In this issue

Non-dominant queens

Social organization in insects is an important model in understanding behavioural instincts and relationships. Primi-

tive eusocials usually lack queen–worker dimorphism (e.g. wasps); whereas advanced eusocials usually exhibit a queen–worker dimorphism in large colony sizes (e.g. ants). Sumana and Gadagkar (**page 1464**) describe the social behaviour of their favourite insect, *Ropalidia marginata*, a primitive eusocial. Surprisingly, *R. marginata* queens are docile, and can serve as a model organism to understand the differences between physical inhibition and chemical pheromonal regulation determining the mechanism of worker reproduction, as it occurs during evolutionary transition from the primitive to the advanced eusocial.

duction while the remaining female wasps in the colony function as sterile workers. Unlike in other species however, *R. marginata* queens are strikingly docile individuals who show little or no physical dominance. When such a behaviourally docile queen is removed from a colony, one of the remaining individuals becomes extremely aggressive, and is known from previous work, to go on to become the next queen if the original queen is not returned. When the original queen is returned after a day's absence, she re-establishes herself as the queen and she usually manages to do so with little or no aggression. We hypothesize that *R. marginata* queens use dominance behaviour to suppress worker reproduction in the beginning, and that they use pheromones to regulate worker reproduction once they establish themselves and start laying eggs. If this hypothesis is correct, *R. marginata* would be an ideal model system to study the possible evolutionary transition from physical inhibition to chemical regulation of worker reproduction, and the transition from primitively eusocial to highly eusocial in general.

MANY insect species live in societies of varying degrees of complexity. To differentiate relatively loose social aggregations from true societies and to concentrate on the highest levels of social evolution, attention is usually focused on a subset of social species which are said to have achieved eusociality. Eusocial species are defined as those that exhibit overlap of generations, cooperative brood care and reproductive caste differentiation. Among these, primitively eusocial species are characterized by small colony sizes, absence of queen–worker dimorphism and physical inhibition of worker reproduction by the queens (e.g. many species of wasps and bees). On the other hand, highly or advanced eusocial species are characterized by large colony sizes, clear-cut queen–worker dimorphism and chemical (pheromonal) regulation of worker reproduction by queens (e.g. ants, termites and honeybees)[1].

Ropalidia marginata (Lep.) (Hymenoptera: Vespidae), widely distributed in Peninsular India has been considered a primitively eusocial species because of the absence of morphological differentiation between queens and workers and because many, if not all, female wasps can mate, develop their ovaries and function as solitary nest foundresses or as queens of multiple foundress nests[2]. There is growing evidence that *R. marginata* is different from other primitively eusocial species. In all other primitively eusocial species studied so far, queens are the most behaviourally dominant and active individuals and are thus expected to be capable of using physical aggression (dominance behaviour) to suppress worker reproduction[3,4]. In contrast, an *R. marginata* queen has been described as a behaviourally non-dominant, docile sitter, who cannot possibly inhibit worker reproduction by physical aggression and who probably uses pheromones to do so[2,5–7]. In

Ropalidia marginata – a primitively eusocial wasp society headed by behaviourally non-dominant queens

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Ropalidia marginata is a primitively eusocial polistine wasp widely distributed in peninsular India. As in most other primitively eusocial insects studied so far, colonies are headed by a single queen who monopolizes repro-

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view of this very unusual nature of *R. marginata*, here we systematically document and contrast the dominance behaviour of the queen with those of her workers and also of the workers themselves, in the presence and absence of the queen.

The results described in this paper have been obtained from queen removal experiments conducted on 12 post-emergence colonies of *R. marginata*. Each experiment consisted of three consecutive days of behavioural observations. On day 1, the colonies were observed in their un-manipulated state and were designated as being in the queen-right stage. On day 2, the same colonies were observed starting within 30 min after their queens had been removed. Day 2 was designated as the queen determination stage because the process of the emergence of a new queen begins immediately after the original queen is removed (see below). Thus during the queen determination stage we had the opportunity to observe the behaviour of workers who were in the process of becoming new queens. On day 3, the same colonies were observed again after the original queen was reintroduced. Day 3 was designated as the queen re-establishment stage because the returned queen always re-established herself as the queen of the colony (see below). The colonies used in this study were located in 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 were transplanted to the vespiary[2] at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India, between February and July 2000. Wasps from these nests foraged from natural sources and were free to leave their nests or join other nests. All individuals in each colony were uniquely marked with small spots of quick drying paints of different colours. Each behavioural observation session lasted for 5 min and was followed by a 1 min break before the beginning of the next session. Observations were
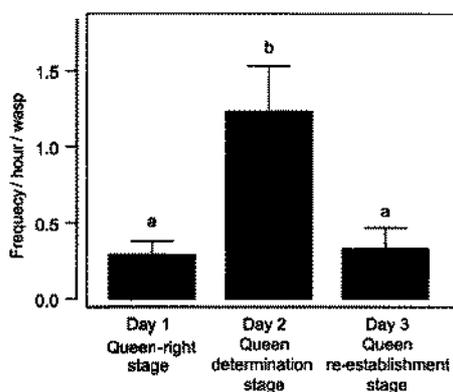
of two kinds: instantaneous scans, to record the behavioural state of each individual and 'all   s-sions where every performance of a set of selected behaviours by every individual was recorded. The scans and all occurrence sessions were randomly intermingled. Eight hours of observations were carried out on each of the 3 consecutive days, by the same observer, between 0800 and 1800 h (in 3 sessions of 3, 3 and 2 h each), yielding 24 h of data per colony, with 20 scans and 60 all occurrence sessions per day.

