

Kin recognition in a semi-natural context: Behaviour towards foreign conspecifics in the social wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae)

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Summary

Female wasps of the tropical primitively eusocial species *Ropalidia marginata* are known to discriminate unfamiliar nestmates from unfamiliar non-nestmates outside the context of their nests. Here, we show that when foreign conspecifics are introduced in the context of a nest in laboratory cages, genetic relatives among them are treated by nest inhabitants more tolerantly than non-relatives, but that no foreign conspecifics are accepted into the nests. However, some wasps may leave their nest and join the foreign relatives and non-relatives to found new colonies cooperatively. Very few of the introduced animals are severely attacked or killed; most are allowed to remain in parts of the cage away from the nest. These results suggest that factors other than genetic relatedness may be involved in regulating tolerance and acceptance of foreign conspecifics on a nest and its vicinity. Our results are different from those of similar experiments with ants, which have demonstrated that former nestmates that are removed as pupae and later introduced as adults are either accepted into the nest or attacked and killed. We attribute this difference to the fact that in a primitively eusocial species such as *R. marginata*, the rules governing tolerance and acceptance of foreign conspecifics must be quite different from those in highly eusocial species. We also attempt to test some predictions of the conspecific acceptance threshold models of Reeve (*Am. Nat.* 133:407–435, 1989). Our results uphold the predictions of his “fitness consequence submodel” but do not support those of his “interaction frequency sub-model”.

Introduction

Experiments using a blind laboratory behavioural assay have demonstrated that unfamiliar nestmates are discriminated from unfamiliar non-nestmates in the primitively eusocial tropical wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae). For such discrimination, it was essential that both the discriminated animals as well as the discriminating animals were exposed to their natal nest and a subset of nestmates after their eclosion. We concluded from these experiments that

both labels and templates used in recognition are acquired by the wasps, either from their nests or from their nestmates (Venkataraman 1990; Venkataraman et al., 1988, 1990; Venkataraman and Gadagkar, 1990). All animals in a colony are therefore likely to have common labels and templates. Several studies on other polistine genera have led to similar conclusions (reviewed in Gadagkar, 1985; Gamboa et al., 1986). Numerous studies on other social insects have revealed the involvement of acquired odours in kin or nestmate recognition (e.g., Stuart, 1987a; Morel, 1988; Crosland, 1989a; Jaisson, 1991).

These results suggest that even an unrelated conspecific can potentially sneak into a colony, acquire that colony's labels and templates, and be accepted into it. Conversely, even a genetically closely related individual may not be accepted into a colony if it has failed to acquire the colony-specific labels and templates. In primitively eusocial species such as *R. marginata*, where it is suspected that mutualistic interactions (Lin and Michener, 1972) may be important in facilitating social evolution (Gadagkar, 1991), factors other than genetic relatedness may play a role in modulating tolerance and acceptance of foreign conspecifics.

What determines whether an individual will be accepted into a colony? In an attempt to answer this question, we introduced various kinds of conspecifics (relatives and non-relatives, who have or who have not acquired labels and templates, etc.) into laboratory cages containing colonies of the social wasp *R. marginata*. These animals will be referred to as "foreign" or "introduced" to distinguish them from members of the colony associated with the nest prior to introduction. The latter will be referred to as "nest" animals. Here, we report the results of one set of such experiments, which show that in the context of a nest, genetically related individuals are treated significantly more tolerantly by the nest animals than unrelated individuals, but that neither are usually accepted onto the nest. However, in some cases, animals co-operatively found new colonies and build new nests together with introduced animals.

Materials and methods

Study animal

All experiments were conducted using female *Ropalidia marginata*, a primitively eusocial tropical wasp. *R. marginata* builds simple, open, single- or multiple-combed, paper carton nests, and may contain from one to a little over a hundred morphologically identical adult wasps (Gadagkar, 1980, 1985a, 1991). Only one of these wasps is the queen or egg layer at any given time, although there may be frequent queen replacements (Gadagkar 1991; Gadagkar et al., 1982, Gadagkar and Joshi, 1983). The colonies may be very long-lived and exhibit what has been called a perennial, indeterminate colony cycle (Gadagkar, 1991).

