The mechanism of nestmate discrimination in the tropical social wasp *Ropalidia marginata* and its implications for the evolution of sociality

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Summary. We have demonstrated that females of the primitively eusocial tropical wasp *Ropalidia marginata* can discriminate nestmates from non-nestmates outside the context of their nests. This was accomplished by recording all behavioural interactions in a neutral arena and comparing tolerance levels. In order for these wasps to make such a discrimination, however, it was essential that after eclosion both the discriminated and the discriminating animals were exposed to their respective natal nests and nestmates. The results suggest that both recognition labels and templates are acquired by the animals from sources outside their body, perhaps from their nest or nestmates. It is thus unlikely that different genetic lines within a colony can be distinguished. We conclude, therefore, that genetic asymmetries created by haplodiploidy, but often broken down by multiple mating and polygyny, are not restored by preferential altruism towards full rather than half sisters by means of kin recognition. Hence we recommend caution in ascribing the multiple origins of eusociality in the Hymenoptera to haplodiploidy.

Introduction

The prominent role ascribed by Hamilton (1964) to kinship in any interaction between animals has rapidly been accepted and widely applied (Hamilton 1972; West-Eberhard 1975; Wilson 1971, 1975). Although the question of whether animals have any direct means of assessing kinship among 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 marine invertebrates, subsocial arthropods, sweat bees, honey bees, several species of ants and wasps, fishes, frogs, toads, iguanas, birds and a variety of mammals (reviews in Fletcher and Michener 1986; Gadagkar 1985b; Gamboa et al. 1986a; Hepper 1986; Hölldobler and Michener 1980; Linsenmair 1985; Sherman and Holmes 1985; Waldman 1987); for the iguana see Werner et al. (1987); for the fish see Quinn and Busack (1985); for the marine invertebrates see Grosberg and Quinn (1986) and Keough (1984).

The mechanism of kin recognition has important implications for the theory of kin selection (Gadagkar 1985b). Particularly compelling evidence for this theory has been the strong correlation between (a) haplodiploidy in the Hymenoptera, which creates asymmetries in genetic relatedness potentially favouring the evolution of sociality and (b) the observed multiple origins of eusociality in that insect order (Wilson 1971). This particular subset of kin selection can be called the haplodiploidy hypothesis. These asymmetries in genetic relatedness break down, however, if queens of social insect colonies mate multiply and produce different patrilines of daughters using sperm from different males or if more than one female lays eggs in the colony, thus considerably weakening the haplodiploidy hypothesis. Multiple mating followed by sperm mixing has now been reported in several species of ants, bees and wasps (reviewed in Gadagkar 1985b; Page 1986; Starr 1984). If workers in social insect colonies preferentially aid their full sisters, the original asymmetries in genetic relatedness can be restored, thus supporting the haplodiploidy hypothesis. To do this, however, requires an ability on the part of workers to discriminate between full and half sisters in spite of being habitu-

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ated to both. It has been argued (Gadagkar 1985b) that this is possible only if recognition labels are self-produced and recognition templates are self-based, that is, both labels and templates used in recognition must be produced by each animal as a result of its own genetic and metabolic machinery. If labels and templates are acquired or learned as a result of exposure to nestmates, the nest or other material in the environment, it is unlikely that different genetic lines within a colony will be discriminated. Recent evidence indicates that in the tropical wasp *Ropalidia marginata,* queens mate multiply and use sperm from at least two to three males simultaneously (Muralidharan et al. 1986). Serial polygyny or queen turnover also appears to be quite common (K. Chandrashekara and R. Gadagkar, unpublished observations). It is therefore of considerable importance to know if the asymmetries in genetic relatedness in this species can be restored by kin recognition. Since *R. marginata* is primitively eusocial, this question is even more pertinent. Such questions have often been discussed largely in the context of more highly eusocial insects, such as honey bees and ants. In these insects workers have lost nearly all reproductive options, and it can therefore be argued that asymmetries in genetic relatedness are no longer that crucial.

Our findings show that the female wasp *R. marginata* can discriminate nestmates (normally expected to be close kin) from non-nestmates (normally expected to be unrelated), provided the female has been exposed after eclosion to its natal nest and a subset of nestmates. Our results suggest, however, that the labels used in such discrimination are not self-produced and the templates are non-self-based, thus making it unlikely that different genetic lines within a colony are distinguished.

Methods

The study animal

Ropalidia marginata (Hymenoptera:Vespidae:Polistinae) is a common paper wasp in peninsular India. Its biology and social organization have been the subject of recent investigations (Gadagkar 1980, 1985a; Gadagkar et al. 1982; Gadagkar and Joshi 1983).

Collection of nests

Thirty naturally occurring nests of *R. marginata* were collected from four different localities situated within a radius of 150 km from Bangalore (13°00′ N and 77°32′ E). The adults were individually removed from each nest, which was then removed from its substratum. At the time of collection the nests contained 1–93 adults (37 ± 20), 7–237 pupae (45 ± 45), 6–244 larvae (58 ± 44), 7–260 eggs (80 ± 52) and 21–506 cells (200 ± 136).

Preparation of experimental animals

Wasps selected as experimental animals were subjected to one of the following four treatments.

Treatment I. Females removed from the nest at the time of collection were brought to the laboratory and individually isolated for 8–45 days (28 ± 17; $n = 52$) in $22 \times 11 \times 11$ cm ventilated plastic boxes.

Treatment II. Females that emerged from nests that had been cut into halves and placed in a plastic box identical to the one mentioned above were allowed to remain on their respective halves for 4–28 days (12 ± 10; $n = 90$). Afterwards, they were individually isolated in plastic boxes for 3–62 days (16 ± 10; $n = 90$).

