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## Cooperative nest building and brood care by nestmates and non nestmates in *Ropalidia marginata*: implications for the evolution of eusociality

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**Abstract** In the primitively eusocial wasp, *Ropalidia marginata*, low levels of intra-colony genetic relatedness, lack of intra-colony kin discrimination and acceptance of young wasps into alien colonies, prompted us to investigate whether or not there exists a cost of such high genetic variability. Freshly eclosed wasps were paired either with their nestmates or with their non nestmates and their performance in nest building and brood care were compared. There was no demonstrable difference between nestmate and non nestmate pairs in terms of success in raising adult offspring, time required for nest initiation, brood developmental period and productivity. There was also no difference in the efficiency of cooperation and division of labour between the nestmate pairs and non nestmate pairs. These results reinforce the idea that the haplodiploidy hypothesis is insufficient to explain the prevalence of worker behaviour in *R. marginata* and emphasize the importance of factors other than genetic relatedness in the evolution of eusociality.

**Key words** Primitively eusocial wasps · Eusociality · Cooperation · Genetic variability · *Ropalidia marginata*

### Introduction

Eusocial insects such as ants, bees and wasps, are characterised by overlap of generations, cooperative brood care and reproductive caste differentiation. Eusocial species therefore exhibit an extreme degree of altruism because only one or a small number of individuals in a colony (queens) reproduce while the remaining individuals (workers) work for the welfare of the colony and die without reproducing (Wilson 1971; Michener 1974; Hölldobler and Wilson 1990; Ross and Matthews 1991; Bourke and Franks 1995). The evolution of such altruistic behaviour and hence of eusociality, was considered paradoxical until Hamilton (1964a,b) proposed the theory of inclusive fitness according to which altruism can evolve if directed towards close genetic relatives. Hamilton's rule states that an altruistic allele can be favoured by natural selection if the benefit to the recipient, devalued by the coefficient of genetic relatedness between the altruist and the recipient, is greater than the cost to the altruist. Because of their haplodiploid genetics, which makes full sisters more closely related to each other than parents would be to their offspring, Hamilton's rule predicts that eusociality should evolve more often in the Hymenoptera. This is often referred to as the haplodiploidy hypothesis.

We have employed the primitively eusocial wasp, *Ropalidia marginata* (Gadagkar 1991a) to test whether the expected high genetic relatedness between workers and their brood is indeed obtained in natural colonies. It turns out that because *R. marginata* queens mate multiply (Muralidharan et al. 1986; Gadagkar 1990a) and because of frequent queen replacements (serial polygyny), the genetic asymmetries created by haplodiploidy are broken down, leading to worker-brood genetic relatedness values ranging from 0.20 to 0.38 (Gadagkar et al. 1991a, 1993). The observed movement of foundresses between nests during colony initiation is likely to further reduce intra-colony genetic relatedness (Shakarad and Gadagkar 1995).

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In spite of such low levels of genetic relatedness, intra-colony kin discrimination can potentially rescue the haplodiploidy hypothesis. Although *R. marginata* has a well developed system of nestmate discrimination, labels and templates used in discrimination are acquired from the natal nest and/or nestmates after the eclosion of adult wasps, making it unlikely that intra-colony kin discrimination can take place (Venkataraman et al. 1988). Furthermore, young wasps (<8 days old) are readily accepted into alien colonies (Venkataraman and Gadagkar 1995; Arathi et al. 1997a). We have also shown that genetically mixed colonies created by such introduction of alien wasps into unrelated colonies, do not differ in any demonstrable way in their social organisation and division of labour as compared to the normal colonies. Indeed, introduced wasps in such genetically mixed colonies become replacement queens at rates indistinguishable from those at which natal wasps do so (Arathi et al. 1997b).

The above mentioned studies however, cannot rule out the possibility that there is a subtle form of intra-colony kin discrimination leading perhaps to poor cooperation and lower productivities in genetically mixed colonies. Here, we explicitly test the possibility that poor cooperation and lower productivity constitute a cost of living in colonies with high genetic variability.

