NESTMATE DISCRIMINATION IN THE SOCIAL WASP *Ropalidia marginata*.

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INTRODUCTION

The genetical theory of evolution (Hamilton, 1964a,b) has revolutionized our approach to the study of interaction between animals. The prominent role ascribed by Hamilton to kinship between the partners in any interaction was rapidly appreciated and widely applied (Wilson 1975). Although the question of whether animals have any direct means of assesing kinship amongst themselves remained untested for many years, it is now quite clear that kin recognition is a very widespread phenomenon. The ability to discriminate kin from non kin has been reported in a marine invertebrate, subsocial arthropods,a sweat bee, honey bees, several species of ants and wasps, frogs, toads and a variety of mammals (reviews in, Fletcher and Michener, 1986; Gadagkar,1985; Gamboa *et al* 1986; Hepper, 1986; Holldobler and Michener, 1980; Linnshenmeir, 1985; Page, 1986),for marine invertebrate see Grosberg and Quinn 1986;

In many North American species of the social wasp *Polistes*, inseminated females overwinter in hibernacula away from their natal nests. Some species such as *P. exclamans* hibernate in groups of nestmates (Allen *et al* 1982). In others such as *P. fuscatus* at least some former nestmates are known to come together to found multiple foundress colonies in the following spring;(Klahn, 1979; Noonan,1981; West-Eberhard 1969). The ability to discriminate nestmates from non nestmates outside the context of the nest is therefore clearly of advantage and such ability has now been demonstrated in several North American species of wasps (Allen *et al* 1982; Bornais *et al* 1983; Gamboa *et al* 1985; 1986; Pfennig *et al* 1983a,b; Post and Jeanne, 1972; Ross and Gamboa, 1981; Ryan *et al* 1985; Shellman and Gamboa 1982). In the tropics on the other hand, there is normally no need for wasps to recognize their nestmates outside their nests. Nor is there typically any long period of isolation from nestmates. Any advantage of the ability to discriminate nestmates outside the context of their natal nests is thus not readily obvious and such an ability has not so far been demonstarted for any tropical wasp. We have therefore investigated the possibility that wasps of the tropical species *Ropalidia marginata* discriminate nestmates from non nestmates outside the context of their nests. We show in this report that *R. marginata* females can indeed make such a discrimination provided they have been exposed upon emegence to their natal nests and nestmates. Wasps who have undergone such exposure will then also recognize those nestmates with whom they may have never come into contact.

MATERIALS AND METHODS

The study animal

Ropalida marginata (Hymenoptera: Vespidae: Polistinae) is a very common paper wasp in peninsular India whose biology and social organization have been the subject of recent investigations (Gadagkar *et al* 1982; Gadagkar, 1980; Gadagkar and Joshi, 1982; 1983)

Collection of Nests.

23 naturally occuring nests of *R. marginata* were collected from 4 different localities situated within a radius of 150 km around Bangalore ($13^{o}00'$N and $77^{o}32'$E).The adults were individually picked off the nest which was then was removed from its substratum. At the time of collection the nests had 1-68 adults (mean ± s.d = 33 ± 18 adults)and

7-126 pupae (36 ± 25).

Preparation of Experimental animals.

Wasps to be used as experimental animals were then subjected to one of the following four treatments.

(i) The females picked off the nest at the time of collection were brought back to the laboratory and individually isolated for 8-45 days (mean ± s.d = 28 ± 17; n=52) in 22 X 11 X 11 cm ventilated plastic jars.

(ii) Some nests with the brood were cut into 2 halves and each half was positioned in a plastic box identical to the one mentioned above. Females emerging from these nest fragments were allowed to remain on their respective halves until the last animal emerging was at least five days old. Following this treatment animals were individually isolated in plastic boxes for at least a week before the experiments began.

(iii) Other nests were monitored continuously and the females eclosing were immediately (within 1 or 2 minutes) separated and individually isolated for 6-48 days (mean ± s.d = 20 ± 13; n= 42).

(iv) For yet other nests, females were artificially removed from their pupal cases, about 24 hours prior to their time of expected natural eclosion. These animals were covered with tissue paper in a petri-plate and allowed to complete their development in an incubator maintained at a temperature of 27 ± 2°C. Such femlales remained in the incubator for 16-49 hours (25 ± 7; n=51) and were then individually isolated for 6-20 days (12 ± 4; n=50).