Treatment III. Females that eclosed from continuously monitored nests were immediately (within 1 or 2 min) separated and individually isolated for 6–48 days (20 ± 13; $n = 42$).

Treatment IV. Females artificially removed from their pupal cases (about 24 h prior to their time of expected natural eclosion) were covered with tissue paper, placed in a petri plate and allowed to complete their development in an incubator maintained at a temperature of $26° \pm 2°$ C. Such females remained in the incubator for 11–49 h (23 ± 6; $n = 98$) and then were individually isolated for 6–62 days (20 ± 12; $n = 97$).

All animals were fed an ad libitum diet of *Corcyra cephalonica* larvae, honey and tap water. Care was taken to ensure that the animals in each experiment were provided honey, water and tissue paper (where necessary) from the same source.

The triplet assay

A triplet assay similar to the one used by Shellman and Gamboa (1982) was used to assay nestmate discrimination. Each triplet consisted of two nestmates and one non-nestmate marked with small spots of quick-drying enamel paint of a single color. The non-nestmates were from nests collected 8 km or more from each other. One hour prior to the commencement of observations the three animals of each experiment were introduced into a freshly ventilated plastic box similar to the one mentioned above. Behavioural interactions between all pairs of animals were recorded continuously for 3 h at a time between 9 a.m. and 6 p.m. for 2 consecutive days (total time: 12 h). In all observations the observer was unaware as to which animals were nestmates and which non-nestmates. We recorded 15 types of behavioural interactions and ranked them qualitatively in increasing order of tolerance (or decreasing order of intensity of aggression). Our findings were based on more than 200 h of observation of this species in nature and in the laboratory (Table 1). A total of 92 experiments (triplets) was performed, 14 using wasps present on the nest at the time of collection but isolated later (treatment I), 15 with wasps that eclosed in the laboratory and were exposed to their natal nests and nestmates (treatment II), 15 with wasps isolated immediately upon eclosion (treatment III), 17 with wasps isolated prior to their natural eclosion and allowed to complete their development in an incubator (treatment IV), and 15 and 16 experiments, respectively, of two kinds of mixed triplets (see below). This amounted to 1104 h of observation, during which a total of 15706 behavioural interactions were recorded. In experiments using wasps that eclosed in the laboratory and were exposed to their natal nests and nestmates (treatment II), the two nestmates in each experiment were from the same natal nests but

Table 1. The 15 behaviours observed ranked by increasing order of tolerance

Rank	Behaviour
1	*Aggressive bite:* The most extreme form of aggression seen in this species; sometimes leads to injuries
2	*Attack:* A ritual act of aggression where the dominant animal climbs onto the subordinate and attempts to bite its mouth parts
3	*Peck*
4	*Chase*
5	**Aggressive mutual antennation:* A kind of sparring contest
6	*Nibble:* Relatively mild with little chance of injury
7	*Crash:* The act of crashing into a sitting animal resulting in one or both falling to the ground; very brief and appears much milder than behaviours 1–6
8	**Falling fight:* Two animals grappling with each other and falling to the ground; very brief and appears much milder than behaviours 1–6
9	*Avoid*
10	*Solicit*
11	**Mutual Approach with withdrawal*
12	*Approach I:* The other withdraws
13	*Approach II:* The other does not withdraw
14	*Antennate*
15	**Mutual Antennation*

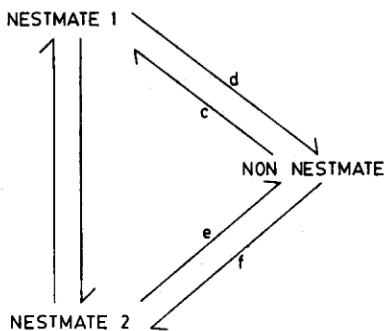* Indicates bidirectional behaviours

Fig. 1. The triplet assay. The behavioural interactions seen in all experiments are classified into six categories, designated a, b, c, d, e, f, such that all interactions initiated by nestmate 2 towards nestmate 1 are assigned to a, all interactions initiated by nestmate 1 towards nestmate 2 are assigned to b, and so on

had not interacted with each other prior to the experiment because they were from two different halves of the nest. Nests were collected as needed to ensure that wasps used for later experiments were not necessarily older and had not necessarily been isolated for longer periods than those used for early experiments. No wasp was used for more than one experiment.

Definitions of the parameters used

The behavioural interactions seen in the experiments can be classified into six categories labelled a–f, as shown in Fig. 1. When bidirectional behaviours occurred, both animals were said to have performed them. Nestmate discrimination was tested by looking for differences in tolerance between nestmates 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 possible categories, were calculated as shown in the example below:

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

where p_i is the proportion of the ith behaviour in a, and r_i is the tolerance rank (Table 1) of the ith behaviour. From the six dyadic tolerance indices thus obtained, two additional tolerance indices were derived as follows: tolerance among nestmates, $T_X = T_a + T_b$; and tolerance among non-nestmates, $T_\alpha = (T_Y + T_Z)/2$, where $T_Y = T_c + T_d$ is the tolerance between one pair of non-nestmates, and $T_Z = T_e + T_f$ is the tolerance between the second pair of non-nestmates.

We used the tolerance index as a measure of discrimination because it combines information from all the behavioural interactions. Such a method has previously been shown to be very sensitive in detecting recognition ability in social wasps (Gamboa et al. 1986a). Our purpose here was not necessarily to assess the importance of nestmate discrimination in the natural life of these wasps but to develop an assay that could be used to compare "normal" animals with those deprived of certain cues. The ultimate aim of this study was to understand the ontogeny of labels and templates that might be used in discrimination.