## Materials and methods

Naturally occurring nests of *R. marginata* with about 20 to 30 pupae were collected, the resident adults and larvae were removed and pupae were monitored every day for eclosions. Adults that eclosed from these nests were removed in less than 12 h after eclosion, marked uniquely with quick drying paint and paired in 22 cm × 11 cm × 11 cm fresh, well ventilated plastic boxes with another wasp that had also eclosed on the same day, either from the same nest or from another nest (located at least 10 km apart). A total of 96 nestmate pairs and 103 non nestmate pairs were thus set up and provided with *ad libitum* food (*Corcyra cephalonica* larvae), water, honey and soft wood (as a source of building material). Under these conditions females of *R. marginata* are known to initiate nests and produce offspring (Gadagkar et al. 1988, 1990, 1991b).

Both nestmate and non nestmate pairs were monitored at least once in 2 days for signs of nest building. After initiation, the nests were checked to record their brood composition until the eclosion of an adult or until one of the wasps died. A pair of wasps that raised at least one adult offspring was termed successful. The time taken to initiate nests and brood developmental periods were calculated from these records. The productivities of the nests initiated by nestmate and non nestmate pairs in terms of the number of eggs, larvae and pupae at the time of eclosion of the first adult offspring were also computed from these records. In a sub-set of 28 nestmate pairs and 27 non nestmate pairs, behavioural observations were made for periods ranging from 12 to 36 hours, at the rate of 4 hours per day between 1030 to 1230 hours and 1430 to 1630 hours, taking care to spread the observations during the egg stage, larval stage and pupal stage as uniformly as possible. All observations were performed in the blind: the observer was not aware of the identity of the nestmate and non nestmate pairs. The observations consisted of "instantaneous scans" and recording of all occurrences of chosen behaviours (see below) as described by Gadagkar and Joshi (1983). Fifteen instantaneous scans and 15 "all occurrences" sessions of 5 min duration each were randomly intermingled during every 2 hour period. The proportion of time spent in the most common behaviours namely sit and groom, sit with raised anten-

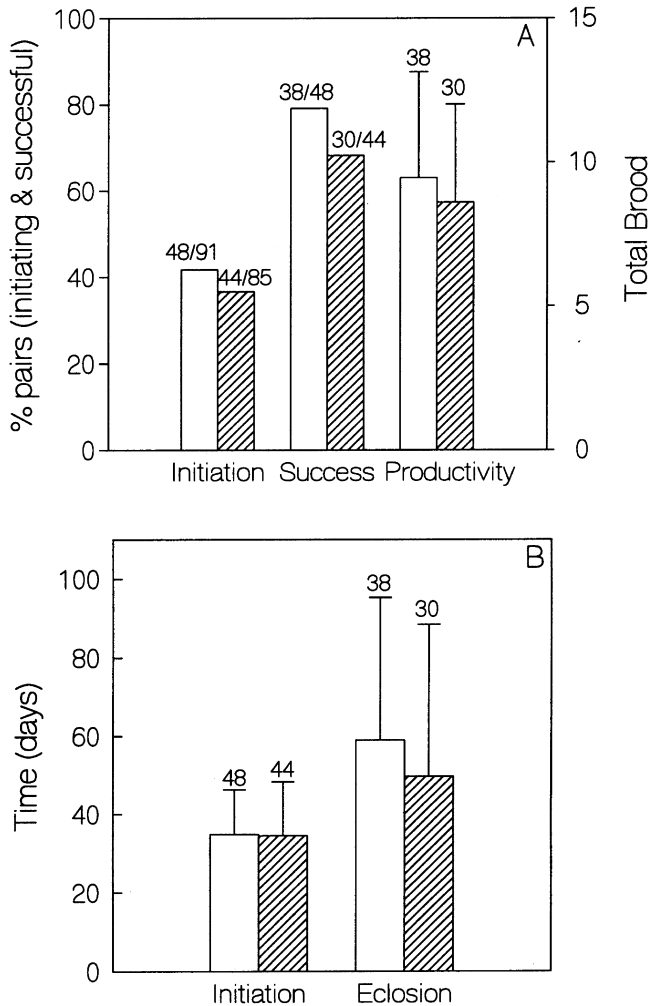
nae, walk, inspect cells and being absent from the nest were computed from the instantaneous scans. Rates of performance per animal per hour of relatively rare behaviours such as bring food, feed larvae, maintenance (construction activities on the nest), dominance behaviours and solicit were computed from the "all occurrences" sessions.