As reported earlier[2], dominance behaviours shown by *R. marginata* wasps consisted of aggressive biting, attack, being offered liquid, chase, crash, hold another individual in mouth, nibble, peck and sit on another individual. The sum of all the dominant behaviours listed above was used to compute the frequency of dominance behaviour per hour, for each individual. From these data, dominance hierarchies were constructed using an index of dominance[2,8] and individuals were arranged in descending order of their values of dominance index. The individual with the highest value was assigned rank 1 and the remaining individuals were assigned consecutively increasing ranks. To correct for variation in the number of
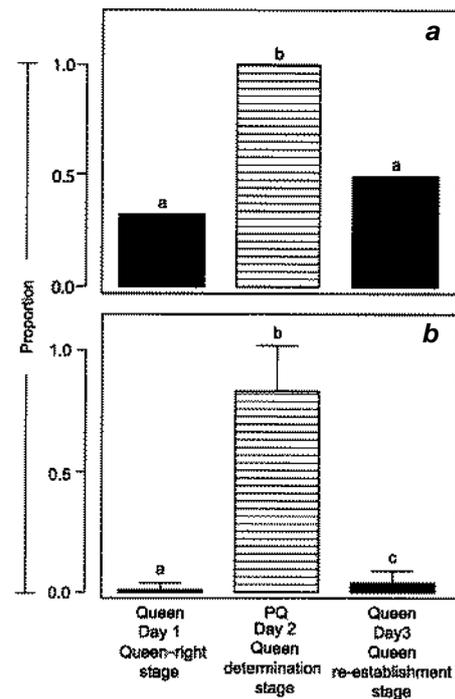

Figure 2. *a*, Proportion of colonies in which the queen/potential queen participated in at least one act of dominance behaviour in the queen-right stage (day 1), queen determination stage (day 2) and queen re-establishment stage (day 3). Bars carrying different alphabets represent significantly different values (*G* test of proportions, *P* < 0.05). *b*, Mean and standard deviation of the proportion of dominance behaviour contributed by the queen in queen-right stage (day 1), and the potential queen in the determination stage (day 2) and the queen in the queen re-establishment stage (day 3). Bars carrying different alphabets represent significantly different values (Wilcoxon matched-pairs signed-ranks test, *P* < 0.05).

Figure 1. Mean and standard deviation of the frequency per hour per wasp of dominance behaviour observed in 12 colonies of *R. marginata*, on day 1 (queen-right stage), day 2 (queen determination stage) and day 3 (queen re-establishment stage). Bars carrying different alphabets represent significantly different values (Wilcoxon matched-pairs signed-ranks test *P* < 0.05).

individuals in different colonies, each individual's dominance rank was normalized as follows: Normalized rank = ((rank × 100)/number of individuals in the colony). Thus, individuals in the colony were ranked from 1 to 100, irrespective of the actual number of wasps in the colony; the top ranking individuals obtained normalized ranks close to 1, while low ranking individuals obtained normalized ranks close to 100. The frequency of dominance behaviour per hour per wasp was calculated for each colony. Frequency of dominance behaviour per hour was also computed separately for the following individuals: 'Queen' – the sole egg layer in the colony. 'Potential queen' – the worker who became very aggressive when the queen was removed from the colony, and who is known to go on to become the next queen of the colony, if the original queen is not returned[2,9]. 'Max. worker' – that worker, other than the potential queen, who showed the highest value of dominance behaviour. 'Min. worker' – that worker, other than potential queen, who showed the lowest value of dominance behaviour. 'Average worker' – mean value of dominance behaviour for all workers, including max. worker and min. worker, but excluding the queen and potential queen.