Mixed triplets

Gadagkar (1985b) outlined a general experimental protocol for distinguishing between self-produced and non-self-produced labels and between self-based and non-self-based templates. The equivalent experimental design is illustrated in Fig. 2 for a triplet assay that assesses the ability of an animal to discriminate between its nestmate and a non-nestmate. In all mixed triplets only two kinds of animals were used: "exposed" animals, exposed to their natal nests and nestmates, and "isolated" animals, isolated from their natal nests and nestmates prior to their expected natural eclosion.

Data analysis

The ability to discriminate nestmates from non-nestmates was assessed by comparison of tolerance indices among nestmates and non-nestmates using the Wilcoxon matched-pairs signed-ranks test and the Mann-Whitney U-test (Siegel 1956).

In all experiments our null hypothesis was that tolerance among nestmates (T_X or T_b) is not significantly different from tolerance among non-nestmates (T_α or T_d). A reasonable alternative hypothesis was that tolerance among nestmates is greater than that among non-nestmates; therefore, we performed one-tailed tests.

We matched the animals used in each experiment as closely as possible in age and body size (as measured by wing length) to ensure that differences in these factors would not influence our results. Such matching was, however, never perfect. We therefore ruled out any residual effects of age and body size by checking for the possibility that discrimination was actually being made on the basis of residual differences in these factors rather than on nestmateship. Here our null hypotheses were
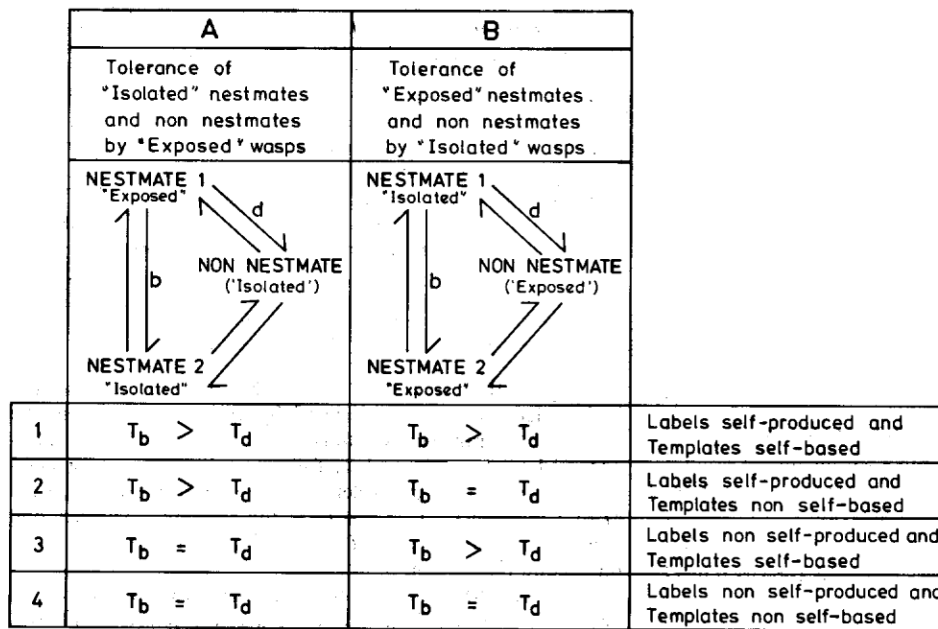
	A	B	
	Tolerance of "Isolated" nestmates and non nestmates by "Exposed" wasps	Tolerance of "Exposed" nestmates and non nestmates by "Isolated" wasps	
	NESTMATE 1 "Exposed" → d → NON NESTMATE ('Isolated') → b → NESTMATE 2 "Isolated"	NESTMATE 1 "Isolated" → d → NON NESTMATE ('Exposed') → b → NESTMATE 2 "Exposed"	
1	$T_b > T_d$	$T_b > T_d$	Labels self-produced and Templates self-based
2	$T_b > T_d$	$T_b = T_d$	Labels self-produced and Templates non self-based
3	$T_b = T_d$	$T_b > T_d$	Labels non self-produced and Templates self-based
4	$T_b = T_d$	$T_b = T_d$	Labels non self-produced and Templates non self-based

Fig. 2. Experimental design of mixed triplets aimed at distinguishing between self-produced and non-self-produced labels and self-based and non-self-based templates. In part A the ability of an "Exposed" wasp to discriminate between a nestmate and a non-nestmate both of whom are "Isolated" is assessed. Conversely, in part B the ability of an "Isolated" wasp to discriminate between a nestmate and a non-nestmate both of whom are "Exposed" is assessed. The six possible types of interactions between the three animals in these triplets are similar to those in Fig. 1, but only the relevant interactions, i.e., b and d, are labelled. Discrimination is again assessed by comparison of tolerance indices calculated for b and d as in the previous set of experiments. "Exposed" wasps are expected to be normal and thus possess both labels and templates regardless of whether or not the labels and templates are self-produced and self-based. "Isolated" animals on the other hand, are expected to possess the appropriate labels and templates only if they are self-produced and self-based. These animals are assumed to lack the labels and templates if these have to be learned or acquired after eclosion from sources outside one's own body.

Here we disregard the possibility that labels and templates may be acquired from sources outside one's own body prior to eclosion as this is clearly not the case in our system (see Results). Thus if "Exposed" wasps can discriminate between "Isolated" nestmates and non-nestmates, labels must be self-produced (column A, rows 1 and 2) and if they cannot, they must be non-self-produced (column A, rows 3 and 4). This is because "Exposed" animals are expected to have both labels and templates, and their inability to discriminate suggests lack of labels on the bodies of the "Isolated" animals. Similarly, if "Isolated" wasps can discriminate between "Exposed" nestmates and non-nestmates, templates must be self-based (column B, rows 1 and 3), and if they cannot discriminate, then templates must be non-self-based (column B, rows 2 and 4). This is once again because "Exposed" wasps are expected to have both labels and templates and if they are not discriminated, it suggests lack of templates in the "Isolated" wasps. This experimental design therefore permits distinction among all four possible combinations of labels being self-produced or non-self-produced and templates being self-based or non-self-based

that (a) the older or larger of the two wasps available for interaction was not treated differently from the younger or smaller of the two, (b) a wasp did not treat animals older or larger than itself differently from those younger or smaller than itself, and, (c) a wasp did not treat animals closer to itself in age or body size differently from those distant to itself in age or body size. These hypotheses were tested by performing two-tailed Wilcoxon matched-pairs signed-ranks tests.

