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RESEARCH ARTICLE

Middle aged wasps mate through most of the year, without regard to body size, ovarian development and nestmateship: a laboratory study of the primitively eusocial wasp *Ropalidia marginata*

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Abstract We studied the mating behaviour of the primitively eusocial wasp *Ropalidia marginata* and the factors that may influence sperm transfer. By introducing a male and a female *R. marginata* into ventilated transparent plastic boxes, we were able to observe mating behaviour, and it involved mounting and short or long conjugation of the wasps. Dissection of female wasps after the observation indicated that long conjugation is a good behavioural predictor of sperm transfer. This finding makes it possible to obtain mated females without dissecting them every time. We tested the effect of age, season, relatedness, body size and female's ovarian status on mating. Under laboratory conditions, mating success declined rapidly below and above the ages 5–20 days. Within this age range mating success was significantly low in December compared to other months tested. There was no nestmate discrimination, and there was no influence of male and female body size or of the ovarian state of the female on the probability of sperm transfer.

Keywords Sperm transfer · Age · Nestmate discrimination · Male wasps

Introduction

Although social behaviour of females has captured most of the attention of researchers studying social wasps, mating behaviour has also been studied sporadically and opportunistically. Such studies have been mostly done on the genus *Polistes* (Beani, 1996; Beani and Lorenzi, 1992; Beani and Turillazzi, 1988; Keeping et al., 1986; Kojima and Suzuki, 1986; Post and Jeanne, 1983a, b; Romani et al., 2005; Wenzel, 1987), but some studies have also been carried out on the genera *Mischocyttarus* (Litte, 1979), *Belonogaster* (Keeping et al., 1986), *Vespa* (Batra, 1980; Spiewok et al., 2006) and *Vespula* (Goodisman et al., 2002; Kovacs et al., 2008; Ross, 1983). However, no studies have been made so far on the mating behaviour of the genus *Ropalidia*. *Ropalidia marginata* exhibits overlap of generations, cooperative brood care, reproductive division of labour and absence of morphological caste differentiation and is therefore referred to as a primitively eusocial wasp (Wilson, 1971). Males of this wasp leave their natal nests within about a week of their eclosion and lead a nomadic life away from their own or other nests. Mating does not take place on the nest. The nomadic males presumably mate with foraging females because we find that several workers (in addition to the queen) in each nest are mated (Chandrashekara and Gadagkar, 1991). This habit of males mating away from the nest has made it difficult for us to study mating behaviour and has restricted our attention to the role of females in the social biology of these wasps (Gadagkar, 2001). However, in this study we observed mating

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behaviour in a laboratory setup, and here we describe the behavioural correlate of sperm transfer. We tested the effect of age of the male and female wasps on sperm transfer and found the optimal age range, at which *R. marginata* wasps mate more readily and transfer sperm with higher probability. To explore other factors that may influence sperm transfer, such as season, nestmateship, body size and ovarian development of the females, we put forward and test the following four hypotheses:

Hypothesis 1: mating should occur throughout the year

Ropalidia marginata is a tropical, perennial wasp and male production and founding of new nests take place all through the year (Sen and Gadagkar, submitted; Shakarad and Gadagkar, 1995). We therefore expect *R. marginata* males produced in different seasons should have equal capability to mate and transfer sperm.

Hypothesis 2: nestmateship should not influence mating

In hymenopteran social insects mating between closely related individuals (e.g. brothers and sisters) can produce diploid males (Crozier, 1977). To avoid production of such males who neither produce diploid females nor take part in colony maintenance, such insects are expected to have mechanisms to avoid mating between the closely related nestmates. Well developed nestmate recognition has been reported in females of *R. marginata* (Venkataraman et al., 1988). However, since outbreeding is assured by mating taking place away from the nest, a recognition-based mechanism to avoid nestmate mating should not exist in *R. marginata*. Therefore, we hypothesize that nestmateship should not influence mating.

Hypothesis 3: body size should not influence mating

As a primitively eusocial species *R. marginata* lacks morphological caste differentiation, i.e. queens and workers are not significantly different in body size. Although only the queen (with highly developed ovaries) reproduces, most of the workers (at least 50%) of *R. marginata* are potentially capable of reproducing (Gadagkar et al., 1988). If the queen is naturally lost or experimentally removed from the nest, one of the workers become aggressive and if the queen is not returned to the nest within next few days, this individual (potential queen) becomes the next queen (Gadagkar, 2001). The potential queen is not determined by her body size. Males often force-mate with the females and existence of female mate choice does not seem likely in this species. We therefore hypothesize that body size should not influence sperm transfer.