Collection of nests and Preparation of experimental animals

Colonies for the study were obtained from Bangalore (13°00' N, 77°32' E) and Mysore (12°25' N, 76°50' E), India. Pairs of nests and the inhabiting adult wasps were

collected from localities separated from each other by at least 10 kms, and brought to the laboratory. This was done to ensure that wasps eclosing from the same nest would be more closely related genetically to each other than they would be to wasps eclosing from the other nest in that pair. We refer to animals eclosing from the same nest as relatives, and those eclosing from different nests in each pair as non-relatives.

One nest from each pair was cut into three parts. The part containing relatively more brood was fixed in a $45 \times 45 \times 45$ -cm wood and wire mesh cage, and the adults present on the nest at the time of collection were released into this cage. Most adults returned to the nest fragment, repaired damaged cells and continued tending brood, a process we refer to as "regeneration". Such nests were maintained on an *ad libitum* diet of *Corcyra cephalonica* (Lepidoptera: Galleridae) larvae, honey and water. A piece of wood was provided as a source of building material. Such nests were maintained for a period of 49–54 days (mean \pm sd = 51 ± 2.6), by which time an apparently normal colony was established on it. All animals eclosing on the second fragment of the nest were allowed to remain on that fragment with other eclosing wasps for a period of 10–20 days (mean \pm sd = 12.1 ± 1.9). Since they were exposed to a fragment of their nest and to a subset of their nestmates, these animals constituted "exposed" relatives of the animals in the regenerated nest. They were then transferred to individual $22 \times 11 \times 11$ -cm ventilated plastic jars until the experiments were begun. Animals from the third fragment of the nest were removed from their pupal cases about 24 h prior to their expected time of natural eclosion and maintained singly in plastic jars. These wasps were not exposed to their natal nests or nestmates, and constituted "isolated" relatives of the animals in the regenerated nest.

The second nest in each pair was cut into two halves which were used to obtain "exposed" non-relatives and "isolated" non-relatives of the animals on the regenerated nest by following the procedures used for the second and third fragments of the first nest respectively. It should be noted that "exposed" animals were always exposed to a fragment of their own nest and to a subset of their own nestmates.

The Assay

The assay consisted of behavioural observations following the introduction of "exposed" relatives, "isolated" relatives, "exposed" non-relatives and "isolated" non-relatives into cages containing regenerated nests. Approximately 3 to 4 animals of each of the 4 categories were simultaneously introduced into a cage containing a regenerated nest. In all, 3 such experiments were performed. The animals were 26–52 days old (mean \pm sd = 40.1 ± 6.3 days) at the time of introduction. All introduced animals were marked with a small spot of coloured paint to indicate the categories ("exposed" relative etc.) to which they belonged. The observer, however, was unaware of the colour codes until the experiment was over.

The Observations

Observations were begun one hour after the introduction of foreign animals and were carried out from 0830–1200 h and 1430–1800 h for five consecutive days in the first

week following introduction, and again for five consecutive days in the second week. In the third week, observations were made once a day for six consecutive days, either from 0830–1200 h or from 1430–1800 h. Most interactions between nest animals and introduced animals occurred in the vicinity of the nests. All interactions initiated by any nest animal towards any introduced animal within 9 cm from the periphery of the nest were recorded in blocks of 5-min observation sessions. Fifteen such five-minute “all occurrences” observations were performed during a period of 3.5 h either from 0830 to 1200 h or from 1430 to 1800 h. Because some animals entered the vicinity of the nest less often, separate “focal animal” sampling sessions of 5-min duration each were conducted once for each introduced animal in every 3.5-h observation period. The blocks of “all occurrences” and “focal animal” sampling sessions were randomly intermingled during a 3.5-h observation period. The sequence of animals chosen for observation during the “focal animal” sampling sessions was also chosen randomly. In each “focal animal” sampling session, all interactions initiated by any nest animal towards the focal animal anywhere in the cage were recorded.

