

# Nestmateship and body size do not influence mate choice in males and females: A laboratory study of a primitively eusocial wasp *Ropalidia marginata*

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## ABSTRACT

We investigated the effect of nestmateship and body size on mate selection through a choice based assay in the primitively eusocial wasp *Ropalidia marginata*. A recent study has shown that male and female *R. marginata* mate with their nestmates and non-nestmates with equal probability if no choice is available. That study could also not detect any influence of body size on mating probability in the absence of choice. To confirm that the same results can be obtained even when the wasps have a choice, we offered a choice of two virgin partners either to a virgin test male or to a virgin test female and measured the probability that the test individual would mate with any particular partner based on nestmateship or body size. We show here that even when a choice is available, neither male nor female test wasps base their mate choice on the nestmateship or body size of the partner. We therefore suggest that the natural mating habit of these wasps is sufficiently promiscuous and not constrained by such factors as nestmateship and body size.

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## 1. Introduction

In hymenopteran insects, inbreeding can lead to production of diploid, non-reproductive males (reviewed in Crozier and Pamilo, 1996). Therefore, such insects are expected to evolve even stronger mechanisms to avoid inbreeding. Natural selection is expected to favour mate choice as a mechanism that can help increase the probability of mating with an unrelated partner of high quality (Halliday, 1983). Indeed, many studies show that kin-recognition-based mate choice allows avoidance of inbreeding both in vertebrates and invertebrates. This has been demonstrated, in particular, in some insect species (Maynard Smith, 1956; Simmons, 1989) including some eusocial termites and bees (Shellman-Reeve, 2001; Smith and Ayasse, 1987).

The ability of female social insects to recognize their female nestmates is well documented in social wasps, bees and ants (Baer, 2003; Chapman et al., 2008; Cini et al., 2009; Dani et al., 1996; Gadagkar, 1985; Gamboa, 1996; Gamboa et al., 1986; Pfennig et al., 1983; Ross and Gamboa, 1981; Venkataraman et al., 1988). However, very few studies, have investigated nestmate recognition abilities in males and the few that do, have focused on male mating preferences (Larch and Gamboa, 1981; Ryan et al., 1984; Sen et al.,

2010). Greenberg (1982) found that in the laboratory, male sweat bees preferentially mated with nestmates.

In the primitively eusocial wasp *Ropalidia marginata* (Gadagkar, 2001), males live in their natal nest for about a week and then leave to lead a nomadic life. Mating does not take place on the nest and is expected to take place in the foraging areas. In a recent study Sen et al. (2010) described the mating behaviour of *R. marginata* and showed that male and female wasps of 5–20 days age mate under laboratory conditions in a transparent plastic container. Here we used the same set-up to examine whether virgin males and females show any mate preference and/or discrimination based on nestmateship, body size and dominance behaviour, when a choice is available. We proposed and tested the following three hypotheses in an effort to understand the mating biology of *R. marginata*.

### 1.1. Hypothesis 1. Nestmateship should not influence mate choice

Since mating does not take place on or in close proximity of the natal nest, outbreeding can be assured in this system. Although *R. marginata* females are capable of nestmate recognition (Venkataraman et al., 1988), in a no choice situation, neither males nor females seem to use any recognition based mechanism that can influence mating probability (Sen et al., 2010). Assuming that the same holds good when a choice is available, we hypothesize that neither sex would use nestmateship as a basis of choosing a mating partner.

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### 1.2. Hypothesis 2. Body size should not influence mate choice

*R. marginata* is a serially polygynous species; soon after a natural queen loss or experimental queen removal one of the workers become physically aggressive and within a few days (if the original queen does not return), reduces her physical aggression and starts laying eggs. This individual referred to as the potential queen, is not determined by her body size. In other words, body size does not predict the probability of an individual's direct reproductive fitness. Sen et al. (2010) have shown that body size does not influence the mating success in either males or females when choice is not available. Assuming that the same holds good even when a choice is available, we hypothesize that neither sex should use body size as a parameter to choose their partner even when a choice is available.

