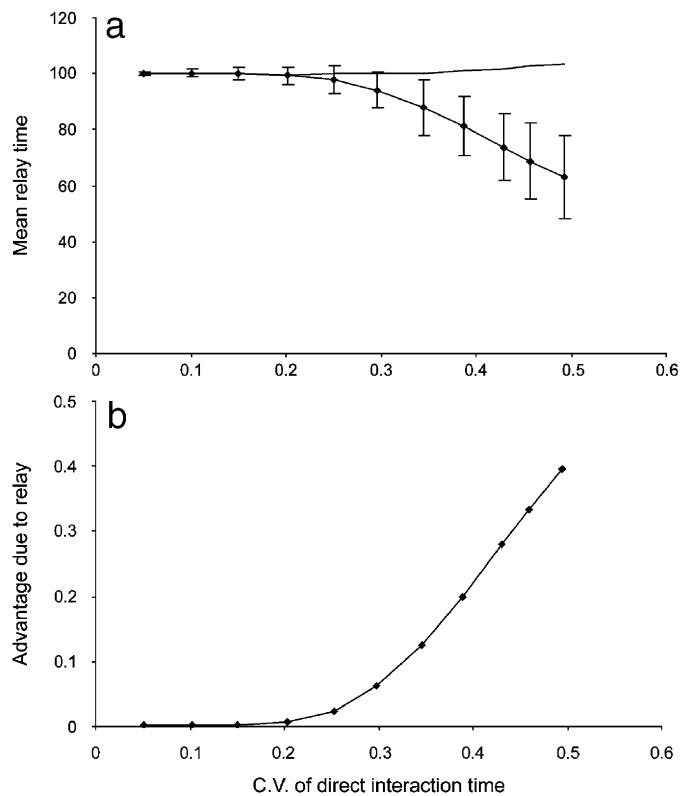


Fig. 5. (a) Variation in mean relay transmission time with CV of the direct transmission time. Random D matrices were generated with mean = 100 and colony size = 40. The standard deviation of the 100 mean values of R were used to plot the 95% normal confidence interval around the average mean obtained, and to hence check if the relay transmission time is significantly different from the direct transmission time. (b) Proportionate advantage due to relay when the CV of direct transmission time varies. The advantage of relay over direct transmission is given by the mean of the difference between D and R matrices as a ratio of the mean direct transmission time ($D-R/D$).

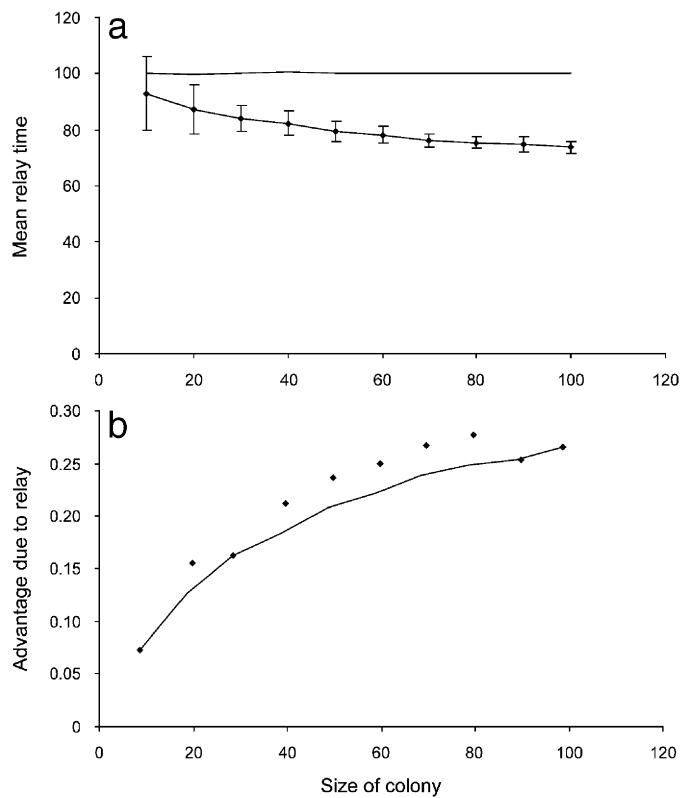


Fig. 6. (a) Variation in mean relay transmission time with size of the colony. Random D matrices were generated with mean = 100 and $CV = 0.4$. The average and confidence interval of mean relay transmission time were calculated over 30 random D matrices generated for each colony size value. (b) Proportionate advantage due to relay when the colony size varies. The advantage of relay over direct transmission is given by the mean of the difference between D and R matrices as a ratio of the mean direct transmission time ($D-R/D$).