Hypothesis 4: ovarian status of the female should not influence mating

Ropalidia marginata workers differ in their ovarian development status. However, mating is neither necessary nor sufficient for a female wasp to develop her ovaries and become the sole egg layer of her colony (Chandrashekara and Gadagkar, 1991; Gadagkar, 2001). Since well-developed ovarian status does not assure reproduction in future, we hypothesize ovarian status should not influence sperm transfer.

Materials and methods

This study was conducted in two parts. In the first part we studied different mating-related behaviours and identified the optimal age for mating and tested hypothesis 1, i.e. effect of season on mating. In the second part we tested hypotheses 2, 3 and 4 and examined the effect of nestmateship, body size of males and females and ovarian condition of females, on mating.

We collected *R. marginata* nests with large numbers of pupae from their sites of natural initiation in Bangalore (13°00′N and 77°32′E). For the first part of the study, we removed all the adults and larvae from the nests, maintained the nests in ventilated plastic boxes and monitored them daily for the presence of newly eclosed wasps. All newly eclosed males and females were removed and kept in isolation in transparent, ventilated plastic bottles (22 cm × 11 cm × 11 cm) with ad libitum food and water until they were used for the experiments. For the second part of the study we collected nests with large numbers of pupae and re-established them in open cages in the vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. All newly eclosed wasps were marked on the day of eclosion and left on the nest (with other nest members and brood) for one day. One-day-old males and females were removed from the nest and kept in isolation as described earlier until they were used for the experiments.

In all replicates for each experiment, one male and one female wasp were taken from their isolation bottles and introduced into an aerated transparent plastic bottle similar to the isolation bottle. Behavioural observations were carried out for 60 min or until the behaviour we call "long conjugation (LC)" (see "Results") was seen, whichever was earlier. Observations were made to record all behavioural interactions between the pair of wasps; for each interaction, we recorded the duration of the interaction and the identity of its initiator. After the observation, the females who exhibited either the behaviour we call "short conjugation (SC)" (see "Results") or long conjugation, were dissected immediately to check for the presence of sperm in their spermathecae.

Since males do not leave their natal nests for about a week after eclosion and females have a mean life span of about 30 days, we arbitrarily chose the age group of 5–20 days for both males and females in the first set of experiments ($n = 47$). We found that many pairs in this age group performed mating behaviour. To examine any possible effect of seasonality on mating we recorded mating behaviour in 30 such additional pairs/month in March, September and December. These data were compared with that of the first set of 47 pairs which were conducted in the month of June/July. All mating experiments were conducted outdoors, on the terrace of the laboratory, under whatever temperatures prevailed there. Subsequently, we also tested males and females younger than 5 days and older than 20 days to see the role of age in mating. Age ranges and average ages of tested males and females of each age group are presented in Table 1.

To test the effect of nestmateship, body size and ovarian status we used males in the age group 5–20 days and paired them randomly with either a nestmate (nestmate pairs, $n = 25$) or a non-nestmate female (non-nestmate pairs, $n = 24$) of the same age group. In constituting nestmate pairs, we avoided pairing individuals who were familiar with each other by virtue of having eclosed on the same nest on the same day. We could not conduct choice experiments for either sex with one nestmate and one non-nestmate partner because whenever two wasps of same sex and one wasp of the opposite sex were placed together in a bottle, instead of any attempt to mate the wasps showed physical aggression to each other. Behavioural observations were carried out using the same methods as stated earlier and after the observation, all 98 wasps were measured for 27 body size parameters (Kardile and Gadagkar, 2005). Data on the 27 body size parameters were subjected to principal components analysis and the position of each wasp was plotted in the space of the first two principal components. All the females were subsequently dissected to check the spermatheca for sperm content and their ovarian condition was evaluated by measuring the following: mean length of the proximal oocytes, mean width of the proximal oocytes, total number of oocytes, total number of mature eggs, total number of oocytes with yolk and total number of resorbing oocytes. The measurements from all the ovarioles (assigning a negative value for each resorbing oocyte) were subjected to principal components analysis and the position of each wasp was plotted in the space of the first two principal components.

Table 1 Proportion of pairs showing sperm transfer in each combination of age groups (1–4, 5–20 and >20)

Male age groups	Female age groups		
	1–4 (2.2 ± 0.9)	5–20 (13.4 ± 4.0)	>20 (35.0 ± 10.7)
1–4 (2.3 ± 1.0)	0/5	0/5	0/5
5–20 (12.0 ± 4.5)	0/5	21/47	2/11
>20 (31.4 ± 8.5)	0/5	1/5	7/38

Mean ± SD for ages (in days) of males and females in each age group are presented

All statistical comparisons were carried out using the software packages StatistiXL Version 1.6 and Statistica Release 7.