### 1.3. Hypothesis 3. Dominance behaviour should influence mate choice

*R. marginata* workers use physical aggression to protect their nest from non-nestmates as well as other predators. A potential queen starts her non-worker life with severe aggression. Sen et al. (2010) suggested that *R. marginata* males often forced females to mate, and found that choice experiments were difficult to conduct due to the high level of aggression between wasps of the same sex. Thus physical aggression plays an important role in survival and fitness of both males and females. We therefore hypothesize that, when a choice is available, the behaviourally dominant individual, rather than the subordinate one (male or female), would be preferentially chosen as the partner by the wasp of other sex.

## 2. Materials and methods

*R. marginata* nests with a large number of pupae were collected in and around Bangalore (13°00'N 77°32'E), India and were re-established in open cages in the vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The vespiary is a room of dimension 9.3 m × 6 m × 4.8 m covered with a wire mesh screen of dimension 0.75 cm × 0.75 cm, which permits *R. marginata* to fly in and out freely. Thus the wasps were free to forage for food and building material on their own. The nests were monitored twice a day (morning and evening) for new eclosions and all newly eclosed wasps were marked immediately with small white spots of quick drying enamel paint. Wasps eclosing in the morning were marked on the thorax and wasps eclosing in the evening were marked on the abdomen. Wasps marked in the morning and evening, were removed from the nest in the morning and evening respectively of the next day, and thus were allowed to stay on their natal nest for 24 h. These one-day old male and female wasps were then kept in isolation for 5–20 days (until the onset of the experiment) in transparent, ventilated plastic bottles (22 cm × 11 cm × 11 cm) with *ad libitum* food, water and building material. Males and females from the same nest were considered as nestmates and while pairing nestmates, familiarity was avoided by pairing wasps eclosing on different dates. Non-nestmates were taken from the nests whose sites of initiations were more than 10 km apart.

To explore male mate choice, we introduced a test male, and two females, one his nestmate the other his non-nestmate, simultaneously in a fresh plastic bottle (as described above). We studied 51 such one male two females (1M2F) triads (Table 1a). To explore female mate choice we introduced one test female, and two males, one her nestmate and the other her non-nestmate. We studied 42 such one female two males (1F2M) triads (Table 1b). To differentiate two wasps of same sex, with similar initial markings,

**Table 1a**

Nest details of one male two females (1M2F) triads. Numbers of matings with nestmates were not significantly different than numbers of matings with non-nestmates (Wilcoxon paired sample test,  $P=0.94$ ).

Nest number	Mating with nestmates	Mating with non-nestmates	Not mated
V.973	1	0	3
V.974	1	1	1
V.975	2	4	2
V.976	1	0	1
V.983	0	2	0
V.984	0	3	4
V.1042	1	1	4
V.1044	0	2	2
V.1046	0	1	2
V.1053	1	3	1
V.1054	2	2	1
V.1055	1	1	0

**Table 1b**

Nest details of one female two males (1F2M) triads. Numbers of matings with nestmates were not significantly different than numbers of matings with non-nestmates (Wilcoxon paired sample test,  $P>0.99$ ).

Nest number	Mating with nestmates	Mating with non-nestmates	Not mated
V.973	3	3	1
V.974	0	0	1
V.975	3	3	2
V.976	2	1	0
V.984	0	0	5
V.1042	1	1	2
V.1044	0	2	0
V.1046	0	0	1
V.1053	0	0	2
V.1054	2	3	0
V.1055	3	0	1

an additional spot of white colour was applied on one of them. Behavioural observations were made for 1 h or till the first occurrence of long conjugation behaviour (Sen et al., 2010), whichever was earlier. We recorded every occurrence of dominance behaviour such as aggressive bite, crash, falling fight, sit on another individual, being offered liquid, hold in mouth, attack, nibble, peck and chase (Gadagkar, 2001), as well as every occurrence and duration of the mating behaviours such as unsuccessful attempt to mount (UAM), mount (MO), short conjugation (SC) and long conjugation (LC) (Sen et al., 2010). Observations were conducted in the blind, i.e., the observer was unaware of the identity of the wasps.