Nestmate and non nestmate pairs were compared for potential differences in productivity and brood developmental periods. Queens and workers and nestmate and non nestmate wasps were compared for potential differences in their behaviours. Principal components analysis of time-budgets and of rates of performance of behaviours was used to compare the positions of queens and workers among nestmate and non nestmate pairs in their behavioural parameter space.

## Results and discussion

Of the 96 nestmate pairs and 103 non nestmate pairs set up, 91 nestmate pairs and 85 non nestmate pairs were followed until the eclosion of the first adult offspring or the death of one of the wasps. These pairs of wasps therefore permit comparison of the success and productivity of the nestmate and non nestmate pairs. Of the 91 nestmate pairs studied, 48 initiated nests and of the 85 non nestmate pairs studied, 44 initiated nests. The proportion of pairs initiating nests among nestmates and non nestmates are not significantly different from each other (Fig. 1A). Of the 48 nestmate and 44 non nestmate pairs that initiated nests, 38 and 30 respectively, were successful. These proportions of successful nests among the initiated nests were not significantly different between nestmates and non nestmates (Fig. 1A). The 38 nestmate pairs that had successful nests produced  $9.4 \pm 3.7$  items of brood (egg + larva + pupa) on the day of eclosion of the first adult offspring. The corresponding value for the 30 non nestmate pairs was  $8.6 \pm 3.4$ . These productivity values are also not significantly different from each other (Fig. 1A). The nestmate and non nestmate pairs initiated nests and produced brood at about the same rates. Nestmates took  $34.9 \pm 11.5$  days to initiate nests while the non nestmates took  $34.5 \pm 13.8$  days to do so. These durations are not significantly different from each other (Fig. 1B). Nestmates required  $58.9 \pm 36.2$  days from initiation to produce the first adult offspring. The corresponding values for non nestmates was  $49.8 \pm 38.6$  days. These egg-to-adult developmental duration were also not significantly different from each other (Fig. 1B).

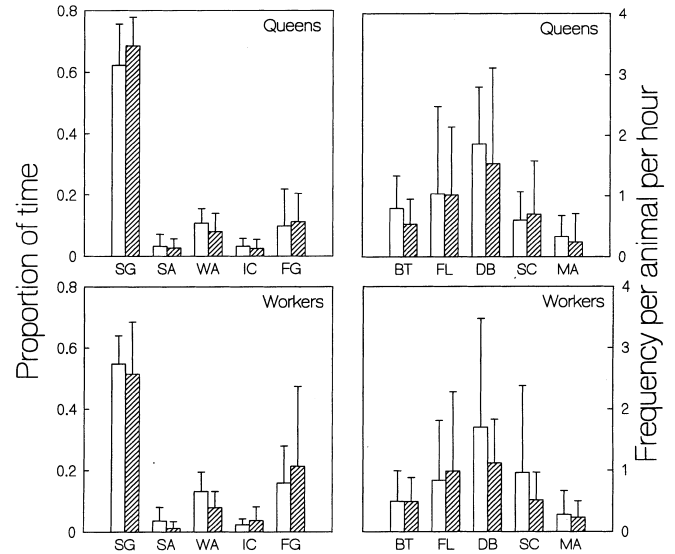
Thus, there are no demonstrable differences between the performance of the nestmate pairs and the non nestmate pairs. This suggests that perhaps there is no difference in the efficiency of cooperation and division of labour between the nestmate and non nestmate pairs. For such a conclusion to be valid however, it is necessary to demonstrate that, in the nestmate pairs and in the non nestmate pairs, both wasps participated in nest building and brood care. If only one wasp in each pair was involved in nest building and brood care, these would be equivalent to single foundress nests and we should then not expect any difference between the nestmate and non nestmate pairs. To confirm that nest



