

**Research article**

**Social organization in experimentally assembled colonies of *Ropalidia marginata*: comparison of introduced and natal wasps**

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**Summary**

In the primitively eusocial wasp, *Ropalidia marginata* worker behaviour cannot be explained satisfactorily by the haplodiploidy hypothesis due to the existence of polyandry, serial polygyny and movement of wasps between nests, which reduce intra-colony genetic relatedness to levels lower than the value expected between a solitary foundress and her offspring. We introduced wasps eclosing from one set of colonies into other colonies separated by a distance of 10 km or more, to examine the possibility of kin recognition and task specialization under conditions of low intra-colony relatedness. Introduced wasps were readily accepted into unrelated foster colonies, where they performed most of the behaviours and tasks shown by the natal wasps. We found no evidence of kin recognition or task specialization among natal and introduced wasps. Introduced wasps sometimes became replacement queens in spite of the presence of natal wasps. Taken together with previous observations, these results lend support to the idea that factors other than genetic relatedness must play a prominent role in the evolution of worker behaviour in *Ropalidia marginata*.

**Introduction**

*Ropalidia marginata* (Lep.) (Hymenoptera, Vespidae) is a primitively eusocial polistine wasp abundantly distributed in peninsular India. New nests may be founded by one or a group of females. In multiple foundress nests only one individual is

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the egg layer (queen) at any given time while the remaining act as subordinate workers. The flexibility in the social roles that individual wasps can adopt and its perennial nesting cycle make this species an attractive model system to investigate the question of why many individuals prefer to give up reproduction and become workers rather than go off on their own to initiate single foundress nests (Gadagkar, 1991 a).

Previous work suggests that genetic asymmetries expected due to haplodiploidy (Hamilton, 1964a, b) are not likely to provide a satisfactory explanation to this question because worker-brood genetic relatedness values are lower than those expected between solitary foundresses and their offspring. This is due to polyandry (multiple mating by the queens) (Muralidharan et al., 1986; Gadakar, 1990a) and serial polygyny (successive queen replacements) (Gadagkar et al., 1991; Gadagkar et al., 1993). Besides, a recent study of newly founded nests showed that there is considerable movement of wasps between nests. There is evidence that at least 16 of the 145 newly initiated nests consisted of individuals from two or more source nests, at least three nests consisted of individuals from three or more source nests and at least one nest consisted of individuals from four or more source nests (Shakarad and Gadagkar, 1995). These are likely to be underestimates because an additional 49 of the 145 nests received at least one joiner, but the source of these wasps was not known.

These observations raise the question of how cooperation and division of labour are maintained by natural selection in colonies with low genetic relatedness. We created mixed colonies of natal and introduced wasps in the laboratory under semi-natural conditions to test the effects of low intra-colony relatedness. In doing so, we made the reasonable assumption that two wasps eclosing on the same nest are likely to be more closely related to each other than two wasps eclosing on two different nests, separated by 10 km or more. Wasps which are 6–8 days old or younger have a significant probability of being accepted into alien nests (Venkataraman and Gadagkar, 1995; Arathi et al. in press). Using 12 such artificially created mixed colonies we have studied social organization to test for kin recognition and task specialization among the natal and introduced wasps present in the assembled colonies.

### Materials and methods

Twelve nests of *R. marginata* with approximately 10–15 pupae and 10–12 adults each were selected to serve as recipient nests. These nests were either naturally initiated or transplanted and maintained for several weeks in a vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore (13°00' N and 77°32' E). The vespiary is a room measuring 9.3 m × 6 m × 4.8 m, covered with a wire mesh of dimension 0.75 cm × 0.75 cm. Donor nests with about twenty pupae were collected for the purpose of obtaining unrelated wasps for introduction onto the recipient nests. Care was taken to ensure that the donor nests were located at least 10 km away from the site of collection or initiation of the recipient nests. The adults and larvae were removed and the nests with only the pupae were maintained at room temperature and monitored daily for eclosion of adults. All adults

on the recipient nests were marked using quick drying paints for individual identification.