Whenever long conjugation behaviour took place, we measured all three wasps of that triad for 27 body size parameters [inter-ocular distance (IOD), right oculo-ocular distance (OODR), left oculo-ocular distance (OODL), head width (HW), head length (HL), clypeus width (CW), clypeus length (CL), width of first segment of right antenna (W1FSR), length of first segment of right antenna (L1FSR), width of first segment of left antenna (W1FSL), length of first segment of left antenna (L1FSL), inter-antennal socket distance (IASD), width of mesoscutum (MSCW), length of mesoscutum (MSCL), alitrunk length (ATL), length of right wing (WLR), length of left wing (WLL), length of 1st marginal cell of right wing (LM1R), length of 1st marginal cell of left wing (LM1L), number of hammuli on right wing, number of hammuli on left wing, width of 1st gastral segment (W1GS), length of 1st gastral segment (L1GS), height of 1st gastral segment (H1GS), width of 2nd gastral segment (W2GS), length of 2nd gastral segment (L2GS) and height of 2nd gastral segment (H2GS) (as described in Kardile and Gadagkar, 2005; Sen and Gadagkar in preparation)]. The body size data for each parameter were then subjected to univariate comparison (paired *t*-test). Data for all 27 parameters were subjected to principal components analysis and the position of each wasp was plotted in the space of

**Table 2**  
Comparison between nestmate and non-nestmate matings of male choice and female choice experiments in *R. marginata*.

Choice experiments	# nestmate matings	# non-nestmate matings	Chi square test ( <i>P</i> value)	Nestmate discrimination. Index
One male two females triads ( <i>n</i> = 30)	10	20	0.06	−0.33
One female two males triads ( <i>n</i> = 27)	14	13	0.84	0.03

the first two principal components. The first principal component was used as the body size index. We estimated the magnitude of size difference of the two potential mates offered to a test wasp by using head width. In 1F2M triads, the average difference of head width in two male wasps was 4.8% and in 1M2F triads the average difference of head width in two female wasps was 2.8%. To ascertain that the two wasps available were significantly different in body size, we compared the body size indices of the pairs of wasps made available to the test wasps and showed that they were indeed significantly different from each other (Wilcoxon signed-rank matched-pair,  $P < 0.0001$  for both comparisons, male choice and female choice). Thus ascertaining that one of the potential mates was bigger in size than the other, we compared the body size indices of the mated wasps to that of the unmated wasps.

In order to compute the degree of discrimination between the two potential partners offered (if any), we calculated a discrimination index using the formula of (Merrel, 1950) with small modifications.

Nestmate discrimination index

$$= \frac{\text{No. of nestmate matings} - \text{No. of non-nestmate matings}}{\text{Total No. of matings}}$$

The nestmate discrimination index scales from  $-1$  to  $+1$ ;  $0$  = random mating between nestmate and non-nestmate matings;  $+1$  = discrimination is complete and only nestmate matings occurs;  $-1$  = discrimination is complete and only non-nestmate matings occurs.