Results

Among the 47 pairs of wasps of 5–20 days age category, four pairs did not interact. In the 43 interacting pairs, it was always the male that initiated all interactions. Males attempted to mount the females, but the females sometimes escaped without permitting the males to grasp them. In seven pairs, behavioural interactions were restricted to such unsuccessful attempts to mount (UAM). In the remaining 36 pairs mounting (MO) was achieved, although this usually happened after one or more UAMs. MO often led to conjugation of the two wasps, involving interlocking of their abdominal tips. Two types of conjugations were observed—short conjugation (SC) and long conjugation (LC). These two forms of conjugation are different not only in duration of interlocking of the abdominal tips but also in the posture of the wasps. When MO does not lead to LC and stops at SC, it appeared to be the male who took an active part in prematurely terminating the conjugation. This is contrary to the situation in *Polistes major*, where the females are seen to actively terminate the copulation (Wenzel, 1987) and *P. fuscatus*, where females can exercise choice by actively controlling insemination (Post and Jeanne, 1983b). A more detailed description of these behaviours is given in Appendix and for the schematic drawing see Fig. 1.

Behavioural correlate of sperm transfer

Among the 36 pairs that performed mounting, 11 pairs performed only SC and 24 pairs performed only LC or both SC and LC. There was a significantly greater probability of sperm transfer in pairs with LC than in pairs without LC, independent of whether SC occurred or not (Fisher's exact test, $P = 8.72\text{e-}7$) and none of the 11 females who underwent only SC were mated. We therefore conclude that SC is neither necessary nor sufficient for sperm

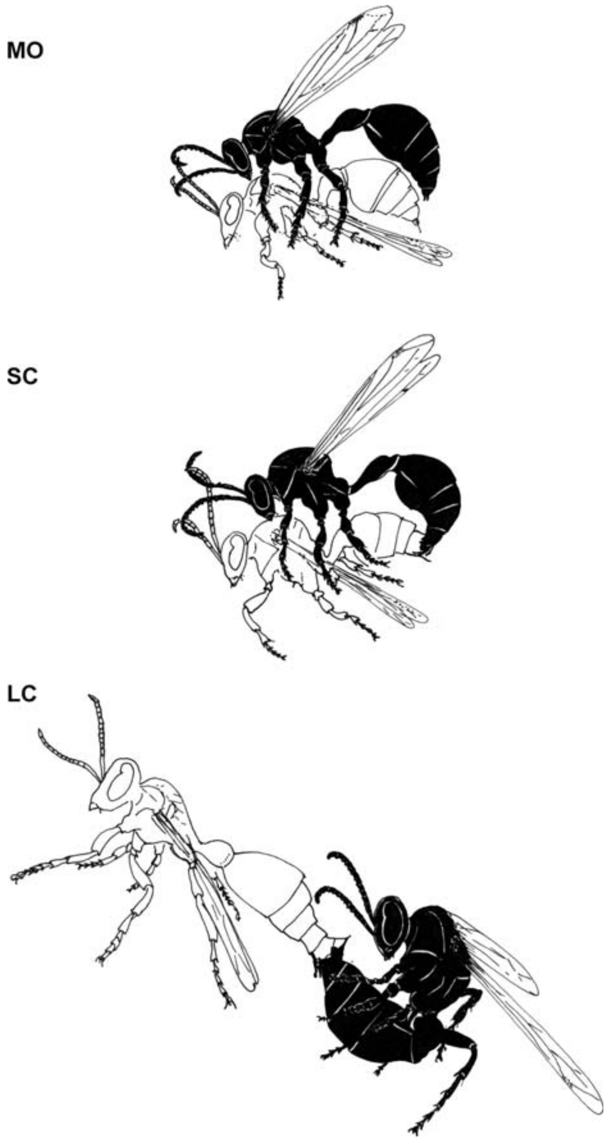


Fig. 1 Schematic drawing of the behaviours involved in mating in *R. marginata*: mount *MO*, short conjugation *SC* and long conjugation *LC*. The longest and most prominent posture of long conjugation behaviour is depicted in the figure. The male attaches its genitalia with the female and turns on its back quickly and remains in that posture till the release. In all figures the male is depicted in *black* and the female is depicted in *white*. Drawing: Thresiamma Varghese. See Appendix for description of the behaviours

transfer. Since 21 out of 24 females who underwent LC were mated, we conclude that LC is a very strong predictor of sperm transfer. We found no other clear behavioural correlate of sperm transfer; numbers and durations of MO and SC and the duration of LC were not significantly different between sperm transferring and non sperm transferring pairs (Mann–Whitney *U* test, $P > 0.008$; α set to 0.008 after Bonferroni correction due to six comparisons; Table 2).