**Fig. 1(A)** The percentage (primary Y-axis) of nestmate pairs that initiated nests is not significantly different from the percentage of non nestmate pairs that did so ( $G = 0.017$ ;  $P > 0.05$ ). Percentage of these that successfully produced an adult offspring was not significantly different between nestmate and non nestmate pairs ( $G = 1.43$ ;  $P > 0.05$ ). Total brood (secondary Y-axis) at the time of eclosion of the first adult in nests of nestmate pairs was not significantly different from that of non nestmate pairs (Mann-Whitney  $U$  test,  $U = 485$ ;  $P > 0.05$ ). **(B)** Time taken to initiate nests was not significantly different between the nestmate and non nestmate pairs (Mann-Whitney  $U$  test,  $U = 1023$ ;  $P > 0.05$ ). Similarly, the developmental period from egg to adult was also not significantly different between the nestmate and non nestmate pairs (Mann-Whitney  $U$  test,  $U = 589$ ;  $P > 0.05$ ). (Open bars = nestmate pairs; Hatched bars = non nestmate pairs. Numbers above the bars are the sample sizes.)

building and brood care was indeed a cooperative effort of both members of each pair, we conducted behavioural observations, as described in the methods, on 28 nestmate pairs and 27 non nestmate pairs.

The behavioural profiles of queens and workers among nestmate pairs and non nestmate pairs are depicted in Fig. 2. Using data on proportions of time spent in the five common behaviours and the rates of performance of the five rare behaviours (see Materials and methods), we compared the behavioural profiles of

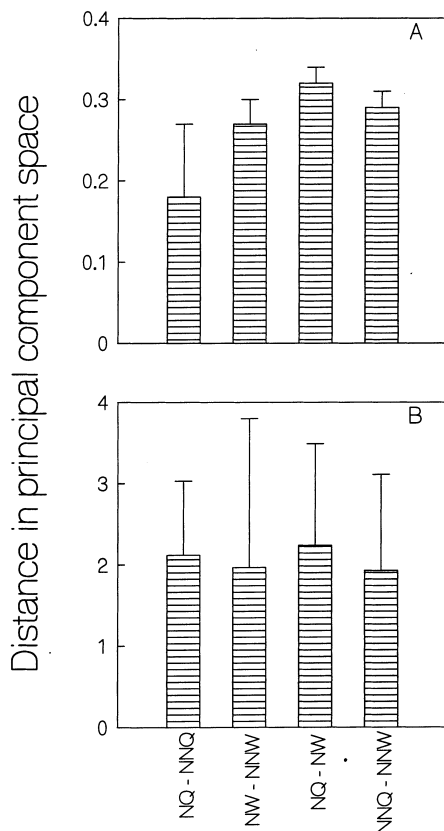


**Fig. 2** Proportion of time spent in common behaviours and frequency of performance of rare behaviours by queens and workers in the nestmate pairs and non nestmate pairs. Behaviours studied are SG = sit and groom; SA = sit with raised antennae; WA = walking; IC = inspect cells; FG = absent from the nest; BT = bring things including food, water and pulp; FL = feed larvae; DB = dominance behaviours; SC = solicit; MA = nest maintenance. For both the queens and workers, nestmates are not significantly different from non nestmates (Mann-Whitney  $U$  test,  $U$  ranges from 635 to 1043;  $P > 0.05$ ). Except in the case of SG where queens have significantly higher values than the workers (Wilcoxon's matched pairs signed ranks test,  $T_s = 23$ ;  $P < 0.05$ ), in all the remaining nine behaviours there is no significant difference between queens and workers, both in nestmate pairs as well as in non nestmate pairs (Wilcoxon's matched pairs signed ranks test,  $T_s$  ranges from 122.5 to 233;  $P > 0.05$ ). (Open bars = nestmate pairs; Hatched bars = non nestmate pairs)