Results

Female wasps of *R. marginata* present on the nest at the time of collection but later isolated from their nest for several days (treatment I) strongly discriminated nestmates from non-nestmates. Nestmates were significantly more tolerant of each other than non-nestmates $(T_x > T_a)$ $(P = 0.006,$

Wilcoxon matched-pairs signed-ranks test and $P = 0.001$, Mann-Whitney U-test; $n = 14$; Table 2). In these experiments nestmates were familiar with each other (by virtue of having been present on the same nest at the time of collection) and were genetically related; they also had past exposure to common cues (by virtue of having been nestmates) to aid in discrimination. The experiments involving wasps that eclosed in the laboratory and then were exposed to their natal nests and nestmates (treatment II) showed that genetic relatedness and past exposure to common cues alone are sufficient for discrimination and that familiarity is not necessary. Here nestmates were genetically related and had past exposure to at least some common cues (by virtue of having eclosed from the same nest)

Table 2. Comparison of tolerance indices by the Wilcoxon matched-pairs signed-ranks and Mann-Whitney U-tests. T_x = tolerance among nestmates. T_α = tolerance among non-nestmates. In both treatments T_x is significantly greater than T_α in both statistical tests. Sample sizes, means, standard deviations and levels of significance are given at the end of the table

Wasps present on the nest at the time of collection and subsequently isolated for several days (treatment I)			Wasps that eclosed in the laboratory but were exposed to half of their nest and a subset of nestmates (treatment II)		
Experiment no. T_x	T_α	T_x-T_α	Experiment no. T_x	T_α	T_x-T_α
1 16.68	16.51	+ 0.17	44 17.27	9.63	+ 7.64
2 22.00	11.88	+10.12	45 19.04	18.67	+ 0.37
3 14.17	13.58	+ 0.59	46 19.16	5.47	+13.69
4 16.92	12.53	+ 4.39	47 15.13	16.20	− 1.07
7 18.21	17.26	+ 0.95	48 21.47	17.21	+ 4.26
9 16.00	9.98	+ 6.02	49 17.36	18.80	− 1.44
12 14.67	13.12	+ 1.55	50 20.01	16.08	+ 3.93
13 17.29	13.76	+ 3.53	51 16.27	12.43	+ 3.84
30 21.57	6.30	+15.27	52 13.08	12.90	+ 0.18
33 22.66	9.61	+13.05	58 20.18	15.28	+ 4.90
34 8.61	12.72	− 4.11	59 20.00	13.79	+ 6.21
39 18.13	10.98	+ 7.15	60 17.00	15.09	+ 1.91
53 21.29	15.14	+ 6.15	61 12.50	16.24	− 3.74
55 12.98	15.85	− 2.87	62 14.06	20.89	− 6.83
			77 17.43	18.26	− 0.83
			78 19.08	17.46	+ 1.62
14 17.23	12.80	4.42	16 17.44	15.28	2.16
±3.92	±2.97	±5.67	±2.67	±3.82	±4.84

$T=12$, $P=0.006$, Wilcoxon matched-pairs signed-ranks test; $U=32$, $P=0.001$, Mann-Whitney U-test	$T=34$, $P=0.039$, Wilcoxon matched-pairs signed-ranks test; $U=79$, $P<0.05$, Mann-Whitney U-test

but were not familiar with each other (by virtue of having eclosed on separate halves of the same nest). Yet, nestmates clearly recognized each other since nestmates were significantly more tolerant of each other than non-nestmates ($T_x > T_\alpha$; $P=0.039$, Wilcoxon matched-pairs signed-ranks test and $P < 0.05$, Mann-Whitney U-test; $n=16$; Table 2).

In order to ascribe the observed differences in behaviour between nestmates and non-nestmates to differences in nestmateship, we attempted to rule out other interfering factors. Although the animals chosen for each experiment were matched as closely as possible in age and body size (in the case of animals present on the nest at the time of collection, matching was possible with respect to body size only, as the age of the animals was unknown), we ascertained that residual differences in these factors could not explain our results.

Reanalysis of the above data, ignoring nestmateship but considering residual differences in age and body size, shows that: (a) the older or larger of the two wasps available for interacting was not treated differently than the younger or smaller one ($0.438 < P < 0.499$, Wilcoxon matched-pairs signed-ranks test and $0.1 < P < 0.382$, Mann-Whitney U-test); (b) a wasp did not treat animals older or larger than itself differently from those younger or smaller than itself ($0.109 < P < 0.807$, Wilcoxon matched-pairs signed-ranks test and $P > 0.1$, Mann-Whitney U-test); (c) a wasp did not treat animals closer to itself in age or body size differently from those distant in age or body size ($0.334 < P < 0.826$, Wilcoxon matched-pairs signed-ranks test and $0.1 < P < 0.26$, Mann-Whitney U-test). We therefore concluded that differences in nestmateship, and not residual differences in age or body size, were being recognized.

