Wasp who would be queen: A comparative study of two primitively eusocial species

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***Ropalidia marginata* and *Ropalidia cyathiformis* are two Old World, primitively eusocial, tropical polistine wasps that exhibit perennial, aseasonal, indeterminate nesting cycles. Queens are periodically ousted and replaced by one of the workers, whom we refer to as the potential queen. Here we identify the characters of the potential queens by experimentally removing queens from several colonies of both species. Potential queens in *R. marginata* are unspecialized, worker-like individuals, not unique in their dominance ranks. In contrast, potential queens in *R. cyathiformis* are queen-like individuals and unique in always holding the top dominance rank among the workers. We suggest that this striking difference in the behaviour of the potential queens of the two species has to do with the very different mechanisms that queens of the two species use to suppress worker reproduction. In regard to their ovarian development, potential queens of neither species are unique and they are only one among several individuals with partially developed ovaries. This may have to do with the fact that queen replacements are frequent in tropical, aseasonal climates, making it adaptive for several individuals to be prepared to take over the position of the queen at short notice.**

Keywords: Dominance behaviour, potential queen, primitively eusocial wasps, queen succession, *Ropalidia cyathiformis*, *Ropalidia marginata*.

HYMENOPTERAN societies may be regarded as feminine monarchies because their colonies consist of one or a small number of fertile queens and a large number of sterile workers, while the males typically do not participate in social life[1]. In any monarchy, the identity of the successor to the king/queen is of obvious interest. In highly eusocial insects, where caste determination is pre-imaginal and queens and workers are morphologically differentiated, new queens are produced by an elaborate process of differential larval nourishment[2]. On the other hand, in primitively eusocial insects, where caste determination is post-imaginal, queens and workers are morphologically identical and where most individuals are totipotent, adult workers can replace their queens[1,3,4]. In newly founded colonies of primitively eusocial species that follow annual nesting cycles in temperate regions, subordinate cofoundresses may, after some period of working, challenge and replace the dominant cofoundress (queen)[5–9]. Queen replacement is not restricted to the founding stage; occasionally some of the eclosing female wasps can also take over the role of a queen if the original queen dies. These females mate with early-produced males and can lay diploid eggs[10,11]. However, eclosing daughters who stay back on their natal nests may often be unable to become replacement queens either due to intrinsic physiological limitations or due to the absence of mating opportunities at the appropriate time[12].

The most widespread opportunities for workers to kill or drive away existing queens and take over their positions are to be found in primitively eusocial species in the tropics, especially those that follow aseasonal, perennial, indeterminate nesting cycles[13–15]. *Ropalidia marginata* and *Ropalidia cyathiformis* are two examples that fit the above description. Frequent queen turnover has been observed in natural colonies of both these species. Because these colonies can be long-lived and undergo successive queen replacements, working for sometime and then replacing the existing queen may be as common or more common a strategy to become queens than founding a new colony[14]. As might be expected from the high frequency of queen replacements, there is little disruption in colony activity, foraging and brood care, following queen replacements[16,17]. *R. marginata* and *R. cyathiformis*, therefore, offer an excellent opportunity to understand the characters of workers who successfully replace their queens.

Materials and methods

Behaviour

Behavioural observations were made on 12 *R. marginata* and 12 *R. cyathiformis* post-emergence colonies during February 2000 and April 2001 (Figure 1). All adult wasps

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Figure 1. Typical nests of *Ropalidia marginata* (left) and *Ropalidia cyathiformis* (right). Photo credit: Thresiamma Varghese.

were marked with paints for individual identification. Behavioural observations consisted of instantaneous scans and 5 min 'all occurrences' sessions, randomly intermingled and spread uniformly between 0700 to 1800 h. Each colony was observed for 6–9 h on the first day of the experiment. On the day following these observations, the queen was removed from each colony. Upon removal of the queen, a single individual becomes very aggressive and is known to become the next queen if the original queen is not replaced. This individual is referred to as 'potential queen'[14].