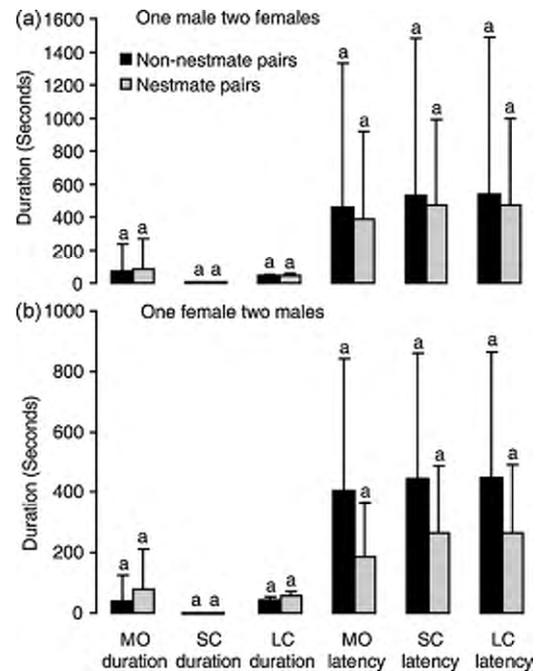
### 3. Results

Out of 51 observed '1M2F' triads, mating occurred in 30 cases and out of 42 observed '1F2M' triads mating took place in 27 cases (Tables 1a and 1b).

#### 3.1. Nestmateship does not influence mate choice—hypothesis 1 was supported

Out of 30 '1M2F' mated triads, mating occurred between nestmates in 10 cases. Out of 27 '1F2M' mated triads, mating occurred between nestmates in 14 cases. In both types of triads, the proportions of nestmate mating were not significantly different from the proportions of non-nestmate mating (Chi square test, Table 2).

When males were offered with two females, discrimination index analysis revealed that discrimination is not complete but there is a tendency towards non-nestmate matings. When females were offered with two males, there was no evidence of discrimination towards nestmates or non-nestmates (Table 2). There was also no significant difference in the durations of mating behaviours (MO, SC or LC) between the nestmate pairs and the non-nestmate pairs (Fig. 1). The latency (time difference between starting of observation and start of a particular behaviour) to MO, SC and LC were also indistinguishable between the nestmate and non-nestmate pairs (Fig. 1). Nor was there any significant difference between the nestmates and non-nestmates for either type of triad, in the number of unsuccessful attempts to mount. Thus *R. marginata* males and females showed no significant behavioural evidence of preference or non-preference towards nestmates, even when a choice was available.



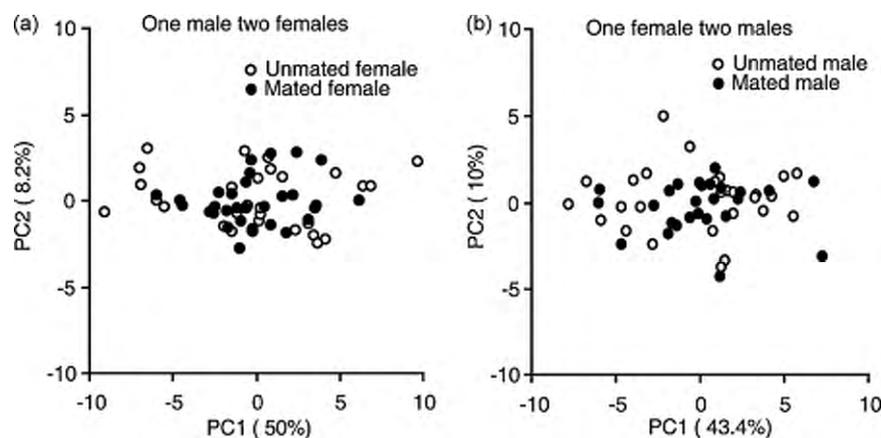
**Fig. 1.** Comparison of nestmate pairs and non-nestmate pairs with regard to the durations of mount (MO), short conjugation (SC) and long conjugation (LC) behaviours and latency to MO, SC and LC in the (a) 'one male two females' triads and (b) '1F2M' triads. Bars carrying same letters are not significantly different from each other (Mann–Whitney test,  $P > 0.008$ , after Bonferroni correction).

#### 3.2. Body size does not influence mate choice—hypothesis 2 was supported

Since there was no effect of nestmateship, we used the body size data from the same experiment to test if the males and/or females showed any form of mate choice based on body size. None of the 27 measured parameters (see Section 2) were significantly different between the mated or unmated individuals in our univariate analysis (paired *t*-test, all *P* values were more than 0.05). The mated and unmated individuals in either type of triad did not form any distinct cluster in two dimensional principal components space (Fig. 2 a and b). There was no significant difference in body size indices of mated versus unmated individuals for either type of triad (pair *t*-test,  $P > 0.05$ ). Thus body size did not influence male or female mate choice, even when choice was available and when the body sizes of the available partners were significantly different.