The results obtained with wasps isolated immediately upon eclosion and not exposed to their natal nests or nestmates were equival. Our findings showed that these animals did not appear to discriminate between nestmates and non-nestmates since the tolerance indices, as compared by the Wilcoxon matched-pairs signed-ranks test, did not differ significantly ($P=0.107$; $n=16$). However, discrimination did appear to occur, as determined by the Mann-Whitney U-test ($P<0.05$; $n=16$; Table 3). Fully developed wasps sometimes chewed their pupal caps (completely or partially) and stayed inside their cells for several minutes, thereby possibly gaining a certain amount of exposure to the nest. When we prepared wasps for experiments in treatment III, we discarded such animals in an attempt to avoid this problem but were obviously not entirely successful. To solve this problem altogether, we also conducted experiments with wasps that were artificially removed from their pupal cases prior to their natural eclosion and allowed to complete their development in an incubator (treatment IV). Our findings showed that such animals clearly failed to discriminate between their nestmates and non-nestmates since the tolerances among nestmates and among non-nestmates were not significantly different from each other, as determined by both statistical tests ($P=0.261$, Wilcoxon matched-pairs signed-ranks test and $P > 0.05$, Mann-Whitney U-test; $n=17$).

The experiments mentioned above suggest that either labels or templates or both are lacking in animals isolated about a day prior to or immediately after their expected natural eclosion. The mixed triplet experiments made a distinction between these different possibilities. Wasps exposed

Table 3. Comparison of tolerance indices by the Wilcoxon matched-pairs signed-ranks and Mann-Whitney U-tests. Parameters as in Table 2. In treatment III T_x is not significantly different from T_a in the Wilcoxon matched-pairs signed-ranks test whereas in the Mann-Whitney U-test T_x is significantly greater than T_a. Sample sizes, means, standard deviations, and levels of significance are given at the end of the table

Wasps isolated from nest and nestmates immediately after eclosion (treatment III)				Wasps isolated from their nest and nestmates prior to their natural eclosion (treatment IV)			
Experiment no.	T_x	T_a	T_x-T_a	Experiment no.	T_x	T_a	T_x-T_a
5	16.00	9.30	+ 6.70	89	17.26	17.97	−0.71
8	13.25	12.43	+ 0.82	90	18.05	12.68	+5.37
11	7.69	11.01	− 3.32	92	16.71	23.04	−6.33
14	11.11	9.44	+ 1.67	94	15.64	15.21	+0.43
36	16.78	9.84	+ 6.94	95	20.24	16.30	+3.94
37	14.62	15.72	− 1.10	96	20.58	17.16	+3.42
38	13.56	10.08	+ 3.48	97	19.54	19.04	+0.50
40	13.33	12.30	+ 1.03	98	14.48	13.52	+0.96
41	8.37	10.55	− 2.18	103	19.40	20.36	−0.96
42	18.93	13.28	+ 5.65	104	14.14	15.95	−1.81
43	18.00	3.53	+14.47	105	20.87	13.66	+7.21
63	19.18	12.77	+ 6.41	106	15.68	19.58	−3.90
65	17.05	16.70	+ 0.35	107	17.10	19.06	−1.96
66	14.50	17.42	− 2.92	108	22.65	16.73	+5.92
85	3.16	11.43	− 8.27	109	15.36	18.54	−3.18
86	16.16	15.54	+ 0.62	110	15.31	16.58	−1.27
				111	21.00	14.70	+6.30
16	13.86	11.96	1.86	17	17.87	17.06	0.82
	±4.41	±3.44	± 5.42		±2.6	±2.72	±3.94
$T=44$, $P=0.107$, Wilcoxon matched-pairs signed-ranks test; U=82, $P<0.05$, Mann-Whitney U-test				$T=63$, $P=0.261$, Wilcoxon matched-pairs signed-ranks test; U=118, $P>0.05$, Mann-Whitney U-test			

Table 4. Comparison of tolerance indices by Wilcoxon matched-pairs signed-ranks and Mann-Whitney U-tests. $T_b=$ tolerance of the nestmate and $T_d=$ tolerance of the non-nestmate. In both cases T_b is not significantly different from T_d in both statistical tests. Sample sizes, means, standard deviations and levels of significance are given at the end of the table

Tolerance of "Isolated" wasps by "Exposed" wasps				Tolerance of "Exposed" wasps by "Isolated" wasps			
Experiment no.	T_b	T_d	T_b-T_d	Experiment no.	T_b	T_d	T_b-T_d
134	8.67	9.60	− 0.93	138	11.71	13.25	−1.54
135	8.83	10.50	− 1.67	139	12.00	8.38	+3.62
136	7.58	4.04	+ 3.54	140	9.00	9.00	0.00
137	8.56	6.23	+ 2.33	141	8.86	5.16	+3.70
142	9.56	11.10	− 1.54	143	12.00	8.93	+3.07
144	11.10	4.50	+ 6.50	145	10.50	7.33	+3.17
148	9.10	8.75	+ 0.35	146	7.67	5.65	+2.02
149	11.72	6.29	+ 5.43	147	8.89	9.00	−0.11
153	5.84	6.36	− 0.52	151	9.50	13.50	−4.00
156	4.80	8.12	− 3.32	152	4.31	4.31	0.00
157	10.43	9.13	+ 1.30	154	9.40	9.50	−0.10
158	11.25	9.53	+ 1.72	155	8.20	8.70	−0.50
160	9.00	10.50	− 1.50	159	6.45	6.50	−0.05
162	9.00	9.40	− 0.40	161	5.25	4.48	+0.77
163	0.00	10.00	−10.00	164	10.33	9.11	+1.22
165	0.00	9.00	− 9.00				
16	7.84	8.32	− 0.48	15 (13[a])	8.94	8.19	0.75
	±3.56	±2.17	± 4.41		±2.31	±2.76	±2.11
$T=64$, $P=0.418$, Wilcoxon matched-pairs signed-ranks test; U=122.5, $P>0.05$, Mann-Whitney U-test				$T=30$, $P=0.139$, Wilcoxon matched-pairs signed-ranks test; U=90, $P>0.05$, Mann-Whitney U-test			