the queens among the nestmate pairs with the queens among the non nestmate pairs using a Mann-Whitney  $U$  test. We find no significant differences between nestmate queens and non nestmate queens in any of the ten behaviours (Fig. 2). Similarly, we find no significant differences between the nestmate workers and non nestmate workers in any of the ten behaviours (Fig. 2). We also compared, for each behaviour, queens with their workers in the nestmate pairs and in the non nestmate pairs, using the Wilcoxon's matched pairs signed ranks test. In both nestmate pairs and non nestmate pairs, the queens spent significantly more time in 'Sit and Groom' (Fig. 2) as is to be expected from previous studies (Gadagkar and Joshi 1983). Of more interest was the finding that in all the remaining nine behaviours, the queens did not differ significantly from the workers both in nestmate pairs and non nestmate pairs (Fig. 2). These results show that both wasps cooperated in nest building and brood care, to the same extent in the nestmate pairs as in the non nestmate pairs.

To rule out a more subtle difference between queens and workers in the nestmate pairs as compared to the non nestmate pairs, we performed a principal components analysis (separately for the time budget data on

the common behaviours and the frequency data on the rare behaviours) and plotted the positions of the 28 nestmate queens and 28 nestmate workers as well as 27 non nestmate queens and 27 non nestmate workers in the parameter space of the first two principal components (the behavioural parameter space). From these plots we computed the distances in behavioural parameter space, between nestmate queens and non nestmate queens on the one hand and distance between nestmate workers and non nestmate workers on the other hand. We compared these queen-queen distances with the corresponding worker-worker distances using a Wilcoxon's matched pairs signed ranks test and failed to detect any significant difference (Fig. 3). Similarly, we compared queen-worker distances for the nestmates with queen-worker distances for the non nestmates and again failed to detect any significant difference (Fig. 3). These results suggest that the behavioural relationships



**Fig. 3** Distance in behavioural parameter space obtained from principal component analysis, of the proportions of time spent in common behaviours by the queens and workers in the nestmate and non nestmate pairs (A) and of the frequency per animal per hour of the rare behaviours exhibited by the queens and workers in the nestmate and non nestmate pairs (B). Distance between nestmate queens (NQ) and non nestmate queens (NNQ) is not significantly different from distance between nestmate workers (NW) and non nestmate workers (NNW). Distance between nestmate queens and their workers is not significantly different from the distance between non nestmate queens and their workers (Wilcoxon's matched pairs signed ranks test;  $T_s$  ranges from 121.5 to 232;  $P > 0.05$ )

between queens and their workers did not depend on whether they were nestmates or non nestmates. It is clear therefore, that both members of the pair participated in nest building and brood care, in the nestmates as well as in the non nestmates.

Even though the nestmates in our experiments may not have been full sisters, on account of multiple mating and serial polygyny in their natal nests, it is reasonable to expect that the nestmates would be more closely related to each other than to their non nestmates who eclosed on different nests, separated by more than 10 km. The absence of any significant difference between the nestmate pairs and non nestmate pairs in their rates of nest initiation, success, productivity and developmental periods can therefore be interpreted to mean that there was no apparent cost of living with distantly related individuals. Thus, low values of intra-colony genetic relatedness, absence of intra-colony kin discrimination and absence of any apparent cost of living and cooperating with unrelated individuals, all underscore the importance of factors other than genetic relatedness for the evolutionary maintenance of worker behaviour in *R. marginata* (Gadagkar 1990b,c, 1991a,b, 1996, 1997; Shakarad and Gadagkar 1997). We speculate therefore, that the overriding advantage of group living over solitary existence, has selected in this species an ability to efficiently cooperate with conspecifics, in spite of varying degrees of genetic relatedness.

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