Normal colonies of *R. marginata* with established queens (queen-right stage) were characterized by relatively low levels of dominance behaviour. On day 1 of the experiment, we recorded 0.29 ± 0.09 acts of dominance behaviour per hour per wasp (Figure 1). Not only was this frequency of dominance behaviour in the colony low, but the queens' contribution to it was also very low indeed. In 8 out of 12 colonies, the queens did not show a single act of dominance behaviour during the 8 h of observation (Figure 2 *a*). Considering all the 12 colonies, the queens were responsible for only 0.01 ± 0.03 proportion of the total dominance behaviour shown on day 1 (Figure 2 *b*). The queens' rate of dominance behaviour was significantly lower than that of an average worker in the colony and comparable to that of a min. worker; it was much lower than that of the max. worker and that of the individual who would become the next queen (potential queen, see below) (Figure 3 *b*). When the individuals in the colonies were ranked in a dominance hierarchy (rank 1 = most dominant), the queen did not occupy rank 1 in any colony. The queens' ranks ranged from 2 to 30.5, in colonies which had 13 to 46 wasps (Table 1). Considering all 12 colonies together, the queens obtained normalized ranks of 48.3 ± 19.7 showing that, on average, the queens were located at about the middle of the dominance hierarchies of their colonies (data on normalized ranks, not shown).

Removal of the queen from *R. marginata* colonies resulted in immediate, dramatic changes. The number of acts of dominance behaviour increased within minutes of removing the queen and, when measured over the entire second day of the experiment (8 h of observation in the queen determination stage), there was a 4.2 fold increase

in the dominance behaviour seen in the colony as the whole (Figure 1). Most of this increased level of dominance behaviour was because of one individual who, upon removal of the queen, became extremely aggressive and who we know from previous experiments, will go on to become the next queen of the colony, if the original queen is not replaced[2]. We therefore refer to this individual as the potential queen. In the 12 colonies studied here, such potential queens became obvious in every colony in less than an hour of the removal of the queen. Thus, not only did the potential queens contribute to the dominance behaviours of their colonies in all cases (Figure 2 *a*), but the potential queen accounted for a very high proportion (0.83 ± 0.19) of the total dominance behaviour seen in the colony (Figure 2 *b*). It should be mentioned that the identity of the potential queen was not obvious on day 1 in any of the 12 colonies. Having identified the potential queen on day 2, we therefore examined her behaviour on day 1, retrospectively. On an average, potential queens stepped up their levels of dominance behaviour on day 2, 17.7 fold relative to their own levels on day 1. This should be considered in light of the fact that average workers, max. workers and min. workers did not significantly alter their rates of dominance behaviours on day 2, relative to day 1 (Figure 3 *a*, *b*). Perhaps even more striking is the fact that in all 12 colonies, potential queens occupied rank 1 in the dominance hierarchies of their colonies on day 2 (Table 1).

Upon the return of the queen on day 3 the colonies returned to nearly the same state as they were on day 1, as dramatically as they had changed on day 2. The rate of dominance behaviour per hour per wasp on day 3 was significantly lower than the corresponding rate on day 2 and was statistically indistinguishable from the corresponding rates on day 1 (Figure 1). Similar to day 1, the queens' contribution to the dominance behaviour of the

Table 1. Dominance rank of the queen in queen-right stage (day 1), potential queen (PQ) in the queen determination stage (day 2) and the queen in the queen re-establishment stage (day 3) in 12 colonies of *R. marginata*. The ranks of the queens on day 3 are not significantly different from those on day 1 (Wilcoxon matched-pairs signed-ranks test, $P < 0.05$)

Colony code	No. of wasps	Ranks of queens on day 1	Ranks of PQs on day 2	Ranks of queens on day 3
V248	17	8.0	1	6.0
V260	32	14.5	1	15.0
V262A	13	4.5	1	2.0
V262	13	8.0	1	1.0
V267	14	9.0	1	5.0
V268	33	17.0	1	17.0
V269	33	2.0	1	10.0
V270	25	12.5	1	7.0
V272	35	30.5	1	5.0
V273	46	22.0	1	26.0
V276	14	7.5	1	3.5
V277	16	5.0	1	1.0