[a] For the Wilcoxon matched-pairs signed-ranks test, sample size is only 13, as T_b-T_d is 0.00 for experiments 140 and 152

to their natal nest and nestmates failed to discriminate between those nestmates and non-nestmates that were isolated from their respective nests and nestmates (T_b is not distinguishable from T_a; $P=0.418$, Wilcoxon matched-pairs signed-ranks test and $P>0.05$, Mann-Whitney U-test; $n=16$; Table 4). In addition, animals isolated from their nests and nestmates also failed to discriminate between those nestmates and non-nestmates that were exposed to their respective nests and nestmates (T_b is not distinguishable from T_d; $P=0.139$, Wilcoxon matched-pairs signed-ranks test and $P>0.05$, Mann-Whitney U-test; $n=15$; Table 4). Although these conclusions are based on accepting the null hypothesis that nestmates and non-nestmates were not treated differently, it should be emphasized that by using the same assay and similar sample sizes we were able to reject the null hypothesis when animals present on the nest or "exposed" animals were used (Table 2). Our results, therefore, suggest that *both* labels and templates are absent in animals isolated from their nests and nestmates (see Methods). In other words, labels are not self-produced and templates are not self-based (see row 4, Fig. 2)

Discussion

Our results show that *R. marginata* females can discriminate between nestmates and non-nestmates, provided the females have been exposed after eclosion to their natal nests and nestmates. Exposure to the nest alone may be sufficient and exposure to nestmates may not be necessary, as

in the case of *Polistes fuscatus* (Shellman and Gamboa 1982), but we did not investigate this question. Even those nestmates with which a wasp may not have interacted can be recognized due to exposure to the nest and a subset of nestmates. Our most important finding is that it is necessary for both the *discriminating* animals as well as the *discriminated* animals to have been exposed after eclosion to their respective natal nests and nestmates. This conclusion results from the observation that wasps isolated prior to eclosion fail to discriminate between their nestmates and non-nestmates that have not been isolated. Similarly, wasps that have been exposed to their natal nests and nestmates also fail to discriminate between those nestmates and non-nestmates that themselves have been isolated.

One of the questions of interest in studying kin recognition is whether discrimination between different patrilines within the same colony will restore the asymmetries in genetic relatedness, created by haplodiploidy but negated by multiple mating or polygyny, and thus support the argument that Hymenopterans are genetically predisposed to the evolution of sociality by kin selection. Nestmate discrimination or kin recognition may be thought of as a process of comparing recognition templates in the brain of an animal with recognition labels carried on the bodies of animals that are being recognized (Gadagkar 1985b; Lacy and Sherman 1983). Also see Gamboa et al. (1986a) who call these the "perception" and "expression" components, respectively. Because it is necessary for both the discriminating and the discriminated wasps to have been exposed to their natal nest and nestmates in order for discrimination to occur, we conclude that the wasps lack both labels and templates at the time of eclosion. Because exposure to the nest and a subset of nestmates is necessary and sufficient for discrimination to occur, we conclude that labels are acquired and templates are learned from the nest and nestmates after eclosion. Such a situation makes it very unlikely that full and half sisters within the same colony will be discriminated (Gadagkar 1985b).

Nestmate discrimination or kin recognition to date has been investigated in about 12 genera of social Hymenoptera. These include 4 wasp genera, *Polistes* (Post and Jeanne 1982; Pfennig et al. 1983; Shellman and Gamboa 1982; Gamboa et al. 1986b) and numerous other papers reviewed in Gamboa et al. (1986a), *Dolichovespula* (Ryan et al. 1985) and *Ropalidia* (this study), *Vespula* (Ross 1983), 2 bee genera, *Lasioglossum* (Greenberg 1979; Buckle and Greenberg 1981) and *Apis* (Breed 1983; Breed et al. 1985; Evers and Seeley 1986;

Getz and Smith 1983, 1986; Noonan 1986; Page and Erickson 1986; Visscher 1986), and 6 ant genera, *Camponotus* (Carlin and Hölldobler 1986, 1987; Carlin et al. 1987; Jaffe and Sanchez 1984; see review in Jaffe 1987), *Pseudomyrmex* (Mintzer 1982; Mintzer and Vinson 1985), *Acromyrmex* (Jutsum et al. 1979), *Odontomachus* (Jaffe and Marcus 1983), *Cataglyphis* (Isingrini et al. 1985, 1986) and *Leptothorax* (Stuart 1987a, 1987b).

Of these genera, *Apis, Pseudomyrmex,* and *Camponotus* are the only ones where discrimination of different patrilines within a colony is potentially possible. It should be emphasized, however, that there is no conclusive evidence that this potential capacity is actually used to restore levels of genetic relatedness between the actors and recipients in altruistic interactions (Carlin et al. 1987; Visscher, 1986). Although in the ants *Odontomachus bauri* and *Leptothorax curvispinosus* nestmate discrimination is based on endogenous odours probably produced by the workers themselves, there is no evidence that different levels of genetic relatedness within a colony can be recognized (Jaffe and Marcus 1983; Stuart 1987a). Similarly, while both endogenous and exogenous factors seem to be involved in worker recognition in *Acomyrmex octospinosus* (Jutsum et al. 1979), there is no evidence of recognition of different levels of genetic relatedness within a colony. In *Cataglyphis cursor* there is strong evidence that preimaginal learning is the basis of nestmate discrimination and that spatial proximity rather than genetic relatedness per se is responsible for kin recognition (Isingrini et al. 1985, 1986). In *Lasioglossum zephyrum*, Greenberg (1979) reported a significant positive correlation between the level of genetic relatedness between guard and intruder bees and the probability of acceptance of intruders by guards. Subsequent experiments, however, showed that familiarity or the lack of it can interfere with such recognition, making it very unlikely that different lines of genetic relatedness can be discriminated once the bees are habituated to all of them (Buckle and Greenberg 1981). Although no detailed experiments have been conducted with *Dolichovespula* and *Vespula*, the nestmate discrimination system of *Polistes* has been well studied. Here, recognition labels have both genetic and environmental components, but recognition templates are learned in the adult stage from the nest, making it very unlikely that full and half sisters will be distinguished within a colony (Gamboa et al. 1986a). In one experiment nestmate sisters were not distinguished from non-nestmate nieces (Gamboa et al. 1987). The results reported here for *R.*