Optimum age for sperm transfer is 5–20 days

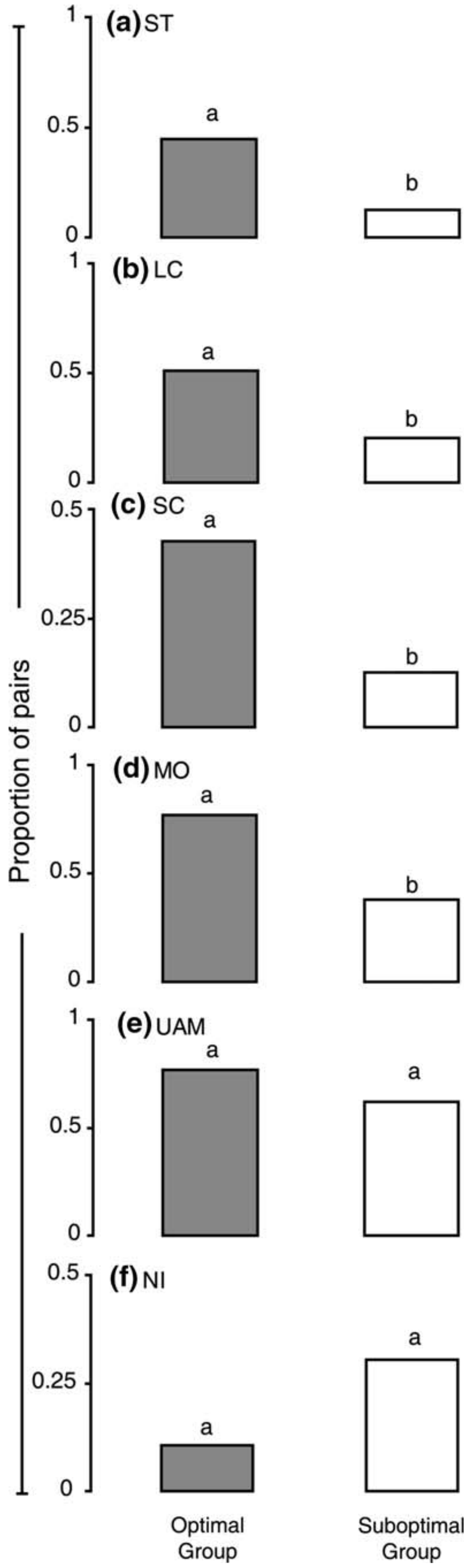
Having observed sperm transfer in 21 out of 47 pairs in which both males and females were in the age group of 5–20 days, we studied an additional set of 79 pairs in which one or both partners were younger (1–4 days) or older (21–65 days). Mating success was not significantly different between any two age group combinations (Fishers exact test, all *P* values more than 0.05). However, since the mating success was visibly higher where both males and females belonged to the 5–20 age groups, than any other combination (Table 1), we compared the mating behaviour of this age group (optimal group) with that of all other age groups pooled (suboptimal group). Considering the full set of 126 pairs (1–65 days), we found that among pairs in which at least one partner was older or younger than 5–20 days (79 pairs), significantly fewer pairs showed sperm transfer, LC, SC as well as MO, compared to the pairs in which both partners were in the age group of 5–20 days (47 pairs) (*G* test for independence; for ST, LC, SC and MO, $P < 0.008$). However, the proportion of pairs not interacting and the proportion of pairs showing UAM were not different in the two groups (*G* test for independence; $P > 0.01$; α set to 0.008 after Bonferroni correction due to six comparisons) (Fig. 2).