All animals were fed on an *ad libitum* diet of *Corcyra cephaloni-*

_ca_ larvae, honey and tap water. Care was taken to ensure that animals used in an experiment were provided honey and water from the same source.

The Triplet Assay.

A triplet assay similar to the one used by Shellman and Gamboa (1983) was used to assay nestmate discrimination. Each triplet consisted of two nestmates and one non nestmate marked with small unique spots of quick drying enamel paints of a single color. The non nestmate was from a nest collected 8 km or more away from the first nest . One hour prior to the commencement of observations the three animals of an experiment were introduced into a ventilated plastic observation box of similar dimension as mentionesd above. Continuous observations were made to record all interactions between all pairs of animals between 9 AM and 6 PM on two consecutive days for a total period of 12 hrs. In all we have recorded 15 behaviours (table 1). A total of 61 experiments (triplets) were performed, 14 using animals present on the nest at the time of collection but isolated later (treatment 1), 15 with animals eclosing in the laboratory and being exposed to their natal nests and nestmates (treatment 2), 15 with animals isolated immediately upon emergence (treatment 3) and 17 with animals isolated prior to their natural eclosion and allowed to complete development in an incubator. This amounted to a total of 732 hours of observation. In experiments using animals eclosing in the laboratory and being exposed to their natal nests and nestmates (treatment 2) the two nestmates in any experiment were from the same natal nests but had not interacted with each other as they were from two different halves of the nest.

Table 1

The 15 behaviours observed ranked by increasing order of of tolerance.

	Behaviour		Rank of Tolerance
Block I	Agressive biting	(AB)	1
	Attack	(AT)	2
Block II	Peck	(PK)	3
	Chase	(CH)	4
	Agressive mutual antennation*	(AM)	5
	Nibble	(NI)	6
	Crash	(CR)	7
	Falling fight*	(FF)	8
Block III	Avoid	(AV)	9
	Solicit	(SC)	10
	Mutual approach with withdrawal	(AA)	11
	Approach (while the other withdraws)	(AW)	12
	Approach (while the other does not withdraw)	(AP)	13
Block IV	Antennate	(AN)	14
	Mutual Antennation*	(MA)	15

*Indicates bidirectional behaviours.

Definitions of Parameters used

The six possible types of interactions (in an experiment with three animals) are designated a_i, b_i, c_i, d_i, e_i, and f_i such that a_i is the number of times the ith behaviour was performed by Nestmate 2 towards Nestmate 1, b_i is the number of times the ith behaviour was performed by Nestmate 1 towards Nestmate 2 and so on as shown in Fig.1. When bidirectional behaviours were involved, both animals were said to have performed them . From the parameters a_i to f_i six other parameters were calculated as follows.