marginata are entirely consistent with this. In summary, some social insects, such as *Apis mellifera, Pseudomyrmex ferruginea* and *Camponotus* species, are potentially capable of discriminating different patrilines within a colony, but the primitively eusocial wasps and bees do not appear to be capable of doing so.

We may therefore conclude that, at least in these primitively eusocial insects, kin recognition is unlikely to restore the prominent role that might be ascribed to kin selection in the absence of multiple mating and polygyny and the consequent low levels of intracolony genetic relatedness. This is not to say that kin selection does not play a role in the evolution of Hymenopteran sociality. Kin selection can always promote sociality in spite of low levels of intracolony genetic relatedness, provided it is relatively more difficult to survive and reproduce in the solitary mode. What *can* be said, however, is that the multiple origins of eusociality in the Hymenoptera cannot readily be attributed to haplodiploidy – West-Eberhard (1978) reached a similar conclusion, although not in the context of kin recognition. The system of discrimination seen in the primitively eusocial wasps and bees and some ants mentioned above has perhaps not evolved for discrimination of different genetic lines within a colony, but merely for nestmate discrimination. It is conceivable that there may be a stronger selection pressure for keeping away aliens that may be potential usurpers or social parasites-in-the-making and thus maintain colony integrity rather than for discriminating between full and half sisters within a colony. The mechanism of nestmate discrimination based on acquiring exogenous odours and post-eclosion learning of templates is perfectly suited to such a situation.

References

Breed MD (1983) Nestmate recognition in honey bees. Anim Behav 31:86–91

Breed MD, Butler L, Stiller TM (1985) Kin discrimination by worker honey bees in genetically mixed groups. Proc Natl Acad Sci USA 82:3058–3061

Buckle GR, Greenberg L (1981) Nestmate recognition in sweat bees (*Lasioglossum zephyrum*): Does an individual recognise its own odour or only odours of its nestmates? Anim Behav 29:802–809

Carlin NF, Hölldobler B (1986) The kin recognition system of carpenter ants (*Camponotus* spp.). I. Hierarchical cues in small colonies. Behav Ecol Sociobiol 19:123–134

Carlin NF, Hölldobler B (1987) The kin recognition system of carpenter ants (*Camponotus* spp.) II. Larger colonies. Behav Ecol Sociobiol 20:209–217

Carlin NF, Hölldobler B, Gladstein DS (1987) The kin recognition system of carpenter ants (*Camponotus* spp.) III. Within-colony discrimination. Behav Ecol Sociobiol 20:219–227

Evers CA, Seeley TD (1986) Kin discrimination and aggression in honey bee colonies with laying workers. Anim Behav 34:924–925

Fletcher DJC, Michener CD (1986) Kin recognition in animals. Wiley, New York, pp X, 465

Gadagkar R (1980) Dominance hierarchy and division of labour in the social wasp *Ropalidia marginata* (Lep.) (Hymenoptera, Vespidae). Curr Sci 49:772–775

Gadagkar R (1985a) Evolution of Insect Sociality-A review of some attempts to test modern theories. Proc Indian Acad Sci [A] 94:309–324

Gadagkar R (1985b) Kin recognition in social insects and other animals-A review of recent findings and a consideration of their relevance for the theory of kin selection. Proc Indian Acad Sci [A] 94:587–621

Gadagkar R, Joshi NV (1983) Quantitative ethology of social wasps: Time-activity budgets and caste differentiation in *Ropalidia marginata* (Hymenoptera, Vespidae). Anim Behav 31:26–31

Gadagkar R, Gadil M, Joshi NV, Mahabal AS (1982) Observations on the natural history and population ecology of the social wasp *Ropalidia marginata* from peninsular India (Hymenoptera, Vespidae). Proc Indian Acad Sci [A] 91:539–552

Gamboa GJ, Klahn JE, Parman AO, Ryan RE (1987) Discrimination between nestmate and non-nestmate kin by social wasps (*Polistes fuscatus*, Hympenoptera:Vespidae). Behav Ecol Sociobiol 21:125–128

Gamboa GJ, Reeve HK, Pfennig DW (1986a) The evolution and ontogeny of nestmate recognition in social wasps. Ann Rev Entomol 31:431–454

Gamboa GJ, Reeve HK, Ferguson ID, Wacker TL (1986b) Nestmate recognition in social wasps: the origin and acquisition of recognition odours. Anim Behav 34:685–695

Getz WM, Smith KB (1983) Genetic kin recognition: Honey bees discriminate between full and half sisters. Nature 302:147–148

Getz WM, Smith KB (1986) Honey bee kin recognition: learning self and nestmate phenotypes. Anim Behav 34:1617–1626

Greenberg L (1979) Genetic component of bee odor in kin recognition. Science 206:1095–1097

Grosberg RK, Quinn JF (1986) The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. Nature 322:456–459

Hamilton WD (1964) The genetical evolution of social behaviour. J Theor Biol 7:1–52

Hamilton WD (1972) Altruism and related phenomena, mainly in social insects. Ann Rev Ecol Syst 3:192–232