Seasonal patterns in mating behaviour

The proportions of pairs that showed LC did not differ during March, June/July and September, but there were significantly fewer matings in December. The same trend

Table 2 Comparison of different behaviours in sperm transferring and non sperm transferring pairs

Behaviour	Sperm transferring pairs (mean ± SD, *n*)	Non sperm transferring pairs (mean ± SD, *n*)
Number of unsuccessful attempts to mounts (UAM) per pair	2 ± 2.1, *n* = 21	3.4 ± 4.6, *n* = 26
Number of mounts (MO) per pair	1.47 ± 0.81, *n* = 21	0.92 ± 1.1, *n* = 26
Number of short conjugations (SC) per pair	0.6 ± 0.9, *n* = 21	0.9 ± 1.5, *n* = 26
Duration of MO (in s)	40.9 ± 100.3, *n* = 21	134.1 ± 157.2, *n* = 15
Duration of SC (in s)	3.1 ± 1.0, *n* = 7	3.2 ± 0.9, *n* = 12
Duration of long conjugations (LC) (in s)	38.4 ± 10.7, *n* = 21	35.3 ± 6.7, *n* = 3



◀ **Fig. 2** Proportion of pairs performing **a** sperm transfer *ST*, **b** long conjugation *LC*, **c** short conjugation *SC* **d** mount *MO*, **e** unsuccessful attempt to mount *UAM* and **f** no interaction *NI* in optimal group (where, both partners were 5–20 days-old) and suboptimal group (where, one or both partners were either 1–4 or 21–65 days old). *Bars* carrying *different letters* are significantly different at $P < 0.008$ (*G* test, α set to 0.008 after Bonferroni correction, due to six comparisons)

was observed with reference to SC and MO, but the opposite was true for no interaction (NI). Significantly larger proportion of pairs performed unsuccessful attempts to mount (UAM) in June/July compared to other months (Fig. 3). We did not expect mating to be influenced by season, but our hypothesis 1 was not entirely supported because wasps mated through most of the year, but the mating probability significantly reduced in the winter months.

No nestmate discrimination during mating

Among nestmate pairs, 17 out of 25 transferred sperm, and among non-nestmate pairs, 15 out of 24 transferred sperm. These proportions are not significantly different from each other (Fisher's exact test, $P = 0.76$). The nestmate and non-nestmate pairs were also behaviourally indistinguishable; numbers of UAM, MO, and SC per pair, durations of MO and LC and latency to show MO and LC (Table 3) were all similar (Mann–Whitney *U* test, all *P* values greater than 0.2). Therefore, our hypothesis 2 is supported and *R. marginata* wasps do not seem to use any recognition-based mechanism to prevent mating between the nestmates.

Body size did not influence sperm transfer

Since there was no nestmate discrimination during mating, here we pooled the body size data of sperm transferring and non sperm transferring pairs in the 49 nestmate and non-nestmate pairs. Both males and females in the sperm transferring pairs were not different from the corresponding sex in the non sperm transferring pairs, by any measure of body size (see "Materials and methods") (Table 4, Student's *t* test, all *P* values greater than 0.04, α set to 0.001 after Bonferroni correction). Although males and females formed distinct clusters in the two-dimensional principal components space, there were no distinct clusters for males transferring and not transferring sperm or for females receiving and not receiving sperm (Fig. 4). Therefore, our hypothesis 3 is supported and body size does not influence sperm transfer.

Ovarian state of females did not influence sperm transfer

To examine the possible effect of the ovarian state of the female wasps on sperm transfer, we compared the ovarian