200. When the CV was varied from 0.005 to 0.493, colony size was kept at 40 and mean D was 100, the improvement increased from 0 to 39.3%. As the colony size increased from 10 to 100, while the CV was held constant at 0.4 and mean D was 100, the improvement increased from 6 to 26.6%.

9. Is relay transmission adequate to detect the presence of the queen in *R. marginata*?

Using data from the 50 colonies mentioned above, we computed the time interval at which a wasp makes contact with any other wasp, by dividing the total time of observation by the number of physical contacts observed between them. The average direct transmission time for a pair of wasps is then half of this time interval. This was the entry in the D matrix for the pair of wasps in question. We then calculated the R matrix from the D matrix by Dijkstra's algorithm.

In our data matrices from the 50 colonies, we found that on average, only about 30% of all animal pairs interacted directly. The mean of the direct transmission time for these pairs was 79.04 min with a CV of 0.36. About 52% of all

possible pairs interacted only indirectly and the mean relay transmission time (which in this case is identical to the mean shortest indirect transmission time, since there was no direct interaction at all), was 148.93 min with a CV 0.34. The remaining 18% of the pairs did not interact either directly or indirectly. Considering the 82% of pairs that interacted either directly or indirectly, we obtained a mean relay transmission time (i.e., the shortest of the direct and indirect transmission times) of 73.84 min with a CV 0.36. Just as 18% of the possible pairs did not interact at all, an average of about 10% of the wasps did not interact with anybody in their colony, either directly or indirectly (Table 1). Consistent with the results of the simulations, the data also showed that the mean relay transmission time increased with the mean direct transmission time and with a constant proportional advantage of relay over direct transmission. Similarly, the mean relay transmission time decreased with the CV of the direct transmission time with an increasing proportional advantage of relay over direct transmission. However the trend with variation in colony size was inconsistent with the results of the simulations. We speculate that this inconsistency may be due to simultaneous uncontrolled variation of mean and CV of the direct transmission time along with colony size in the data as opposed to the simulations where we had held the mean and CV of the direct transmission time constant while varying the colony size.

To address the question of whether relay is adequate for the PQ to detect the presence of the queen we should consider the relay transmission time for the queen-PQ pairs. Of the 50 colonies studied, the queen and the PQ interacted directly in only 24 colonies (Table 2). In these colonies the range of the relay transmission time between the queen and PQ was 25.0–125.0 min. In the remaining 26 colonies where the queen and PQ did not interact directly, the relay transmission time ranged from 54.7–340.0 min.

Table 1
Colony and interaction parameters

| Parameter | Inter colony mean | Inter colony s.d. |
|---|-------------------|-------------------|
| Colony size | 26.2 | 13.7 |
| Mean direct interaction time | 79.04 min | 23.25 min |
| CV of direct interaction time | 0.36 | 0.12 |
| Mean relay interaction time | 73.84 min | 22.11 min |
| CV of relay interaction time | 0.36 | 0.11 |
| Proportion of interactions that occur only directly | 0.30 | 0.18 |
| Proportion of interactions made possibly by relay | 0.76 | 0.22 |
| Mean of R from infinities | 148.93 min | 54.08 min |
| CV of R from infinities | 0.34 | 0.06 |
| Proportion of inert wasps | 0.10 | 0.10 |
| $(D-R)/D$ | 0.06 | 0.05 |

R = relay interaction time; D = direct interaction time; $(D-R)/D$ = advantage of relay over direct interaction time.

Table 2
Queen—PQ interactions with relay

| Parameter | Nests with finite D | Nests with infinite D | Total |
|------------------------------|-----------------------|-------------------------|-----------------|
| # of nests | 24 | 26 | 50 |
| Mean direct interaction time | 59.63 min | | 59.63 min |
| s.d. direct interaction time | 37.17 min | | 37.17 min |
| Mean relay interaction time | 51.66 min | 150.13 min | 102.86 min |
| s.d. relay interaction time | 28.77 min | 74.33 min | 74.59 min |
| Range (D) | 25–150 min | | 25 min–Infinity |
| Range (R) | 25–125 min | 54.667–340 min | 25–340 min |
| $(D-R)/D$ | 0.0819 | | |

Considering all 50 colonies together, the mean relay transmission time was 102.9 min.