The experiment described above was repeated three times with different pairs of nests. This amounted to 1144 5-min “all occurrences” observation blocks and 1002 5-min “focal animal” observation blocks, amounting to a total of 178 h 50 min of observations.

Data Analysis

In a previous study of nestmate discrimination in *R. marginata*, we had recorded 15 different behavioural interactions, and ranked them in increasing order of tolerance (Venkataraman et al., 1988). In this study, we recorded only eleven of those behaviours, but ranked them in the same order, namely: 1) aggressive bite (AB), 2) attack (AT), 3) peck (PK), 4) chase (CH), 5) aggressive mutual antennation (AM), 6) nibble (NI), 7) being avoided (BV), 8) mutual approach with withdrawal (AA), 9) approach with withdrawal (AW), 10) approach (AP) and 11) antennation (AN). The remaining behaviours did not occur; for definitions see Venkataraman et al. (1988). From these data, a family of tolerance indices, namely $T1_{ER}$, $T1_{IR}$, $T1_{ENR}$ and $T1_{INR}$, corresponding to the behaviours shown by the nest animals towards “exposed” relatives, “isolated” relatives, “exposed” non-relatives and “isolated” non-relatives respectively were computed as shown in the example below:

$$T1_{ER} = \sum_{i=1}^{11} p_i r_i$$

where p_i is the proportion of the i th behaviour amongst the set of behaviours directed towards “exposed” relatives, and r_i is the rank of the i th behaviour. We have reason to believe that such a tolerance index is a good measure of discrimination, because it combines information from all behavioural interactions, and has been shown to be very sensitive in detecting discrimination ability in social wasps (Gamboa et al., 1986; Venkataraman et al., 1988). On the other hand, our ranking of the eleven behaviours

in increasing order of tolerance, being subjective, may contain some inherent bias. To be reassured that our results are not too sensitive to this subjective ranking, we have classified the eleven behaviours into three sub-groups, namely a) aggressive behaviours (which include the first three in the above list of behaviours), b) relatively non-aggressive behaviours (which include behaviours 4–9 in the above list) and c) very tolerant behaviours (which include the last two in the above list). Giving a rank of 1 for all behaviours belonging to the first sub-group, a rank of 2 for all behaviours belonging to the second sub-group and a rank of 3 for those that belong to the third sub-group, a second family of tolerance indices, namely $T2_{ER}$, $T2_{IR}$, $T2_{ENR}$ and $T2_{INR}$, corresponding to the behaviours shown by the nest animals towards the four categories of foreign animals respectively, were computed as shown in the example below:

$$T2_{ER} = \sum_{i=1}^{11} p_i r_i$$

where p_i is the proportion of the i th behaviour among the set of behaviours directed towards "exposed" relatives, and r_i is the new rank of the i th behaviour (see above). Such an index may be somewhat less informative, since it uses only 3 distinct ranks, but it is also likely to be less subjective, because the classification of behaviours into the three broad sub-groups is likely to be less subjective.

Behaviours belonging to the first of the above sub-groups, namely aggressive behaviours, were used to compute a family of aggressiveness indices, namely $T3_{ER}$, $T3_{IR}$, $T3_{ENR}$ and $T3_{INR}$, corresponding to the four categories of foreign animals, respectively, as shown in the example below:

$$T3_{ER} = \sum_{i=1}^3 p_i$$

where p_i is the proportion of the i th behaviour among the set of behaviours shown towards "exposed" relatives, but only behaviours belonging to the first sub-group are included in the summation.

