

Age Specific Acceptance of Unrelated Conspecifics on Nests of the Primitively Eusocial Wasp, *Ropalidia marginata*

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To investigate the possible role of the age of an individual on its acceptance onto unrelated colonies, several individually marked foreign conspecifics that had been isolated immediately upon eclosion from their natal nests, were introduced into laboratory cages containing apparently healthy colonies of *R. marginata*. Foreign conspecifics, if less than or equal to 8 days of age, had a finite probability of being accepted onto unrelated colonies. Young wasps appeared to be accepted preferentially over older ones, both because young individuals made more attempts to join as well as because they met with greater success per attempt. The resident wasps vigorously nibbled and groomed young foreign conspecifics once they were accepted onto nests, just as they did for newly eclosed wasps from their own nests. In both cases, this may result in the transfer of nestmate discrimination odours from mature to young wasps. Three confirmed records of unrelated accepted foreign conspecifics becoming foragers in their foster colonies are presented. We hypothesize that although young foreign conspecifics may be accepted for the proximate reason they may not be efficiently discriminated from newly eclosed nestmates, such acceptance may have an ultimate adaptive value, or at least may not be detrimental to the accepting colony. If the basis of social life is mutualistic interaction among individuals with varying levels of relatedness, as has been postulated for such primitively eusocial wasps, then the advantage of accepting a young foreign conspecific who can be subdued into a subordinate worker role with relative ease, is not difficult to appreciate.

Key Words: Social wasps, *Ropalidia marginata*, Kin recognition, Mutualism, Eusociality, Hymenoptera

Introduction

Females of the primitively eusocial wasp *Ropalidia marginata* can discriminate nestmates from non-nestmates even outside the context of their nests, provided the discriminating as well as the discriminated individuals have previously been exposed to a fragment of their natal nests and a subset of their nestmates. This suggests that labels and templates used in such recognition are acquired and learned, after eclosion of the individuals concerned, from a common

source such as the nest or nestmates (Venkataraman 1990, Venkataraman et al. 1988, 1990, Venkataraman & Gadagkar 1990). Extensive studies of other primitively eusocial wasps have led to similar conclusions (Reviews in, Gadagkar 1985, Gamboa et al. 1986). The use of acquired odours in kin and nestmate discrimination is also common in other social insects (Stuart 1987, Morel et al. 1988, Crosland 1989, Jaisson 1991).

Such a mechanism of nestmate discrimination should permit the occasional acceptance of genetically unrelated conspecifics provided the lat-

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ter can acquire the odours of unrelated colonies. Conversely even a genetically related individual may not be accepted if it fails to acquire the colony-specific odours. In primitively eusocial species such as *R. marginata*, it appears that the genetic asymmetry created by haplodiploidy may be less important in moulding the evolution of social behaviour than mutualistic interactions among somewhat less related individuals (Gadagkar 1991a). The reasons for such a speculation are many. Polyandry and serial polygyny are common characteristics of *R. marginata* leading to a substantial reduction in worker-brood genetic relatedness (Muralidharan et al. 1986, Gadagkar 1990c, Gadagkar et al. 1991, 1993). There is a considerable amount of drifting of individuals and usurpation of colonies in the pre-emergence phase (Shakarad & Gadagkar, in press). Workers thus rear brood that are rather distantly related to them and appear to do so without an ability to discriminate among different levels of relatedness within a colony (Venkataraman et al. 1988).

It was therefore of interest to investigate the factors that determine whether a foreign conspecific will be accepted onto a colony. In one set of experiments designed to answer this question, we introduced different 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 experiments demonstrated that in the context of a nest, genetically related foreign conspecifics are treated significantly more tolerantly than unrelated ones but neither are accepted onto the nest. Yet in some cases, insects from the resident colony cooperatively founded new colonies with the foreign conspecifics (Venkataraman & Gadagkar 1992). The latter result suggested that cooperation with unrelated individuals is possible at least under some circumstances. Both anecdotal evidence from an earlier study (Venkataraman &

Gadagkar 1992) and numerous instances in the literature from diverse families of social insects, including ants, bees and wasps, show that callow individuals are readily accepted onto unrelated colonies (Fields 1905, Forel 1928, Wilson 1971, Jaisson 1972, Holldobler & Michener 1980, Carlin & Holldobler 1986, Morel et al. 1988).