Since the upper limit for the relay transmission time is 340 min, this implies that the decay time of the pheromone (t_d) is at least 340 min. Hence Eq. (1) yields a mean realization time (t_r) of at least $340 - 102.9 = 237.1$ min. Recall that we have set the upper limit of the realization time as 30 min in Section 4. Thus the present value of at least 237.1 min for the realization time is, as in the case of direct transmission of pheromone, inadequate to account for the rapidity with which the PQ realizes the absence of the queen. It is therefore clear that the queen could not possibly be using physical interactions, whether direct or indirect, to transmit her pheromone to the PQ.

10. Rub abdomen behaviour

Our experiments show that physical interactions are not sufficient for the queen in *R. marginata* to transmit her pheromone to her workers. If the pheromone is non-volatile, it is possible that the queen applies it on the nest material, and the workers perceive it through receptors on their antennae and/or legs. A characteristic behaviour, performed mostly by the queens of *R. marginata*, which we refer to as ‘rub abdomen’ behaviour, can potentially be used to apply the pheromone on the nest surface if it is produced by a gland that opens on the ventral surface of the abdomen near its tip. An individual performing this behaviour rubs the ventral surface of the tip of her abdomen on the nest surface while walking on the nest. In this section we take a closer look at the rub abdomen behaviour (RA), and consider the possibility that the queen uses this behaviour to apply her pheromone on the nest surface.

Focal behaviour sampling for RA was performed on 8 post-emergence nests of *R. marginata*. Observations were done in 4 sessions of 2.5 h, between 8 am and 6 pm. Two alternate sessions were carried out on each day, thereby covering 10 h in two days. A nest map was made prior to observations. Each session of 2.5 h was divided into 50 sessions of 5 min each. The nest was observed, and whenever an act of RA was seen, the path taken by the individual during the behaviour was traced out on the nest map, and the identity of the individual performing RA,

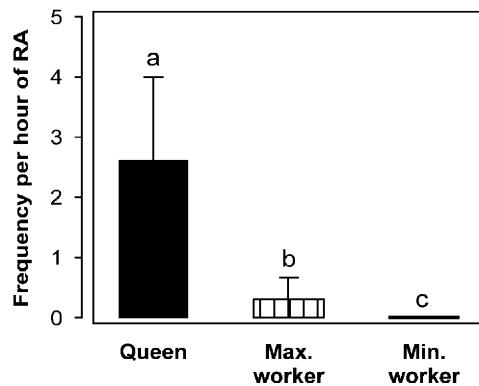


Fig. 7. Mean and standard deviation of the frequency per hour of rub abdomen behaviour shown by the queen (filled bar), Max worker (vertical bars) (defined as the wasp which shows the highest rate) and Min worker (defined as the wasp which shows the lowest rate) in the RA focal behaviour experiment ($n = 8$ colonies). Comparisons are by Wilcoxon matched-pairs signed-ranks test; bars with different letters are significantly different ($p < 0.05$) from each other.

along with the starting time and ending time of the behaviour was recorded.

In 3 out of 8 nests, only the queen showed RA. In the rest of the 5 nests, the queen showed the highest rates of RA. On an average, the queen’s frequency of RA was 2.61 ± 1.4 per h, which was significantly higher than the 0.31 ± 0.36 per h showed by workers showing the highest rates of RA (Wilcoxon matched pairs signed ranks test, $p = 0.018$) (Fig. 7). Thus the queen performs RA once in about 23 min. If she uses this behaviour to apply her pheromone on the nest surface, then she would be applying a fresh coat on an average in every 23 min. Thus if the queen is removed, the PQ should realize her absence in about 23 min. We have calculated the realization time to be a maximum of 30 min. So, it seems reasonable that the queen of *R. marginata* could use the rub abdomen behaviour to apply her pheromone on the nest material.

11. Discussion

We have shown that in *R. marginata*, the emergence of the potential queen takes less than half an hour, which implies that t_r is at most 30 min.