colony was small (although statistically significantly more than on day 1, Figure 2 *b*). In 6 out of 12 colonies, queens re-established themselves without showing a single act of dominance behaviour (in the remaining six colonies, queens re-established themselves with small number of dominance behaviours). This was not significantly different from the corresponding situation on day 1 when the queen did not show any dominance behaviour in 8 out of 12 colonies (Figure 2 *a*). The phenomenon of the queens not requiring to show a single act of dominance on day 3 is far more significant than their not showing no dominance on day 1. This is because, on day 3, queens re-establish themselves in colonies from which they have been absent for a whole day (note that queens usually do not leave their colonies even for a few minutes) and that too in the presence of potential queens who have stepped-up their own levels of dominance behaviour 17.7 fold relative to their own levels on day 1 and 269 fold relative to the queens' level of dominance behaviour on day 1. It maybe mentioned that when queens re-establish themselves on day 3, they re-occupy approximately the same middle and low ranks in the dominance hierarchies, that they did on day 1; the ranks of the queens on day 3 were statistically indistinguishable from that on day 1 (Table 1). On day 2, queens obtained normalized ranks of 34.71 ± 20.61 which was indistinguishable from their normalized ranks in the queen-right colony (day 1) (Wilcoxon matched-pairs signed-ranks test, $P > 0.05$) (data not shown). While there is a small (but statistically significant) increase in the level of dominance behaviour of the queen on day 3 relative to day 1 (Figure 3 *a*, *c*), much more spectacular is the sharp reduction in the dominance behaviour of the potential queen on day 3, relative to day 2 (Figure 3 *b*, *c*). Once again the average, max. and min. workers were not different on day 3, relative to days 1 and 2 (Figure 3 *a*–*c*).

We cannot entirely rule out the possibility that *R. marginata* queens use visual or vibrational cues to inhibit/ regulate worker reproduction. The absence of morphological caste differentiation and of any obvious cuticular markings or of consistent size differences between queens and workers, make the use of visual cues seem unlikely. There also appear to be no obvious special behaviours shown only by the queens that could indicate their presence in an honest way. Besides, queens in highly eusocial species are well-known to use pheromones to regulate worker reproduction. Based on these arguments and on the results reported here and in previous studies of *R. marginata*[2,3,6,7,10], our best hypothesis for the mechanism/s by which *Ropalidia marginata* queens achieve and maintain reproductive monopoly in their colonies is the following. In the beginning (queen determination stage), queens use high levels of physical aggression to suppress worker reproduction. Later, as their ovaries develop, they begin to use pheromones to signal their presence to their workers. It has been argued that while physical aggression can be used to suppress reproduction of workers against their evolutionary interest, pheromones cannot be thought of as doing the same. Pheromonal inhibition is likely to be evolutionarily unstable because workers should retaliate by developing resistance to the pheromones. Instead, pheromonal regulation of worker reproduction is more appropriately interpreted as adaptive behaviour by the workers who refrain from reproduction in response to an honest signal of the queen's presence. That queens need to rely on physical aggression in the beginning (before they start laying eggs) but can afford to stop being aggressive later (after they start laying eggs) suggests that the production of the pheromone/s that they might use, might be intimately and metabolically linked to ovarian activity itself and therein could lie its honesty. Physical inhibition of worker reproduction is more likely to be found in small colonies of primitively eusocial species while chemical regulation is more likely
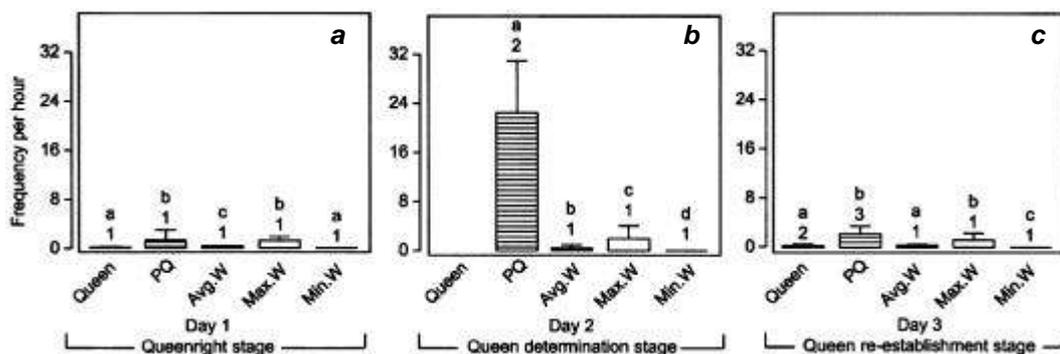

Figure 3. Mean and standard deviation of the frequency of dominance behaviour of the queen (filled bars), potential queen (horizontally striped bars), average worker (vertically striped bars), max. worker (empty bars) and min. worker (chequered bars) in the queen-right stage (day 1) (*a*), the queen determination stage (day 2) (*b*) and the queen re-establishment stage (day 3) (*c*). Bars with significantly different values within a day carry different alphabets, while different numbers represent significantly different values for comparisons across days (Wilcoxon matched- pairs signed-ranks test, $P < 0.05$).

to be found in large colonies of highly eusocial species[11–13]. According to our hypothesis, *R. marginata* is unusual in that the transition from physical inhibition to chemical regulation occurs in the same species. It follows therefore that *R. marginata* is an attractive model system to investigate the evolutionary transition from physical inhibition to chemical control (of worker reproduction) in particular and the transition from the primitively eusocial state to the highly eusocial state in general.

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