Behavioral observations were carried out on each of the 12 recipient nests for a period of 20 hours prior to the introduction of unrelated wasps. Sampling methods consisted of “instantaneous scans” and recording all occurrence of rare behaviors as described by Gadagkar and Joshi (1983). Each “all occurrence” session lasted for 5 minutes. 102 instantaneous scans and 102 “all occurrence” sessions were randomly intermingled during the period of observations, which was spread uniformly between 08.00 and 18.00 h in 2 to 4 days.

Adults from the donor nests were removed immediately upon eclosion, isolated into clean 15 ml glass vials. These wasps were also marked with unique colour codes and introduced onto the recipient nests within 24 h after eclosion. As far as possible, the introduction of unrelated (introduced) wasps was matched with eclosion of adults on the recipient nests so that the matched natal wasps could be treated as controls. Introductions were continued until there were at least six introduced wasps on each recipient nests. The period of introduction ranged from 7 to 10 days. Twenty hours of post-introduction observations were conducted as before, one week after the introduction of the last wasp. The post-introduction observations were carried out blind, i.e., the observer was not aware of the identity of the introduced and natal wasps on the nest. The number of introduced wasps, natal wasps and total brood on each of the twelve nests used in the study are given in Table 1. Wasps that had eclosed prior to the start of introduction and which remained for the duration of the experiment were termed the “resident” wasps and were considered separately for data analysis (not included with “natal” wasps).

Data from instantaneous scans were used to calculate the proportions of time spent by individuals in various behaviours, from which the time activity budgets were obtained for individual wasps. Data from the “all occurrence” sessions were used to calculate the rates at which individuals performed various behaviours as well as rates of interactions between individuals, both in terms of frequency per hour and in terms of frequency per animal or pair of animals per hour. These

**Table 1.** Characteristics of the nests during the post-introduction observations

Nest code	Number of resident wasps	Number of natal wasps	Number of introduced wasps	Total brood (Egg + Larva + Pupa)
L 65	3	9	8	66
L 39	7	3	6	54
L 29	12	12	11	81
L 76	6	4	11	60
L 63	9	7	9	133
L 52	13	4	11	89
V 101	11	5	4	84
L 77	8	3	7	69
V 105	7	6	4	103
V 104	4	6	4	50
V 106	13	5	5	93
V 107	13	5	5	99

frequencies and interaction rates were compared between the pre- and post-introduction observations, and also between the natal, introduced and resident wasps.

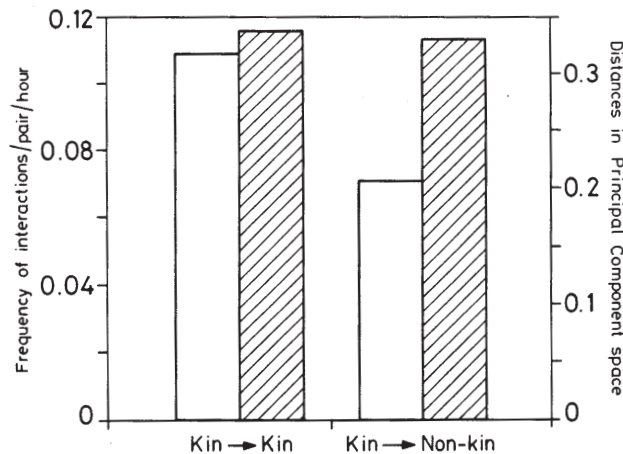
To test whether the introduced and the natal wasps formed distinct behavioral groups, the time activity budgets of the individual wasps were subjected to principal components analysis as described previously (Gadagkar and Joshi, 1983). The distance between pairs of wasps in the principal component space was calculated from a scatter plot obtained using the first two principal components.