Tolerance shown by the nest animals towards various categories of foreign animals was assessed by pair-wise comparison of tolerance/aggressiveness indices, using z tests as described below. While comparing the first family of tolerance indices between "exposed" relatives and "isolated" relatives, for instance, we tested the hypothesis that $T1_{ER} = T1_{IR}$. A z score was calculated by the expression:

$$z = \frac{T1_{ER} - T1_{IR}}{\sqrt{V(T1_{ER}) + V(T1_{IR})}}$$

where

$$V(T1_{ER}) = \sum_{i=1}^{11} r_i^2 \sigma_{ii} + \sum_{i=1}^{11} \sum_{j=1}^{11} r_i r_j \sigma_{ij}, \quad \sigma_{ii} = p_i(1-p_i)/n1$$

and $\sigma_{ij} = -p_i p_j^* / n_1$. In this example p_j^* are proportions of the 11 behaviours shown towards "isolated" relatives, r_j are the ranks of these 11 behaviours, and n_1 is the total number of behaviours shown towards "exposed" relatives. $V(T1_{IR})$ was analogously defined by interchanging p_i s and p_j^* s and replacing n_1 with n_2 , which is the total number of behaviours shown towards "isolated" relatives. Only behaviours with non-zero p_i s and p_j^* s were included in the comparison. $V(T1_{ER})$ and $V(T1_{IR})$ were considered approximations of the variances of $T1_{ER}$ and $T1_{IR}$. Such z scores thus permit a large sample normal test. In order to perform a two-tailed test, the null hypothesis was rejected if the z score was less than -1.96 or greater than 1.96 ($p < 0.05$). An identical procedure was used while comparing the second family of tolerance indices.

While comparing the family of aggressiveness indices which represent proportions of aggressive interactions (not requiring ranking of behaviours), z scores were calculated more simply, as in the example shown below:

$$z = \frac{T3_{ER} - T3_{IR}}{\sqrt{\frac{T3_{ER}(1 - T3_{ER})}{n_1} + \frac{T3_{IR}(1 - T3_{IR})}{n_2}}}$$

Results

Of the 46 foreign female wasps belonging to the "exposed" relative, "isolated" relative, "exposed" non-relative and "isolated" non-relative categories introduced into a total of three cages containing nests, none was ever accepted on the nests (Table 1). Because these experiments were conducted concurrently with others, where foreign animals (young animals less than 8 days old) were sometimes accepted on the nests (Venkataraman, 1990), acceptance or non-acceptance of introduced animals was easy to recognise. Animals were considered accepted if they were seen for more than 5 min on the nest during the day, or if they were seen on the nest on any night. "Accepted" animals became part of the colonies in their cage of introduction, and were seen moving between the nest and other areas of the cage like the original nest animals. They were also usually present on the nest at night. "Unaccepted" animals sometimes alighted on the nest during the day, but elicited high levels of aggression from the nest animals, resulting in their immediate departure. Such unaccepted animals were never seen on the nest at night.

We recorded 18 instances of a brief presence of one of the introduced animals on a nest. These may be regarded as attempts to join the nest. The proportion of such attempts made by relatives (12/18) and that made by non-relatives (6/18) were not significantly different ($p > 0.05$, G test, with William's correction, Sokal & Rohlf 1981). Relatives hence do not seem to make significantly more attempts to join the nests as non-relatives (although it must be remembered that the total number of attempts was very small). The nest animals interacted extensively with the introduced animals in the vicinity of the nest (on 396 occasions as seen from the "all occurrences" sampling sessions). Computation of tolerance indices from these 396 interactions and their pairwise comparison across different categories of foreign animals showed that

Table 1. Number of foreign animals introduced and their fates in the three replicates

	Experiment 1	Experiment 2	Experiment 3
Number of resident adults present at the time of introduction	36	10	72
Number of nest animals that died during the observation period	1	2	1
Number of foreign animals introduced			
<i>Exposed relatives</i>	4	4	4
<i>Exposed non-relatives</i>	4	4	4
<i>Isolated relatives</i>	4	3	4
<i>Isolated non-relatives</i>	4	4	3
Number of foreign animals that died during the observation period			
<i>Exposed relatives</i>	1	0	1
<i>Exposed non-relatives</i>	1	0	2
<i>Isolated relatives</i>	1	1	1
<i>Isolated non-relatives</i>	1	2	1
Number of foreign animals accepted onto the nest	0	0	0

nest animals were significantly more tolerant of "exposed" relatives than they were of "exposed" non-relatives or of "isolated" non-relatives (Table 2). Tolerance towards "isolated" relatives was intermediate, and not significantly different from that towards any other category of animals. Identical results were obtained using all three families of tolerance/aggressiveness indices. Notice, however, that the numerical values of the aggressiveness index will be in the opposite direction compared to the first and second family of tolerance indices. This is simply because the first two are tolerance indices while the third is an aggressiveness index.