All data come from day 1 of the experiment; removal of the queen on day 2 was only for the purpose of retrospectively identifying the potential queen among the wasps studied on day 1. From the instantaneous scans, the proportions of time spent by each wasp in the following five common behaviours were calculated: sit and groom, sit with raised antennae, walk, inspect cells and being absent from nest. From the all occurrences sessions, the frequencies per hour per wasp of the performance of the following 11 behaviours were calculated: dominance behaviour, subordinate behaviour, bring food, snatch food, lose food, feed larvae, solicit, bring building material, snatch building material, lose building material, and build the nest (for description of the behaviours, see Gadagkar[14]). For each colony, a behavioural dominance hierarchy was constructed as follows: using the dominance–subordinate interaction matrix for the colony, a dominance index was calculated for each wasp as described before[14]. All wasps in a colony were arranged in descending order of their dominance index and thus assigned dominance ranks, with the individual having the highest value of dominance index getting rank one. This analysis was carried out separately for each colony. The behavioural similarities and differences between queens, potential queens and average workers (the mean value of all workers except the potential queen, for each variable) were assessed by performing a principal components analysis. Values for each of the 16 variables for the queen, potential queen and average workers of each of the 12 colonies were fed as input variables in the principal components analysis. This analysis was done separately for each species.

Ovarian status

A separate set of 14 *R. marginata* and ten *R. cyathiformis* colonies were used to determine the ovarian status of the wasps. To do so, all individuals, including the queen, potential queen and all workers were dissected to ascertain the state of their ovarian development. The following seven measurements were made: length and width of the largest proximal oocyte, average length and average width of all the six proximal oocytes, total number of oocytes, total number of oocytes with yolk, and total number of mature oocytes. In *R. marginata*, the potential queen was identified by temporarily removing the queen because she cannot be identified in the presence of the queen (see results). In *R. cyathiformis*, the potential queen was identified by the fact that she always has rank two, next to her queen, in the dominance hierarchy of the colony as evidenced from the behavioural observations which were carried out on an independent set of colonies, as described above, before these dissection were made (see results). Values of the above seven variables for each wasp in a colony were subjected to principal components analysis. The value of the first principal component for each wasp was designated as her ovarian index. This analysis was performed separately for each colony. In each colony, the wasps were ranked according to their ovarian index with the individual having the highest ovarian index being assigned rank one.

Results

Behavioural profiles of potential queens

Potential queens of *R. marginata* and *R. cyathiformis* exhibit strikingly contrasting behaviours. In *R. marginata*, the dominance ranks of potential queens ranged from 1 to 22. In only four out of 12 colonies, the potential queen was the most high-ranking worker. In the remaining eight colonies, 1–32 individuals were more high-ranking than the potential queen. In no colony of *R. marginata* did the queen occupy the top rank and 1–29 individuals were more high-ranking than the queen. In contrast, potential queens of *R. cyathiformis* were unique in that they always occupied rank two, next to their queens, who occupied rank 1 in all the 12 colonies (Table 1).

Multivariate analysis using all 16 behaviours shows that the behavioural profiles of potential queens in *R. marginata* are more similar to the behavioural profiles of the average workers of their colonies, rather than to the behavioural profiles of the queens of their colonies (Figure 2 *a*). Potential queens of *R. cyathiformis* exhibit the opposite features. Potential queens in *R. cyathiformis* are, again, more similar to the behavioural profiles of the queens of their colonies rather than to the behavioural profiles of the average workers of their colonies (Figure 2 *b*).