$$X_i = a_i + b_i$$

$$Y_i = c_i + d_i$$

$$Z_i = e_i + f_i$$

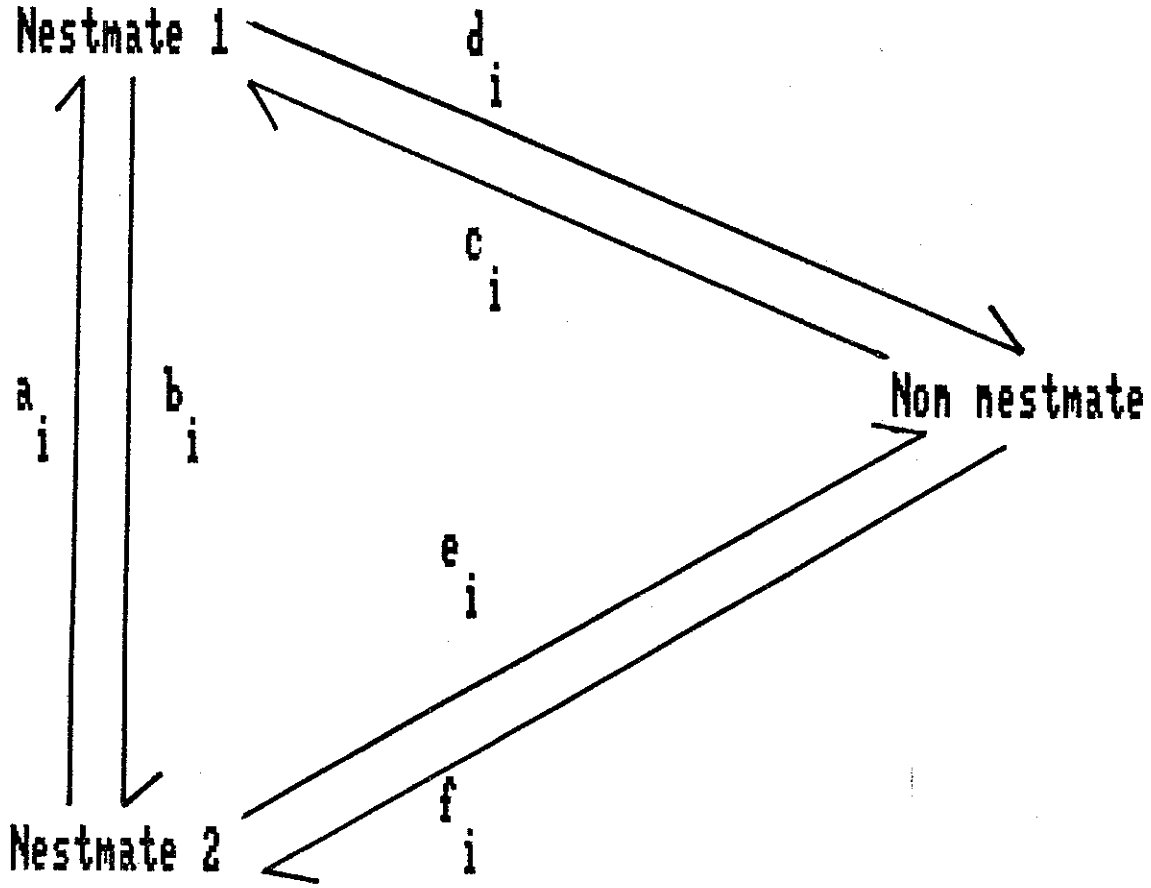
$$\epsilon_i = (Y_i + Z_i)/2$$

$$\beta_i = c_i + f_i$$

$$\gamma_i = d_i + e_i$$

Thus X_i is the sum of interactions between nestmates, Y_i and Z_i are the sums of interactions between each of the two pairs of non nestmates respectively, ϵ_i is the interaction between non nestmates corrected for the fact that there are four types of non nestmate pairs as opposed to a single type of nestmate pair, β_i is the interaction

Fig.1 The Triplet Assay



initiated by the lone non nestmate towards either of two other animals and γ_i is the interaction initiated by either of the two nestmates toward the lone nestmate. These parameters were used in χ^2 analysis (see below). Nestmate discrimination was also tested by looking for differences in tolerance between nesetmates and non nestmates. To do this Tolerance Indices T_a, T_b, T_c, T_d, T_e and T_f corresponding to each of the six types of interactions are calculated as shown in the example below.

$$T_a = \sum_{i=1}^{15} a_i r_i$$

where r_i is the tolerance rank of the ith behaviour (Table 1). From the six tolerance indices thus obtained six other tolerance indices were again calculated as follows

$$T_X = T_a + T_b$$

$$T_Y = T_c + T_d$$

$$T_Z = T_e + T_f$$

$$T_\alpha = (T_Y + T_Z)/2$$

$$T_\beta = T_c + T_f$$

$$T_\gamma = T_d + T_e$$

Data Analysis

The ability to discriminate nestmates from non nestmates was assesed both by examining differences between the patterns of interactions using a test (by pooling data from all experiments pertaining to a particular type of experiment (treatment))and by comparing tolerance indices among the experiments of a particular type (treatment) by the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956; Sokal and Rohlf, 1981).

Our null hypotheses were that interactions among nestmates (X_i) were indistinguishable from interactions among non nestmates. $(\mathcal{C}_i)$ and that tolerance among nestmates (T_x) was not significantly different from tolerance among non nestmates $(T_{\mathcal{C}})$. In the triplet assay two out of three animals are in the company of a nestmate while the third animal is not. To test if the fact of being a lone animal itself causes differences in behaviour we tested additional null hypotheses that the interactions initiated by the lone animal towards the other two (β_i) are indistinguishable from the interactions initiated by the two nestmates towards the lone non nestmate (γ_i) and similarly that the tolerance shown by the lone animal towards the two others (T_β) is not significantly different from the tolerance shown by the two nestmates towards the lone non nestmate (T_γ).

To ensure that differences in age and body size are not sufficient to explain our results we matched the animals used in an experiment as closely as possible to minmize differences in age and body size. Any residual effects of age and body size was examined by testing the following six additional null hypothesis both by the test as well as the Wilcoxon matched pairs signed ranks test.