From the "focal animal" sampling sessions we recorded a total of 118 interactions between the nest animals and the introduced animals. Eighty-seven of these interactions (73.7%) occurred away (more than 9 cm) from the nest. These data hence permit us to ask whether different categories of animals are also distinguished away from the nest. Computation of tolerance/aggressiveness indices from these 87 interactions (those that occurred away from the nest) and their pairwise comparison showed that no category of animal was treated significantly more tolerantly than any other category. It appears, therefore, that no discernible discrimination takes place away from the nest, although it must be mentioned that the sample sizes here are somewhat smaller than those of "all occurrences" sampling sessions, which were used to show that discrimination does take place in the vicinity of the nest. However, our suspicion that relatives and non-relatives are not distinguished away from the nest is strengthened by the observation in one of the three experiments of an unexpected but rather interesting phenomenon. Four of the foreign animals (which included one animal of each category) constructed a satellite nest which was joined by three nest animals. Even more interesting was the fact that one of the foreign animals, an "exposed" relative, established herself as the queen of this satellite nest.

Table 2. Data from "all occurrences" sessions. Frequency of interactions (and their corresponding tolerance/aggressiveness indices) shown by nest animals towards the four categories of foreign animals. T1 and T2 are the two families of tolerance indices, and T3 is the family of aggressiveness indices (see text). Pairs of tolerance indices having common numbers in parentheses are significantly different from each other by a two-tailed z test ($p < 0.05$)

Behaviour	Tolerance rank	"Exposed" relatives			"Exposed" non-relatives			"Isolated" relatives			"Isolated" non-relatives		
		Frequency of interactions											
		Replicate			Replicate			Replicate			Replicate		
		1	2	3	1	2	3	1	2	3	1	2	3
AB	1	2		2			1			1		1	
AT	2									1			
PK	3	25	9	17	14	40	12	16	13	6	27	32	5
CH	4	3			1	1	1	2			2		1
AM	5					1							
NI	6						3	1		2			
BV	7						1						1
AA	8												
AW	9	33	8	6	9	18	4	11	13	5	13	20	
AP	10	6			1	1		1			1		
AN	11												
Total no. of interactions		69	17	25	25	61	22	33	26	14	44	54	6
T1		5.8739 (1,2)			5.0000 (1)			5.5616			5.0000 (2)		
T2		1.5586 (3,4)			1.3989 (3)			1.4932			1.375 (4)		
T3		0.4955 (5,6)			0.6262 (5)			0.5206			0.6346 (6)		

Table 3. Data from "focal animal" sampling sessions. Frequency of interactions (and their corresponding tolerance/aggressiveness indices) shown by nest animals towards the four categories of foreign animals away from the nest. Tolerance/aggression towards any category of animals was not significantly different from that towards any other category of animals by a two-tailed z test ($p > 0.0562$)

Behaviour	Tolerance rank	"Exposed" relatives			"Exposed" non-relatives			"Isolated" relatives			"Isolated" non-relatives		
		Frequency of interactions											
		Replicate			Replicate			Replicate			Replicate		
		1	2	3	1	2	3	1	2	3	1	2	3
AB	1												
AT	2						2						2
PK	3	3	1	2	2		7	8		4	8	5	4
CH	4			1							1		
AM	5						1	1					
NI	6									1			
BV	7			1	1		2	1			1	2	
AA	8					1			1				
AW	9	1	1	1	2	2		1	1	3	2	1	
AP	10					1		1	1	1		3	1
AN	11									1			
Total no. of interactions		4	2	5	5	4	12	12	3	10	12	11	7
T1		5.1817			4.8572			5.9200			4.8999		
T2		1.4545			1.5238			1.6800			1.5000		
T3		0.5454			0.5238			0.4800			0.6333		