#### 3.3. Influence of dominance—hypothesis could not be tested

In three '1M2F' triad replicates, dominance behaviour was observed between the two females. In all three cases the males mated with the subordinate females. In seven '1F2M' triads, dominance behaviour was observed between the two males. Five dominant males and two subordinate males mated with the females. Because of such low sample size we could not test the influence of dominance behaviour.



**Fig. 2.** Body sizes of mated and unmated (a) females and (b) males, plotted in two dimensional principal components space. Neither mated females nor mated males are well separated from their unmated counterparts.

#### 4. Discussion

Our attempts to study mate choice when two virgin potential mates were available, showed that *R. marginata* males and females do not select their mates based on body size and nestmateship. Female *R. marginata* are capable of nestmate recognition after staying for one or more days in their nest (Venkataraman et al., 1988). All of our experimental wasps were allowed to live on the nest for one day. Therefore, the females could potentially use a nestmate recognition based mechanism to choose/prevent nestmate mating. But neither females nor males showed any evidence of using recognition based cue to choose a mate. Such absence of recognition based mate choice was also found in *P. fuscatus* (Larch and Gamboa, 1981) but Ryan and Gamboa (1986) showed that *P. fuscatus* males and gynes mated with their nestmates less often than with non-nestmates.

Although body size plays some role in mating behaviour of some paper wasps (Polak, 1993), it is not surprising that our second hypothesis was also supported and that neither males nor females chose larger or smaller partners as body size does not seem to play a significant role in any part of social life of *R. marginata* (Gadagkar, 2001).

In our experiments, very few males and females showed dominance behaviour. In male choice experiments, the sample size was too small to conduct a statistical test; therefore we cannot support or reject the third hypothesis.

Within the few 1M2F triads, where dominance behaviour was seen, the males mated with subordinate females wherever there was physical aggression between the females. Since mating is not a pre-requisite for a female to develop her ovaries to become the sole egg layer of her colony and even to suppress the ovaries of mated nestmates (Chandrashekara and Gadagkar, 1991), it may be more important for the dominant females in our experiments to dominate the subordinate female than to mate with the male. However, because of the low sample sizes of triads where dominance behaviour was observed, we would not like to over-interpret the results.

Among the few 1F2M triads where dominance behaviour was seen, the dominant males appeared to get the mating opportunity (five out of seven). In most of the cases where the wasps showed physical aggression to each other, there was no mating. But the dominance behaviour in the triads is of importance because when only two wasps were paired, there was no physical aggression (Sen et al., 2010). The dominance between the two females is possibly due to nestmate discrimination while the dominance between the two males could either be nestmate discrimination or

male–male competition. But here again we should be cautious in over-interpreting the results because of low sample size.

In *R. marginata* outbreeding is almost assured because mating takes place away from the nest. Body size does not influence dominance or fitness in this species. *R. marginata* females who are known to have nestmate recognition abilities, do not seem to have an active role in mating (Sen et al., 2010) and they do not necessarily require to be mated to gain direct fitness (Chandrashekara and Gadagkar, 1991). Therefore, this species may not practice mate choice based on the criteria we tested. At least some workers in most *R. marginata* colonies are found mated suggesting that they mate opportunistically (or are inseminated by males, without much of a choice) while on foraging or reconnaissance trips away from their colonies. The chances that these females will use the sperm stored in their spermathecae is rather small, thus lowering the fitness costs of mating with a “sub-optimal” partner, both for males as well as for females. Under all these circumstances, it is not so surprising after all that mating is rather promiscuous, without regard to such factors as nestmateship, body size or dominance status of the partners.

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