Table 1. Dominance ranks of queens (Q) and potential queens (PQ)

Nest code	No. of wasps	Rank of		No. of workers with higher rank than PQ
		Q	PQ	
Ropalidia marginata				
V248	17	8	2	1
V260	33	14.5	8.5	9
V262a	13	4.5	2	1
V262	13	8	1	0
V267	14	9	1	0
V268	34	17	9	8
V269	34	2	12	10
V270	25	12.5	2	1
V272	42	30.5	1	0
V273	43	22	22	32
V276	14	7.5	4	3
V277	16	5	1	0
Ropalidia cyathiformis				
C01	15	1	2	0
C03	28	1	2	0
C07	24	1	2	0
C08	22	1	2	0
C33	18	1	2	0
C34	16	1	2	0
C38	11	1	2	0
C50	23	1	2	0
C58	18	1	2	0
C59	19	1	2	0
C60	12	1	2	0
C86	13	1	2	0

Ovarian status of potential queens

With regard to the state of development of their ovaries, potential queens of *R. marginata* and *R. cyathiformis* are not very different from each other. In both species, potential queens varied greatly in their ovarian development. In *R. marginata*, only in six out of 14 colonies, potential queens had the best-developed ovaries among the workers. In the remaining eight colonies, there were one to 15 workers with better developed ovaries than the potential queens (Table 2). Similarly, in *R. cyathiformis* only in 4–10 colonies, potential queens had the best-developed ovaries among the workers of their colonies. In the remaining seven colonies there were one to 21 workers with better-developed ovaries than the potential queens (Table 3). In all 14 colonies of *R. marginata* and in 9 out of 10 colonies of *R. cyathiformis*, queens had the best-developed ovaries and occupied rank one based on their ovarian index.

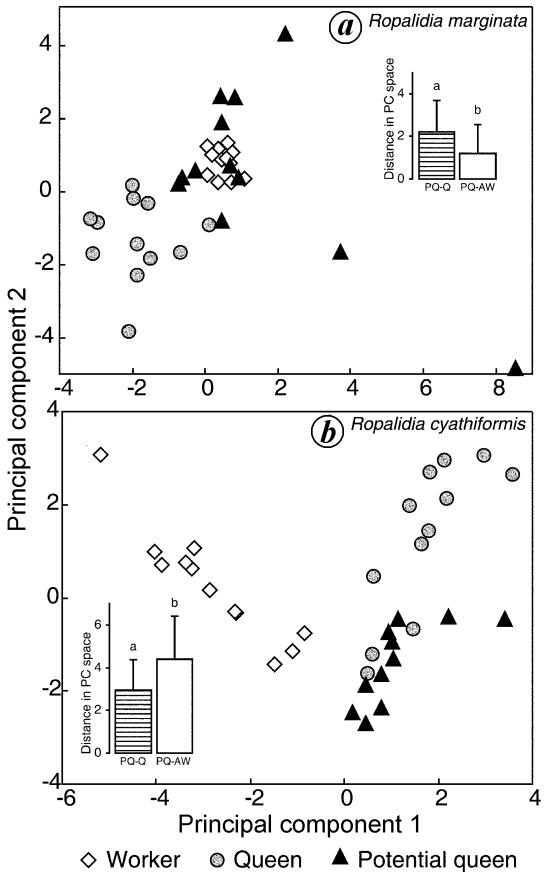

Figure 2. Relative positions of queens (grey circles), potential queens (black triangles) and average workers (white diamonds) in the space of the first two principal components (see methods). (Insets) Mean and standard deviation of the distance between potential queen and queen (PQ–Q) and potential queen and average worker (PQ–AW) in the principal component (PC) space. PQ–Q distance is significantly greater than PQ–AW distance in *R. marginata*. While the opposite is true in *R. cyathiformis* (paired *t*-test, $P < 0.05$). Data used were collected from 12 colonies of *R. marginata* and 12 colonies of *R. cyathiformis* (see subsection 'Behaviour' in Materials and Methods).

Table 2. Ovarian ranks of queens (Q) and potential queens (PQ)

Nest code	No. of wasps	Ranks of		No. of workers with higher rank than PQ
		Q	PQ	
R. marginata				
V426	9	1	3	1
V434	17	1	9.5	15
V439	17	1	4	2
V440	57	1	3	1
V444	25	1	6	4
V455	27	1	2	0
V503	27	1	2	0
V505	43	1	2	0
QR2	22	1	5	3
QR3	9	1	2	0
QR4	14	1	2	0
QR6	11	1	2	0
QR7	73	1	13	11
QR8	74	1	4	2
R. cyathiformis				
C76	10	1	6	4
C79	18	1	14.5	16
C80	23	1	13	21
C81	11	1	2	0
C85	14	1	3	1
C90	16	1	10	14
C93	16	1	2	0
C96	14	1	2	0
C97	19	4*	14.5	17
C98	9	1	2	7

*This is the only exception to the rule that the queen has the best-developed ovaries in her colony. There is evidence that this colony was undergoing a queen replacement at the time of collection.