Although the foreign animals had a slightly higher mortality as compared to the nest animals (mortality rates not significantly different, χ^2 for test of independence = 0.889, $df = 2$, $p > 0.5$; Table 1), very few foreign animals died; most survived in parts of the cage away from the nest. No foreign animal was ever seen being killed. The proportion of animals dying out of the total number of animals of each category introduced is not different between the categories of foreign animals, nor is there any significant difference in the proportion of relatives and non-relatives that died (G test with William's correction, $p > 0.05$; Sokal and Rohlf, 1981).

Discussion

We introduced genetic relatives and non-relatives into laboratory cages containing active nests of the primitively eusocial wasp *R. marginata* and found that no foreign animal, relative or non-relative was accepted into the nest. However, genetic relatives were discriminated from genetic non-relatives in the vicinity of the nest, when both classes of individuals had been exposed to their respective natal nests and a subset of nestmates. This was evident from pairwise comparison of tolerance/aggressiveness indices computed from behaviours shown by the nest animals towards the various categories of foreign animals. These results are consistent with our earlier studies of the mechanism of nestmate discrimination in *R. marginata* (Venkataraman et al., 1988). It should be emphasized that such discrimination is based on recognition labels and templates that are acquired by the wasps from their nests or nestmates after eclosion (Venkataraman et al., 1988). Although relatives were distinguished from non-relatives, the fact that no animal was accepted into the nest confirms our suspicion that genetic relatedness, even if discerned, is not necessarily sufficient for acceptance of an animal into a nest. Acceptance of animals may depend on factors other than genetic relatedness. We have observed other instances of such a dichotomy between acceptance and tolerance. Some wasps which are either rejected or leave their natal nests are not subsequently accepted into the nest, but their presence is tolerated in the cage (unpublished observations). Besides, foreign conspecifics are accepted into natural colonies occasionally in post-emergence colonies, and routinely in small pre-emergence colonies (unpublished observations).

We also observed one instance of nest animals and introduced animals (which included relatives and non-relatives of the nest animals) coming together and constructing a satellite nest. Some nest animals were sufficiently tolerant of the foreign animals to co-operate with them in the construction of a new nest. We have seen other instances of genetically unrelated wasps co-operating in the construction or maintenance of nests, both in the laboratory as well as in the field (unpublished observations). The observation that relatives were treated more tolerantly in the vicinity of the nest compared to non-relatives, but that no more aggression was shown towards relatives than to non-relatives when encountered away from the nest, indicate that complex, context-dependent rules may govern the tolerance and acceptance of foreign animals. An equally significant observation was that very few foreign animals were severely attacked or killed, and that among the few that died

(from unknown causes) there was no significant preponderance of any particular category of foreign animals.

Two studies on ants reported in the literature are sufficiently detailed and have enough parallels with our experiments to permit comparison. Stuart (1987b) removed pupae of the ant species *Leptothorax curvispinosus* and allowed them to eclose and to age for 38–157 days in isolation. Such workers, when returned to their natal nest, were readily accepted onto the nests suggesting, according to Stuart (1987b), that individual workers produce persistent, colony-specific odours. Such workers studied by Stuart (1987b) correspond to the “isolated” relative category of foreign animals used in our study. Our results are different because the “isolated” relatives in our study were not accepted onto the nests. Crosland (1989b) separated newly eclosed workers of the ant species *Rhytidoponera confusa* and reared them in groups of nestmates for 31–81 days. He found that such workers were attacked and often killed when introduced back into their natal nests, suggesting, according to him, that young workers have different odours compared to older workers. The workers used by Crosland (1989b) had undergone a treatment similar to the “exposed” relative category of foreign animals used in our study. Again, our results are different because “exposed” relatives were not severely attacked or killed; indeed, they were treated more tolerantly than any other category of foreign animals used in our study.