All comparisons were carried out across nests using Wilcoxon's matched pairs signed ranks test. Thus, there were twelve matched pairs of observations in each test. However, for the sake of brevity we have presented only the means across colonies. To determine whether introduced wasps became replacement queens with probabilities consistent with their relative abundance in each colony, a computer simulation was performed. Each event of queen turnover was simulated by drawing a random number uniformly distributed between 0 and 1 and assigning it to the natal or introduced category based on the proportion of natal and introduced wasps in that nest. This yielded the simulated value for the number of nests where introduced wasps took over as replacement queens. The simulation was repeated 10,000 times to obtain a frequency distribution of the simulated value for the number of nests where introduced wasps became replacement queens. The observed number of nests where introduced wasps became replacement queens was then compared with this expected distribution. The null hypothesis that introduced wasps became replacement queens based on their relative numbers in the various nests was rejected if the observed value did not lie in the inner 95% of the simulated distribution.

## Results and discussion

All introduced wasps were readily accepted into their respective recipient colonies without any overt signs of aggression, and appeared to become well integrated into their foster colonies. The frequencies of behavioural interactions amongst kin (amongst natal wasps and amongst introduced wasps) and among non kin (between natal and introduced wasps) were not significantly different from each other (Fig. 1). Principal components analysis of time activity budgets showed no obvious clustering of introduced and natal wasps based on behaviour. Mean distances among kin and among non kin in the principal components space were not significantly different either (Fig. 1). These results suggest that kin recognition is absent once wasps are integrated into a single colony. This is in accordance with earlier findings in *R. marginata* as well as in other primitively eusocial wasps (Gadagkar, 1985; Gamboa et al., 1986; Venkataraman et al., 1988; Queller et al., 1990; Venkataraman and Gadagkar, 1992).

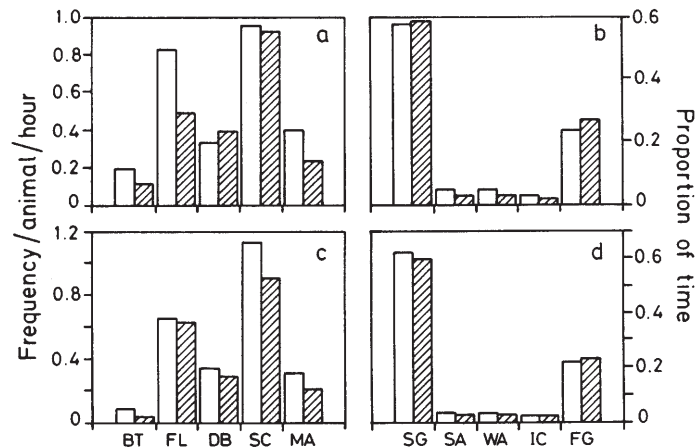
We compared mean behavioural profiles of the wasps during the pre-introduction and post-introduction periods and failed to detect any significant differences (Wilcoxon's matched pairs signed ranks test:  $P > 0.05$ ;  $n = 12$ ; range of  $T_s = 15.5$  to  $37.5$ ; Fig. 2a, b). Introduced wasps participated in all the behaviours recorded on their nests. We found no significant difference in the rates of performance or the proportions of time spent in the different behaviours between introduced and natal



**Figure 1.** Behavioural interactions (open bars) as well as inter-wasp distances in principal components space (hatched bars) among kin (among introduced and among natal wasps) and among non-kin (between introduced and natal wasps) are not significantly different (all  $p$  values  $>0.05$ ). Statistical comparison is based on Wilcoxon's matched pairs signed ranks test using kin-kin and kin-non kin frequencies of interactions or distances for each of the 12 nests as matched values. For the sake of brevity only means across all colonies are presented here

wasps (Wilcoxon's matched pairs signed ranks test:  $P > 0.05$ ;  $n = 12$ ; range of  $T_s = 14$  to 32; Fig. 2c, d). These results suggest that there was no detectable task specialization between introduced and natal wasps. The absence of task specialization is also evident from the observation that 16 out of 69 natal wasps and 12 out of 85 introduced wasps went on to become foragers and successfully brought food to their respective colonies. These proportions are not significantly different from each other ( $G = 2.096$ ;  $p > 0.05$ ). The absence of task specialization between genetically unrelated groups of individuals in artificially created genetically mixed colonies is reminiscent of a similar finding in the ant *Camponotus planatus* (Carlin et al., 1993).