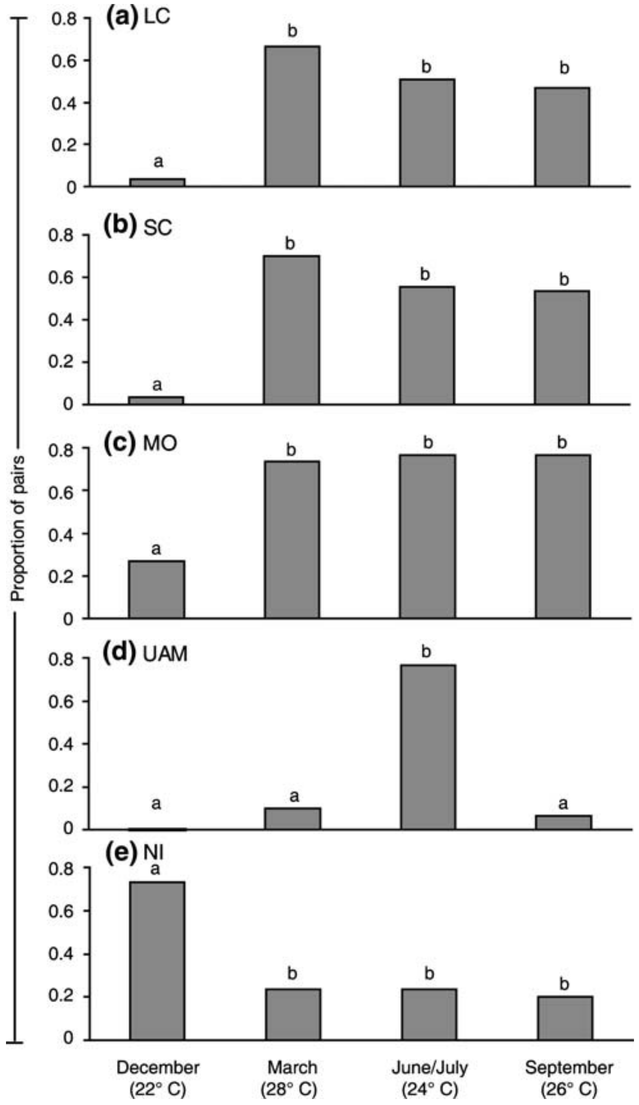


Fig. 3 Proportion of pairs performing **a** long conjugation *LC*, **b** short conjugation *SC* **c** mount *MO*, **d** unsuccessful attempt to mount *UAM* and **e** no interaction *NI* in different times of the year. The mean monthly temperatures for the relevant months, as obtained from the Meteorological Department are shown below the name of the month. *Bars* carrying *different letters* are significantly different at $P < 0.008$ (Fisher's exact test, α set to 0.008 after Bonferroni correction, due to six comparisons)

Table 3 Comparison of different behavioural parameters in nestmate and non-nestmate pairs

Behavioural parameters	Nestmate pairs	Non-nestmate paris
Number of UAM per pair	1.1 ± 0.4	1.9 ± 1.3
Number of MO per pair	1.1 ± 0.2	1.1 ± 0.5
Number of SC per pair	0.6 ± 0.5	1.1 ± 1.6
Duration of MO (in s)	29.3 ± 75.9	56.5 ± 84.9
Duration of LC (in s)	54.5 ± 23.7	44.2 ± 24.7
Latency to MO (in s)	1025.6 ± 603.9	955.7 ± 450.1
Latency to LC (in s)	1051.9 ± 600.7	997.8 ± 471.8

profiles of females in sperm transferring and non sperm transferring pairs in the 49 nestmate and non-nestmate pairs, used above for studying the effect of nestmateship and body size. Females receiving sperm were not different from those not receiving sperm, by any measure of ovarian condition (see "Materials and methods") (Student's *t* test, all *P* values greater than 0.17; α set to 0.007 after Bonferroni correction). In the two-dimensional principal components space, females receiving and not receiving sperm did not cluster separately (Fig. 5). Thus, our hypothesis 4 was supported implying ovarian status of the females does not play a role in sperm transfer.

Discussion

We have shown that behavioural observations permit a reasonably accurate prediction of whether conjugating pairs of *R. marginata* have mated successfully (transferred sperm). It is safe to conclude that pairs showing only SC are not mated. By assuming that pairs showing LC have mated, we have only a small probability of error which can subsequently be eliminated by dissecting the females after completion of the experiment requiring live, mated wasps. Our finding that 5–20 days is the optimal age for mating and that males and females older or younger than 5–20 days have significantly lower probability of mating, made it easier for us to test our four hypotheses.

It is not surprising that males younger than 5 days do not mate because they do not leave their natal nests at this age and mating does not take place on the nest. Females below the age of 5 days do leave the nest for short durations, but they appear to be making reconnaissance flights and not true foraging flights as nothing is brought back. Since there is such a small chance that young males and females would encounter the opportunity to mate, the trait of mating at an early age may not have evolved in this wasp. It is possible that young wasps (1) are not physiologically prepared to mate or (2) are not capable of exhibiting themselves as potential mating partners and thus fail to attract the opposite sex. Why males and females older than 20 days mate at such low probability under our laboratory conditions is less clear. Although many males live longer than 20 days in the laboratory (Sen and Gadagkar, submitted), this may not be true in nature. For females, we expect that selection should favour early mating as this would make a long period of diploid egg laying possible. In *Polistes fuscatus*, males have been reported to be attracted to freshly freeze-killed females of ages ranging from zero to more than 14 days, although neither mating behaviour nor sperm transfer were studied (Post and Jeanne, 1985).