Leptothorax curvispinosus and *Rhytidoponera confusa* appear to represent two extremes in the fate of nestmates that are returned to their natal nests after a period of separation. Our results are intermediate in that the returned animals were neither accepted onto the nests nor were they severely attacked and killed. It may be possible to conclude from the difference between our results and those of Stuart (1987b) and Crosland (1989b) that *R. marginata* is different from *Leptothorax curvispinosus* and *Rhytidoponera confusa* in that individual wasps of *R. marginata* do not produce colony-specific odours, and that young workers do not necessarily have significantly different odours compared to older workers. We believe, however, that there is a more interesting and perhaps more meaningful interpretation of the differences between our results and those of Stuart (1987b) and Crosland (1989b). In highly eusocial insects such as ants, the rules governing acceptance of and tolerance towards foreign animals are probably fairly simple. The *Leptothorax curvispinosus* workers introduced by Stuart (1987b) probably had the required colony-specific odours, and were thus accepted onto the nests. The workers of *Rhytidoponera confusa* introduced by Crosland (1989b) probably had different odours compared to the workers on the nest, so that they were attacked and killed. Such rules should be quite adequate for a highly eusocial insect where there is probably no great danger in mistakenly admitting into the nest a non-nestmate worker who has somehow acquired the colony-specific odour. This is because even if a non-nestmate worker is admitted to a colony, it is unlikely to pose any significant reproductive competition to the members of that colony (except to a small extent in species with reproductive workers). Similarly, there would probably be no great harm in attacking and killing a nestmate worker who, for some reason, lacks the colony-specific odour. This is because of the very large worker force that is usually maintained by highly eusocial insect colonies.

We expect such simple rules to be inadequate in a primitively eusocial species such as *R. marginata*, where many individuals have reproductive options and can become replacement queens. In such a situation, it may be quite detrimental to the fitness of the colony members to admit into their midst an individual of unknown reproductive potential and dominance rank, even if it has the colony-specific odour. Conversely, not every worker would necessarily gain an advantage by attacking and killing individuals who may be present in the vicinity of the nest but lacking the colony-specific odour. Some members of the colony may in fact find it advantageous to leave their natal nest and co-operate with such strangers to build a new nest if there is some chance that they would, at least at a later date, become egg layers on the new nest. Whether or not the mechanism of production of colony-specific odours is different between *Leptothorax curvispinosus* and *Rhytidoponera confusa* on the one hand and *R. marginata* on the other, we believe that the primitively eusocial status of *R. marginata* is likely to make the rules governing tolerance and acceptance of foreign animals sufficiently different to account for such differences in the results of otherwise similar experiments.

Reeve (1989) has modelled situations where evolutionarily stable acceptance thresholds of dissimilarity between an actor's template and those of the recipients vary according to the recognition context. One of the predictions of these models is that universal acceptance of all animals is likely in a context where interactions with undesirable animals are few, universal rejection is expected when encounters with undesirable animals are frequent and differential acceptance is likely to prevail in contexts with intermediate frequencies of such interactions. The results described in this paper can perhaps be used to test these predictions. For this purpose, let us ignore "isolated" animals (relatives and non-relatives) because it is not entirely clear if they are being appropriately recognized. The "exposed" relatives and "exposed" non-relatives, however, are clearly recognized as such, and may be equated to the desirable and undesirable categories of animals, respectively, in the models. There are at least three different recognition contexts in our experiments, namely "on the nest", "in the vicinity of the nest" and "away from the nest".