Of the 12 nests in our study, 2 nests experienced natural queen replacements and in both the cases one of the introduced wasps became the replacement queen in spite of the presence of several natal and resident wasps (Table 2). This prompted us to remove the existing queens and create artificial queen replacements in the 6 nests that were still active at that time. Here an introduced wasp became a replacement queen in one case, natal wasps became replacement queens in three cases and resident wasps became replacement queens in the remaining two cases. Comparing the probabilities with which introduced (3/85) and natal (3/69) wasps became replacement queens we found no significant difference ( $G = 0.049$ ;  $p > 0.05$ ). However, a proper comparison should take into account the relative numbers of introduced and natal wasps available on the day of queen replacement. The computer simulations do just that and the results show that the probability with which introduced and natal wasps became replacement queens were not different from those expected by chance alone, based on their relative numbers in different colonies. However, the point which we wish to highlight here is that introduced wasps not



**Figure 2.** (a) Mean frequencies of performance of various behaviours shown by wasps during pre-introduction (open bars) were not significantly different from the proportions during the post-introduction observations (hatched bars) ( $p > 0.05$ ). (b) Similar comparisons using proportions of time spent in the other behaviours also showed that there was no significant difference between the pre- and post-introduction phase ( $p > 0.05$ ). (c) Mean frequencies of performance of various behaviours shown by natal wasps (open bars) and introduced wasps (hatched bars) in various behaviours were not significantly different ( $p > 0.05$ ). (d) Similar comparisons using proportions of time spent by the natal and introduced wasps in the other behaviours also showed that there was no significant difference ( $p > 0.05$ ). Statistical comparisons are based on Wilcoxon's matched pairs signed ranks test using the respective values for each of the 12 nests as matched values. For the sake of brevity only means across all colonies are presented here. SG = Sit and groom, SA = Sit with raised antennae, WA = Walking, IC = In cells, FG = Foraging, BT = Bring things (includes food, liquid and building material), FL = Feed larvae, SC = Solicit, DB = Dominance behaviours, MA = Nest maintenance

only became well integrated and foraged for their foster colonies, but that they also had a fair chance of becoming replacement queens.

The results reported here confirm the previously observed lack of intra-colony kin recognition in *R. marginata*. Taken together with previously reported low intra-colony genetic relatedness values in this species (Muralidharan et al., 1986; Gadagkar, 1990a; Gadagkar et al., 1991; Gadagkar et al., 1993), these results lend further support to the idea that kin selection (based on genetic asymmetries created by haplodiploidy) alone does not appear to be an adequate explanation for the evolution and maintenance of worker behaviour in this species. On the other hand, they lend credence to the possibility that factors other than genetic relatedness may be important in this regard (Lin and Michener, 1972; Alexander, 1974; West-Eberhard, 1975; Evans, 1977; Gadagkar, 1990b, 1990c, 1991b). Considering that wasps move between newly initiated colonies and the theoretical result of Nonacs (1989) that assessing the competitive abilities of nestmates may sometimes be more important than assessing genetic relatedness, our results suggest the possibility of becoming the queen in future (West-Eberhard, 1975; Gadagkar, 1990d) as yet another factor that might favour the evolution of the worker strategy rather than the solitary nest founding strategy.

**Table 2.** Number of animals of different category in each nest at the time of queen turnover

Nest code	Number of resident wasps	Number of natal wasps	Number of introduced wasps	Source of new queen
L 76*	5	4	10	Introduced
L 63	0	2	3	Introduced
L 52*	12	4	10	Introduced
V 101	6	4	3	Resident
L 77	4	3	5	Resident
V 104	0	3	2	Natal
V 106	0	2	3	Natal
V 107	0	3	1	Natal

\* Nests experienced natural queen turnover.

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