Table 3. Number of colonies in which one or more workers had better than or equally well-developed ovaries compared to the potential queen in *R. marginata* (Rm) and *R. cyathiformis* (Rc)

Measures of ovarian development	No. of colonies with one or more workers with higher rank than PQ	
	Rm (14)	Rc (10)
Length of largest oocyte	11	5
Width of largest oocyte	10	6
Mean length of proximal oocytes	10	5
Mean width of proximal oocyte	8	5
No. of oocytes	8	6
No. of mature oocytes	14	9
No. of oocytes with yolk	12	8
Index of ovarian development	8	7

In the one remaining colony of *R. cyathiformis* the queen occupied rank four, but there is evidence that this colony was undergoing a queen replacement at the time of collection. To exclude the possibility that our results are an artefact of the ovarian index, we have also examined each individuals ovarian measurement separately. In *R. marginata*, depending on the measure of ovarian development, in 8–14 colonies potential queens were not unique among the workers in having the best-developed ovaries (Table 1). Similarly, in *R. cyathiformis*, in 5–9 colonies potential queens were not unique among the workers in having the best-developed ovaries (Table 1).

Discussion

We find that potential queens of *R. marginata* and *R. cyathiformis* are strikingly different in behaviour and remarkably similar in their ovarian status. The striking difference in behaviour of the potential queens has to be viewed in the context of an equally striking difference in the behaviour of their queens. In *R. cyathiformis*, queens are the most behaviourally active and dominant individuals and appear to use physical aggression to suppress worker reproduction[18]. It is therefore not surprising that potential queens of *R. cyathiformis* are queen-like and invariably second to the queen in their behavioural dominance hierarchies. In contrast, *R. marginata* queens are inactive and behaviourally non-dominant and cannot possibly be using physical aggression to regulate worker reproduction[18]. They probably use pheromones or some other non-behavioural mechanism to do so[19]. Besides, dominance–subordinate interactions among workers appear more likely to help in decentralized regulation of worker activities such as foraging[20,21]. It is not surprising, therefore, that potential queens of *R. marginata* do not hold high or consistent ranks in the dominance hierarchies of their colonies. Because new queens of *R. marginata* are aggressive in the beginning and only later become inactive and behaviourally non-dominant[22], it is also not surprising that in the presence of the queen, the potential queens are not queen-like individuals.

The fact that potential queens are not unique in having the best-developed ovaries among the workers and the fact that this is true of both the species, is perhaps related to the colony cycle of these tropical wasps. Because working for sometime and then driving away their queens or leaving to found their own colonies are common in aseasonal tropical climates[14], the ability to develop their ovaries should be quite common among workers. It is probably for this reason that potential queens are only one among many individuals with partially developed ovaries. Which particular individual among the subset of individuals with partially developed ovaries actually gets to replace the lost queen may depend on qualities other than ovarian development. We know that in *R. cyathiformis*, the important quality is physical aggression which allows the potential queen to occupy rank number two. The case of *R. cyathiformis* is more similar to other primitively eusocial species, where the potential queens are known to be holding high dominance ranks[4–6,23,24]. We speculate that in *R. marginata*, the ability to take over the position of the queen may have to do with ability to produce appropriate levels of pheromones, which is therefore not reflected as behavioural uniqueness.

Seniority in age has been shown to be an important determinant of potential queens in some species[9], but this does not appear to be the case either in *R. marginata*[16] or in *R. cyathiformis*[25]. Clearly, identifying the qualities of potential queen is an important component of understanding social organization and evolution of tropical wasps.

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