It is possible that interaction frequencies with desirable and undesirable animals that have prevailed over generations for a given population modulate acceptance thresholds, or that rates of interaction are measured on a day-to-day basis, and used to modulate acceptance thresholds. First, let us consider the possibility that phylogenetically "fixed" rates of interaction are used. For species such as *R. marginata*, that build open combs, nestmate foragers (desirable animals) keep returning to the nest every few minutes, but non-nestmates (undesirable animals) may only land on the nest very rarely (perhaps due to mistaken nest identity). Rates of interaction with returning desirable animals should therefore be much higher than those with undesirable animals on the nest. On the other hand, since foraging is done individually and without recruitment, animals are much more likely to encounter non-nestmates (undesirable animals) than nestmates (desirable animals) when they are away from their nests (Tab. 4, rows 1 and 2). These asymmetries in rates of interaction with desirable and undesirable animals, on the nest and away from it, should on the basis of the model lead to universal acceptance of foreign animals on the nest but universal rejection of foreign animals away from the nest (Tab. 4, row 5).

Table 4. A possible test of conspecific acceptance threshold models (Reeve, 1989). Data in this table are pooled from "all occurrences" and "focal animal" sampling sessions (number of interactions in parenthesis)

Recognition context	On the nest	In the vicinity of the nest	Away from the nest
Frequency of interaction expected in natural colonies, with desirable animals	High	–	Low
Frequency of interaction expected in natural colonies, with undesirable animals	Low	–	High
Frequency/h of interaction between any nest animal and a desirable animal ("exposed" relatives)	0.0946(6)	1.541(115)	0.417(10)
Frequency/h of interaction between any nest animal and an undesirable animal ("exposed" non-relatives)	0.0315(3)	1.354(109)	0.966(21)
Behaviour of nest animals towards foreign animals expected on the basis of the "interaction-frequency" sub-model of Reeve (1989)	Universal acceptance	Differential acceptance	Universal rejection
Behaviour of nest animals towards foreign animals expected on the basis of the "fitness consequence" sub-model of Reeve (1989)	Universal rejection	Differential acceptance	Universal acceptance
Behaviour of nest animals towards foreign animals observed	Universal rejection	Differential acceptance	Universal acceptance

Considering the possibility that interaction frequencies are measured on a day-to-day basis leads to similar conclusions. Although sample sizes are small, we observed a higher rate of encounter by nest animals of relatives than of non-relatives on the nest, nearly the same rates of encounter of the two categories of animals in the vicinity of the nest and a higher rate of encounter of non-relatives than of relatives away from the nest (Tab. 4, rows 3 and 4). These interaction frequencies would also lead to an expectation, on the basis of the model, of universal acceptance on the nest, differential acceptance in the vicinity of the nest and universal rejection away from the nest (Tab. 4, row 5). However, our results showed exactly the opposite trend (Tab. 4, row 6). There was universal rejection of animals on the nest, but nearly universal acceptance away from the nest. In addition, nest animals co-operatively founded a satellite nest with foreign animals which included an "exposed" relative (desirable) and an "exposed" non-relative (undesirable).

We suspect therefore that factors other than interaction frequencies are likely to be important in modulating acceptance thresholds. Reeve's (1989) models consider at

least two other factors. His models predict that the probability of acceptance of undesirable animals will be proportional to the cost (such as that resulting from injury) of rejecting animals. We have no clear way of estimating the costs of rejecting relatives or non-relatives in the three recognition contexts of our experiment. His models also predict that the probability of accepting undesirable animals will be proportional to the fitness consequence of accepting animals. We expect the fitness consequences of accepting non-relatives to become progressively less negative as the scene shifts away from the nest, and in fact to become positive if co-operative nesting with non-relatives is possible. Our results are hence in accordance with these predictions of Reeve's (1989) models. We suspect therefore that fitness consequences are likely to be more important than interaction frequencies in modulating acceptance thresholds, especially in primitively eusocial species such as *R. marginata*. Reeve's (1989) models can perhaps be applied more realistically to primitively eusocial species if one allows the levels of desirability and undesirability of different categories of animals to vary with the recognition context. For instance, non-relatives may be undesirable on the nest, but may become less undesirable when new nests are co-operatively initiated away from the nest, especially if the original nest animals have some finite chances of becoming queens of the satellite nests, either now or in the future.

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