(1,2) The older (larger) of the two animals available for interaction is treated differently from the younger (smaller) of the two.

(3,4) Animals older (larger) than oneself are treated differently then animals younger (smaller) than oneself.

(5,6) Animals closer to oneself in age (body size) are treated differently than animals farther from oneself in age (body size).

Nestmates appear to be more tolerant of each other than non nestmates,provided discrimination takes place> the differences the tolerance indices between nestmates and non nestmates were therefore examined by a one tailed test. All other tests were two-tailed.

Results

Female wasps of R.marginata present on the nest at the time of collection but later isolated from nestmates for several days (treatment 1) strongly discriminate nestmates from non netsmates. Interactions between nestmates (X_i) are significantly different from those between non nestmates ($\mathcal{C}_i$) (= 53.52; df = 7; $p<0.001$) (Table 2). Nestmates are significantly more tolerant of each other than are non nestmates ($T_X > T_{\mathcal{C}}$) ($p = 0.0055$; $n = 14$; $T = 12$) (Table 3). In these experiments nestmates had both familiarity with each other (by virtue of having been present on the nest at the time of collection) as well as genetic relatedness (by virtue of being nestmates) to aid in discrimination. That genetic relatedness alone is sufficient and that familiarity is not necessary for discrimination is shown by experiments involving animals eclosing in the laboratory and then being

Table 2: Comparison of interactions among nestmates with interactions among non nestmates.

TREATMENT	1		2		3		4	
BEHAVIOUR	X	ALPHA	X	ALPHA	X	ALPHA	X	ALPHA
AB	-	-	-	-	-	-	2	2
AT	4	16	51	16	10	4	2	13
PK	106	294	238	416	262	339	315	345
CH	39	119	173	186	188	181	151	115
AM	-	-	-	-	-	-	12	11
NI	5	10	3	0	1	0	1	0
CR	5	22	13	19	1	0	1	0
FF	4	8	44	2	0	3	-	-
AV	-	-	-	-	-	-	120	92
SC	1	2	3	0	2	1	0	2
AA	18	6	12	14	12	4	2	6
AW	101	131	320	473	195	186	143	221
AP	72	52	191	53	57	49	42	52
AN	97	52	194	62	45	28	16	14
MA	154	97	254	74	66	14	24	22
degrees of freedom	7		9		6		6	
chi-square value	53.52		192.05		21.22		6.08	
p value	<0.001		<0.001		<0.005		>0.05	

The behaviours AM,AV,AB were not recorded for treatments 1,2,3. Behaviours were either not observed or not recored for those entries where a dash is indicated. If the expected value for any entry was less than 5 that entries row was deleted from the test.In this table all rows are shown. The degrees of do not include rows where the expected values are lees than 5.

Table 3: Comparison of tolerance among nestmates with tolerance among non nestmates:

Treatment 1

EXPT.NO	X	ALPHA	BETA	GAMMA
1	16.68	16.51	15.99	17.02
2	22.00	11.88	8.88	14.89
3	14.17	13.58	9.49	17.67
4	16.92	12.53	8.68	16.38
7	18.21	17.26	15.48	19.04
9	16.00	9.98	8.86	11.10
12	14.67	13.12	11.84	14.40
13	17.29	13.76	15.60	11.93
30	21.57	6.30	6.60	6.00
33	22.66	9.61	6.98	12.23
34	8.61	12.72	16.42	9.02
39	18.13	10.98	7.90	14.07
53	21.29	15.14	9.62	20.67
55	12.98	15.85	16.39	15.31

X vs alpha	beta vs gamma
N=14	N=14
T=12	T=22
p=0.0055	p=0.0555

exposed to their natal nests and nestmates (treatment 2). Here nestmates were genetically related (by virtue of eclosing from the same nest) but were not familiar with each other (by virtue of eclosing on different halves of the nest). Yet nestmates recognised each other. Interactions between nestmates (X_i) were significantly different from interactions between non nestmates (ξ_i) ($\chi^2 = 192.05$; df = 9; $p<0.001$) (Table 2). Nestmates were again more tolerant of each other than were non nestmates ($T_X > T_\xi$) ($p = 0.0394$; $n = 16$; $T = 34$) (Table 4).