We have also shown that the average age of queen signal available with the PQ by means of direct interactions with the queen is 111 min. However, since the queen and the PQ do not interact for 300 min in some nests, the decay time of the pheromone should be at least 300 min, thus implying that the realization time should be at least 189 min. This is not compatible with the calculated realization time of at least 30 min. So we considered the possibility of transmission of the queen signal by means of a relay mechanism, i.e. through the fastest path out of the direct as well as indirect interactions.

Our model showed that such a relay mechanism could have an advantage of up to 39.31% over direct interactions. Our calculations showed that about 70.02% of the possible pairs of individuals do not interact at all directly, but about 75.74% of them interact through relay. This again shows that relay has an advantage over direct interactions in allowing a large proportion of individuals to interact with each other. The mean advantage due to relay was seen to be $5.75 \pm 4.93\%$ in 50 colonies of sizes ranging from 8–76. When we looked at interactions between the queen and the PQ, we saw that in over half the colonies the PQ never interacted directly with the queen, but in all of them, such interactions were possible through relay. The mean advantage due to relay for queen-PQ interactions was 8.19%.

However, we see that the mean relay transmission time for queen-PQ interactions is 102.86 min, with some nests having a relay transmission time as high as 340 min. This implies that the decay time is at least 340 min, and hence the mean realization time at least $340 - 102.86 = 237.14$ min. This is also incompatible with the observed realization time is at least 30 min, hence we rule out the possibility of the queen using relay interactions to transmit her signal on the colony.

Next we considered the possibility that the queen might be applying her pheromone on the nest material. A particular behaviour shown primarily by the queens in *R. marginata* seemed likely to be used for such a purpose. We call this the rub abdomen behaviour. Focal behaviour sampling for RA showed that the queen rubs her abdomen on the nest once in about 23 min. This implies that the queen could be applying a fresh coat of her pheromone on the nest material once in about 23 min, and thus, if the queen is removed, the PQ can perceive her absence at most in about half an hour.

Queens and workers have been shown to have different cuticular hydrocarbon profiles in many species of ants and wasps, and they have been thought to be involved in queen recognition in these species (Bonavita-Cougourdan et al., 1991; Cuvillier-Hot et al., 2001; Dietemann et al., 2003; Dapporto et al., 2004; Hannonen et al., 2002; Heinze et al., 2002; Liebig et al., 2000; Monnin et al., 1998, 2002; Peeters et al., 1999; Sledge et al., 2001, 2004). In *R. marginata*, the low rates of interaction of the workers with the queen and the rapid emergence of the potential queen make it difficult for us to postulate the involvement of a contact pheromone

in the process of queen recognition, which makes it unlikely that workers detect their queens by directly perceiving their cuticular hydrocarbon profiles. On the other hand, we have evidence that the queen does not use a volatile pheromone to signal her presence to the workers (Suman et al., under review). The rub abdomen behaviour of the queen suggests that she might be applying a chemical on the nest material that can be perceived by the workers as they move on the nest surface. This chemical would have a decay time, and once it decays after the queen is removed, the potential queen would emerge. Work done on *Polistes dominulus* (Dani et al., 1994, 1996) has shown that the contents of the Dufour's gland is similar to the cuticular lipids of these wasps; and the authors have postulated the Dufour's gland as the source of the cuticular lipids, which are known to be correlated with the ovarian status of the wasps (Bonavita-Cougourdan et al., 1991; Dapporto et al., 2004; Sledge et al., 2001, 2004). It is possible that in *R. marginata*, the queen applies the contents of her Dufour's gland on the nest surface, which would explain the non-volatile nature of the queen pheromone, as well as the rapid emergence of the potential queen in spite of the low rates of interaction between the queen and the workers.

Thus we conclude that the queen of *R. marginata* does not use any kind of physical interactions, direct or indirect, to communicate her presence and reproductive status to her workers. However, she perhaps uses the rub abdomen behaviour to apply her pheromone on the nest material. If the queen applies her pheromone on the nest surface by means of the rub abdomen behaviour, then it is likely that the pheromone is produced by a gland opening on the ventral surface of the abdomen near its tip, which is the part that is rubbed on the nest surface. We postulate the source of the queen pheromone to be the Dufour's gland, which produces non-volatile lipids. We are now continuing experiments to investigate the nature of both the cuticular hydrocarbons and the Dufour's gland contents of *R. marginata*, and their correlations with the ovarian and behavioural profiles of the wasps.

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