The probability of mating seems to reduce in laboratory-reared wasps in winter (December). This probably reflects

Table 4 Mean and SD of different body size parameters in males and females who did and did not take part in sperm transfer

Body size parameter	Mated female	Unmated female	Mated male	Unmated male
Interocellar distance (IOD)	0.31 ± 0.02	0.31 ± 0.03	0.33 ± 0.04	0.33 ± 0.03
Right ocello-ocular distance (OODR)	0.56 ± 0.03	0.58 ± 0.02	0.48 ± 0.03	0.47 ± 0.03
Left ocello-ocular distance (OODL)	0.54 ± 0.02	0.55 ± 0.03	0.47 ± 0.04	0.45 ± 0.03
Head width (HW)	3.2 ± 0.14	3.22 ± 0.11	3.1 ± 0.13	3.1 ± 0.08
Head length (HL)	2.77 ± 0.11	2.76 ± 0.1	2.46 ± 0.13	2.46 ± 0.18
Width of mesoscutum (MSCW)	2.18 ± 0.11	2.16 ± 0.13	2.15 ± 0.19	2.09 ± 0.07
Length of mesoscutum (MSCL)	2.45 ± 0.15	2.48 ± 0.13	2.32 ± 0.16	2.30 ± 0.12
Alitrunk length (ATL)	3.4 ± 0.34	3.48 ± 0.23	3.32 ± 0.2	3.36 ± 0.20
Length of right wing (WLR)	9.82 ± 0.60	9.98 ± 0.53	9.67 ± 0.50	9.58 ± 0.47
Length of left wing (WLL)	9.86 ± 0.62	9.98 ± 0.49	9.64 ± 0.57	9.57 ± 0.5
Length of first marginal cell of right wing (LM1R)	4.9 ± 0.27	4 ± 0.21	4.76 ± 0.31	4.7 ± 0.25
Length of first marginal cell of left wing (LM1L)	4.91 ± 0.29	5.03 ± 0.21	4.76 ± 0.32	4.71 ± 0.24
Number of hammuli on right wing	12.2 ± 1.3	12.5 ± 0.7	11.8 ± 1.4	11.6 ± 1.0
Number of hammuli on left wing	12.3 ± 1.3	12.7 ± 1.1	11.9 ± 1.3	11.7 ± 1.1
Length of first gastral segment (L1GS)	2.54 ± 0.18	2.52 ± 0.24	2.52 ± 0.24	2.44 ± 0.14
Width of first gastral segment (W1GS)	1.37 ± 0.09	1.47 ± 0.37	1.35 ± 0.11	1.38 ± 0.18
Height of first gastral segment (H1GS)	1.23 ± 0.09	1.24 ± 0.11	1.20 ± 0.1	1.17 ± 0.10
Length of second gastral segment (L2GS)	3.71 ± 0.38	3.93 ± 0.37	3.59 ± 0.34	3.63 ± 0.28
Width of second gastral segment (W2GS)	3.17 ± 0.19	3.19 ± 0.23	3.17 ± 0.24	3.05 ± 0.14
Height of second gastral segment (H2GS)	3.03 ± 0.24	3.07 ± 0.15	2.99 ± 0.16	2.95 ± 0.11
Clypeus length (CL)	1.36 ± 0.08	1.38 ± 0.08	1.16 ± 0.07	1.17 ± 0.06
Clypeus width (CW)	1.43 ± 0.06	1.46 ± 0.05	1.27 ± 0.06	1.25 ± 0.04
Length of first segment of right antenna (L1FSR)	0.74 ± 0.02	0.74 ± 0.03	0.73 ± 0.03	0.73 ± 0.03
Length of first segment of left antenna (L1FSL)	0.73 ± 0.03	0.74 ± 0.03	0.73 ± 0.03	0.73 ± 0.03
Width of first segment of right antenna (W1FSR)	0.27 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
Width of first segment of left antenna (W1FSL)	0.27 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.26 ± 0.02
Inter-antennal socket distance (IASD)	0.39 ± 0.02	0.39 ± 0.02	0.36 ± 0.02	0.35 ± 0.02

All measurements are represented in millimetres except the numbers of hamuli in left and right wings

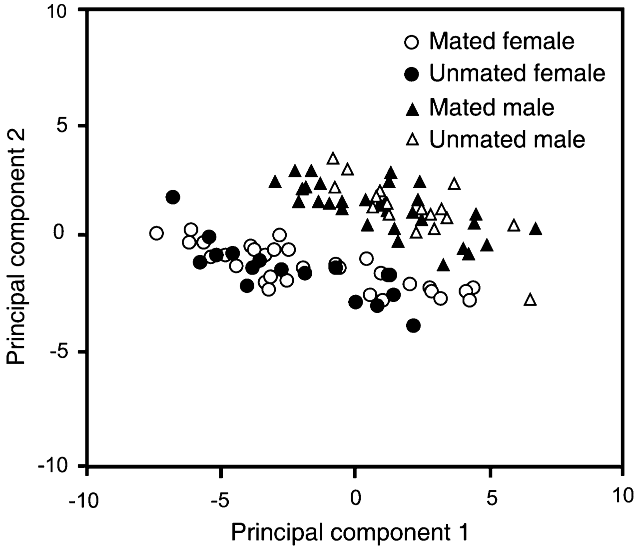


Fig. 4 Body size of mated and unmated males and females, plotted in two-dimensional principal components space. Note that while males and females are clustered separately, neither mated males nor mated females are well separated from their unmated counterparts

the situation in nature. The fact that very few nests are initiated during December to February (Shakarad and Gadagkar, 1995) can be a result of absence of abundant mated females during that time.