Wasps isolated immediately upon eclosion and not exposed to their natal nests nestmates made, if anything, a feeble discrimination between nestmates and non nestmates. Interactons between nestmates (X_i) were distinguishable from those of non nestmates (ξ_i) ($\chi^2 = 21.22$; df = 6; $p<0.005$) (Table 2) but the tolerances of nestmates and non nestmates were not different from each other ($p = 0.1073$; $n = 16$; $T = 44$) (Table 5). The weak discrimination being made may probably be because fully developed wasps may sit inside their pupal cases and gain a certain amount of exposure to their nests before chewing their pupal caps and coming out. To circumvent this problem we have also conducted experiments with wasps artificially removed from their pupal cases prior to their natural eclosion and allowed to complete their development in an incubator. Such animals failed to discriminate their nestmates from non nestmates. Interactions between nestmates (X_i) were indistinguishable from those between non nestmates (ξ_i) ($\chi^2 = 6.08$; df = 6; $p>0.05$) (Table 6). The tolerance indices between nestmates and non nestmates were also not different from each other ($p> 0.2614$; $n = 17$; $T =63$).

Table 4: Comparison of tolerance among nestmates with tolerance among non nestmates:

Treatment 2

EXPT.NO	X	ALPHA	BETA	GAMMA
44	17.27	9.63	9.35	9.91
45	19.04	18.67	21.52	15.82
46	19.16	5.47	1.00	9.93
47	15.13	16.20	19.67	12.74
48	21.47	17.21	19.86	14.57
49	17.36	18.80	21.40	16.20
50	20.01	16.08	19.88	12.28
51	16.27	12.43	9.71	15.14
52	13.08	12.90	13.90	11.90
58	20.18	15.28	17.00	13.55
59	20.00	13.79	8.45	19.12
60	17.00	15.09	14.69	15.50
61	12.50	16.24	16.47	16.00
62	14.06	20.89	19.19	22.58
77	17.43	18.26	19.04	17.48
78	19.08	17.46	20.17	14.76

X vs alpha	beta vs gamma
N=16	N=16
T=34	T=53
p=0.0394	p=0.4380

Table 5: Comparison of tolerance among nestmates with tolerance among non nestmates:

Treatment 3

EXPT.NO	X	ALPHA	BETA	GAMMA
5	16.00	9.30	7.60	11.00
8	13.25	12.43	6.79	18.07
11	7.69	11.01	10.00	12.02
14	11.11	9.44	9.60	9.28
36	16.78	9.84	7.66	12.03
37	14.62	15.72	18.50	12.93
38	13.56	10.08	9.00	11.16
40	13.33	12.30	7.44	17.17
41	8.37	10.55	8.00	13.09
42	18.93	13.28	9.17	17.38
43	18.00	3.53	7.05	0.00
63	19.18	12.77	10.29	15.25
65	17.05	16.70	18.75	14.66
66	14.50	17.42	20.83	14.01
85	3.16	11.43	8.84	14.02
86	16.16	15.54	18.50	12.58

X vs alpha
N=16
T=44
p=0.1073

beta vs gamma
N=16
T=52
p=0.408

Table 6: Comparison of tolerance among nestmates with tolerance among non nestmates:

Treatment 4

EXPT.NO	X	ALPHA	BETA	GAMMA
89	17.26	17.97	13.87	22.07
90	18.05	12.68	9.61	15.75
92	16.71	23.04	22.38	23.70
94	15.64	15.21	15.50	14.93
95	20.24	16.30	12.75	19.85
96	20.58	17.16	15.49	18.82
97	19.54	19.04	20.87	17.21
98	14.48	13.52	13.73	13.31
103	19.40	20.36	19.33	21.39
104	14.14	15.95	19.40	12.50
105	20.87	13.66	9.18	18.15
106	15.68	19.58	19.72	19.44
107	17.10	19.06	20.99	17.12
108	22.65	16.73	18.58	14.88
109	15.36	18.54	20.98	16.11
110	15.31	16.58	19.25	13.91
111	21.00	14.70	10.82	18.57

X vs alpha
N=17
T=63
p=0.2614

beta vs gamma
N=17
T=64
p=0.5540

The tolerance rank assigned to each behaviour in Table 1, though partly subjective are based on more than 200 hours of observation of this species both in nature as well as under laboratory conditions similar to the ones used in these experimients. Yet, to guard against any remaining subjectivity we have taken the following precaution: The 15 behaviours are grouped into 4 blocks as shown in Table 1. The differences in tolerance levels between two blocks are rather gross so that we are far more confident of the ranking of the blocks. The exact ranks of each behaviour within each block is more subjective. We have therefore randomly shuffled the ranks within each block without affecting the sequence of the blocks and reanalyzed our data. This does not alter our conclusions in any way.