Hepper PG (1986) Kin recognition: Functions and mechanisms a review. Biol Rev 61:63–93

Hölldobler B, Michener CD (1980) Mechanisms of identification and discrimination in social Hymenoptera. In: Markl H (ed) Evolution of social behaviour: hypothesis and empirical tests. (Dahlem Konferenzen). Verlag Chemie, Weinheim, pp 35–58

Isingrini M, Jaisson P, Lenoir A (1986) Influence of preimaginal experience on the social behaviour of adult Ants and the

importance of fellowship in nestmate recognition. In: Passera L, Lachaud JP (eds) The individual and society. Privat, I.E.C., Toulouse, pp 49–53

Isingrini M, Lenoir A, Jaisson P (1985) Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. Proc Natl Acad Sci USA 82:8545–8547

Jaffe K (1987) Evolution of territoriality and nestmate-recognition systems in ants. Experientia [Suppl] 54:295–311

Jaffe K, Marcus M (1983) Nestmate recognition and territorial behaviour in the ant *Odontomachus bauri* Emery (Formicidae: Ponerinae). Insectes Soc 30:466–481

Jaffe K, Sanchez C (1984) On the nestmate-recognition system and territorial marking behaviour in the ant *Camponotus rufipes*. Insectes Soc 31:302–315

Jutsum AR, Saunders TS, Cherrett JM (1979) Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. Anim Behav 27:839–844

Keough MJ (1984) Kin recognition and the spatial distribution of larvae of the bryozoan *Bugula neritina* (L.). Evolution 38:142–147

Lacy RC, Sherman PW (1983) Kin recognition by phenotype matching. Am Nat 121:489–511

Linsenmair KE (1985) Individual and family recognition in subsocial arthropods, in particular in the desert isopod *Hemilepistus reaumuri*. In: Hölldobler B, Lindauer M (eds) Experimental behavioural ecology. Fischer, Stuttgart New York, pp 411–435

Mintzer A (1982) Nestmate recognition and incompatibility between colonies of the acacia ant *Pseudomyrmex ferruginea*. Behav Ecol Sociobiol 10:165–168

Mintzer A, Vinson SB (1985) Kinship and incompatibility between colonies of the acacia ant *Pseudomyrmex ferruginea*. Behav Ecol Sociobiol 17:75–78

Muralidharan K, Shaila MS, Gadagkar R (1986) Evidence for multiple mating in the primitively eusocial wasp *Ropalidia marginata* (Lep) (Hymenoptera: Vespidae). J Genet 65:153–158

Noonan KC (1986) Recognition of queen larvae by worker honey bees (*Apis mellifera*). Ethology 73:295–306

Page RE Jr (1986) Sperm utilization in social insects. Ann Rev Entomol 31:297–320

Page RE Jr, Erickson EH Jr (1986) Kin recognition and emergency queen rearing by worker honey bees (Apis mellifera L.) Ann Entomol Soc Am 79:460–467

Pfennig DW, Gamboa GJ, Reeve HK, Shellman-Reeve J, Ferguson ID (1983) The mechanism of nestmate discrimination in social wasps (*Polistes,* Hymenoptera: Vespidae). Behav Ecol Sociobiol 13:299–305

Post DC, Jeanne RL (1982) Recognition of former nestmates during colony founding by the social wasp *Polistes fuscatus* (Hymenoptera: Vespidae). Behav Ecol Sociobiol 11:283–285

Quinn TP, Busack CA (1985) Chemosensory recognition of siblings in juvenile coho salmon (*Oncorhynchus kisutch*) Anim Behav 33:51–56

Ross KG (1983) Laboratory studies of the mating biology of the eastern yellow jacket, *Vespula maculifrons* (Hymenoptera: Vespidae). J Kans Entomol Soc 56:523–537

Ryan RE, Cornell TJ, Gamboa GJ (1985) Nestmate recognition in the bald-faced hornet, *Dolichovespula maculata* (Hymenoptera, Vespidae). Z Tierpsychol 69:19–26

Shellman JS, Gamboa GJ (1982) Nestmate discrimination in social wasps: The role of exposure to nest and nestmates (*Polistes fuscatus,* Hympenoptera: Vespidae). Behav Ecol Sociobiol 11:51–53

Sherman PW, Holmes WG (1985) Kin recognition: issues and evidence. In: Hölldobler B, Lindauer M (eds) Experimental behavioural ecology. Fischer, Stuttgart New York, pp 437–460

Siegel S (1956) Nonparametric statistics for the behavioral sciences. McGraw-Hill, Kogakusha Tokyo, pp 312

Starr CK (1984) Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic Press, pp 427–464

Stuart RJ (1987a) Individual workers produce colony specific nestmate recognition cues in the ant, *Leptothorax curvispinosus*. Anim Behav 35:1062–1069

Stuart RJ (1987b) Transcient nestmate recognition cues contribute to a multicolonial population structure in the ant, *Leptothorax curvispinosus*. Behav Ecol Sociobiol 21:229–235

Visscher PK (1986) Kinship discrimination in queen rearing by honey bees (*Apis mellifera*). Behav Ecol Sociobiol 18:453–460

Waldman B (1987) Mechanisms of kin recognition. J Theor Biol 128:159–185

Werner DI, Baker EM, Gonzalez EDC, Sosa IR (1987) Kinship recognition and grouping in hatchling green iguanas. Behav Ecol Sociobiol 19:83–89

West-Eberhard MJ (1975) The evolution of social behavior by kin selection. Q Rev Biol 50:1–33

West-Eberhard MJ (1978) Polygyny and the evolution of social behaviour in wasps. J Kans Entomol Soc 51:832–856

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge, Massachusetts, pp 548

Wilson EO (1975) Sociobiology: The new synthesis. Harvard University Press, Cambridge, Massachusetts, pp 697