Nestmate discrimination among females is well developed in *R. marginata* (Venkataraman et al., 1988). However, as mating never takes place on the nest and outbreeding is facilitated by mating away from the nest, it is not surprising that these wasps have not developed the ability to discriminate against nestmates during mating in the laboratory context. Similar lack of nestmate discrimination in the context of mating has also been reported in *P. fuscatus* (Larch and Gamboa, 1981; Post and Jeanne, 1983a; Post and Jeanne, 1983b). Our observations suggest that males terminate the mating and also force-mate with the females. Therefore female choice may not exist in this species.

Since the wasps in our experiments did not have a choice between different mating partners, it is not surprising that body size did not influence the probability of

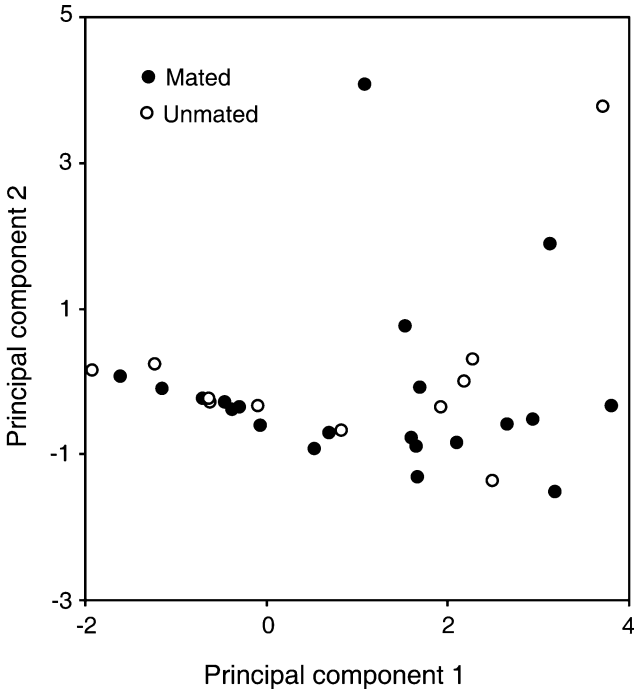


Fig. 5 Ovarian profile of mated and unmated females, plotted in two-dimensional principal components space. Note that mated and unmated females are not separated on the basis of their ovarian profiles

mating. Furthermore, since body size does not play a significant role in a female's chance to become a queen, it probably should not influence the probability of sperm transfer. It may appear surprising that ovarian condition of the females also did not influence the probability of mating. However, in *R. marginata* females, ovarian development and mating appear to be delinked. On the one hand, workers with poorly developed ovaries may be mated. On the other hand, females with fully developed ovaries, indeed females who are functioning as the sole egg layers of their colonies may sometimes be unmated (Chandrashekara and Gadagkar, 1991).

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Appendix: Description of the mating related behaviours

Unsuccessful attempt to mount (UAM): The male approaches the female and tries to climb onto the female but is unsuccessful because the female flies away.

Mount (MO): The male climbs onto the back of the female and holds her with his fore and hind limbs. The female crouches and folds her wings under her abdomen with her antennae pointed towards the substrate. The male vigorously antennates the female's head and antennae, and drums the female's abdomen repeatedly with his stretched abdomen and/or rubs his abdomen against the female's abdomen with clockwise and anticlockwise movements. Subsequently the female's antennae point upwards and the male's antennal tips become curved and brush the flat antennae of the female repeatedly.

Short conjugation (SC): With his curved abdomen the male lifts the female's abdomen upward. The cerci protrude from the male's abdominal tip and their abdominal tips touch briefly (for a maximum of five seconds). Through out this behaviour the male remains mounted on the female and terminates the behaviour by releasing the female and flying away.

Long conjugation (LC): This behaviour starts like the short conjugation but instead of the male releasing the female, he protrudes his aedeagus into the female's abdomen. Subsequently he releases his grip on female and turns on his back and remains locked with the female through an abdominal connection which lasts for at least 20 seconds. The female might move about, dragging the male in this posture but the connection remains intact

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