While the advantage of a triplet assay is that interactions between nestmates and non neatmates can be recorded within the same experiment and using the same animals, a disadvantage is its asymmetry. Two animals are nestmates of each other while the third is alone. If this fact itself alters their behaviour, interpretation of our results would be difficult. We have therefore ascertained that the paired nestmates do not behave differently towards the lone non nestmate compared to how the lone non nestmate behaves towards the two nestmates. Both these interactions are of the non nestmate type and any difference between them may be ascribed to the asymmetry in the situation. In all the 4 types of experiments (treatments) tolerance of the two nestmates by the lone non nestmate (T_β) is not significantly different from the tolerance shown by the two non nestmates towards the lone non nestmate (T_γ) (Table 3-6).

We would like to ascribe the observed differences in behaviour between nestmates and non nestmates to the difference in kinship. In order to do so we should guard against the possibility of other differences between the animals leading to the observed results. Although the animals chosen for an experiment were matched as closely as possible for equivalence in age and body size (in the case of animals present on the nest at the time of collection matching was only possible with respect to body size as the ages of the animals were unknown) we have ascertained that any residual difference in age and body size are not sufficient to explain our results. Reanalysis of our data ignoring nestmateship but considering residual differences in age and body size (again in the case of animals present at the time of collection only the effect of body size could be studied as their ages are unknown)show the following. In all our experiments,older or larger animals are not treated differently compared to younger or smaller animals ($p>0.1252$).Similarly animals which are older or larger than oneself are not treated differently compared to those that are younger or smaller than oneself ($p>0.0663$). Finally animals that are relatively closer to oneself in age and body size are not treated differently from those that are relatively distant from oneself ($p>0.3343$). These results strengthen our conclusion that it is differences in kinship that are being recognized.

Discussion

Female *R. marginata* discriminate between nestmates and non nestmates provided they have been exposed upon eclosion to their natal nests and nestmates. Even those nestmates with whom wasps may not have interacted are recognized as nestmates because of exposure to the nest and a subset of nestmates. This result is entirely consistent with what is known about nestmate discrimination in the better studied North American *Polistes* species where it has been demonstrated that exposure to nest or nestmates is essential for nestmate discrimination (Shellman and Gamboa, 1982;Pfennig *et al* 1983a,b).

Nestmate discrimination or kin recognition may be thought of as a process comparing recognition templates carried in the brains of animals with recognition labels carried on the bodies of animals that are being recognized (Gadagkar 1985b; Lacy and Sherman, 1983). Our results suggest that one or both of these (labels and templates) are lacking in animals isolated immediately after or somewhat before natural eclosion and that one or both of these are acquired as a result of exposure to the nest and nestmates. If labels or templates are acquired from the nest or other nestmates and if this is essential for discrimination between nestmates and non nestmates it seems somewhat unlikely that different levels of genetic relatedness within a colony such as that between full and half sisters can be distinguished. The version of Hamilton´s kin selection theory (1964a,b) that attempts to explain the origin of eusociality in Hymenoptera which has sometimes been called the haplodiploidy hypothesis depends very heavily on the higher level of relatedness of workers to their sisters (coefficient of gentic relatedness, $r = 0.75$) compared to their relatedness to their daughters ($r = 0.5$) (see Gadagkar, 1985a, 1985b; Hamilton, 1972; Trivers and Hare, 1976; West-Eberhard, 1975; Wilson, 1971). This asymmetry breaks down however, if queens mate multiply, use sperm from different males randomly and produce several genetic lines of daughters who would be be half sisters ($r = 0.25$) of each other. Workers would now profit instead from raising their own daughters and kin selection would not be as powerful a force in moulding sociality as expected. Besides the known multiple origins of eusocialty in Hymenoptera would not be such a compelling evidence in favour of kin selection theory. These problems can be overcome if workers can discriminate between full- and half- sisters and aid only full sisters thus restoring the required high level of gentic relatedness between workers and the broob they raise (Gadagkar 1985b, Getz *et al* 1982; Page and Metcalf 1982). It is now clear that *R. marginata* queens mate multiply and use sperm randomly thus creating the kinds of difficulties for kin selection theory mentioned above (Muralidharan *et al* 1986). The results presented here suggest that these difficulties are unlikely to be overcome by kin recognition.

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