We have therefore conducted experiments to study the effect of the age of introduced wasps and demonstrate that young unrelated foreign conspecifics have a finite chance of being accepted onto unrelated colonies.

Materials and Methods

Study Animal

Ropalidia marginata (Lep.) (Hymenoptera: Vespidae) is a primitively eusocial independent founding polistine wasp distributed widely in peninsular India (Gadagkar et al. 1982, Gadagkar 1991a). Nests may be initiated by one or a small group of females only one of which assumes the role of egg-layer (queen) while the others assume the role of workers. Female offspring may either stay back and become workers or leave to found new single or multiple-foundress colonies (Gadagkar 1991a). Those who stay back on their natal nests have a finite chance of replacing the original queen and taking over as the next queen (Gadagkar et al. 1991, 1993).

Collection of Nests and Preparation of Experimental Animals

Ropalidia marginata females were collected from naturally occurring post emergence nests, brought to the lab, released into a 45×45×45 cm³ wood and wire mesh cage and provided an *ad libitum* diet of final instar *Corcyra cephalonica* larvae, honey, water and a few blocks of soft wood as building material. Under these circumstances the wasps usually constructed nests, produced brood and maintained apparently normal social organization (Chandran & Gadagkar 1990). Four such laboratory nests were thus es-

tablished using wasps from four different naturally occurring nests. At the same time, five other post emergence nests were collected from a site at least 10 km away from previous ones. These nests were brought to the laboratory, cleared of all adult wasps and monitored continuously for eclosion of adult wasps. In less than 12 hr after their eclosion, female wasps were isolated into individual ventilated plastic jars of 22×11×11 cm from a few hours to 29 days.

In all, 44 'Isolated' non-relatives of varying ages ranging from 0-29 days (mean \pm s.d = 7.84 \pm 7.4), marked with unique spots of paint were introduced into one of the four cages containing a nest each. All wasps were introduced one hour prior to the commencement of observations and no wasps were introduced subsequently. Introductions were always made from the side of the cage opposite to the location of the colony.

The Observations

Observations began one hour after the introduction of foreign wasps and were carried out from 0830 to 1200 hr and 1430 to 1800 hr 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 to 1200 hr or from 1430 to 1800 hrs. Most of the interactions between resident wasps and introduced wasps occurred in the vicinity of the nest. All interactions initiated by any resident wasp towards any introduced one, within 9 cm from the periphery of the nest, were recorded in blocks of 5 min observation sessions. Fifteen such 5 min 'All Occurrences' observation blocks were performed during a period of 3.5 hr (either from 0830 to 1200 hrs or from 1430 to 1800 hr). Because some wasps did not come to the vicinity of the nest often enough, separate 'Focal Animal' sampling sessions of 5 min duration each were conducted once for each introduced individual in any 3.5 hr observation pe-

riod. In each 'Focal Animal' sampling session, all interactions initiated by any resident wasp towards the focal animal anywhere in the cage were recorded. The blocks of 'All Occurrences' and 'Focal Animal' sampling sessions were randomly intermingled during a 3.5 hr observation period. The sequence of individuals chosen for observation during the 'Focal Animal' sampling sessions was also chosen randomly.

Observations on the four nests together amounted to 1259 blocks of 'All Occurrences' sampling sessions and 775 blocks of 'Focal animal' sampling sessions and in all corresponded to 169 hr and 29 min of observations. In addition, the position of all introduced wasps were recorded on most nights (85 out of 109 nights) until all introduced wasps died or were accepted onto the nest.

Acceptance or otherwise of introduced wasps was easy to define. Individuals were considered accepted if they were seen for more than 5 min on the nest during the day or were seen on the nest on any night. "Accepted" individuals became part of the colonies in their cage of introduction and were seen moving between the nest and other areas of the cage, much like the original resident wasps. They were also usually present on the nest at night. "Unaccepted" individuals sometimes alighted on the nest during the day but elicited high levels of aggression from the resident wasps resulting in their immediate departure. Such unaccepted individuals were never seen on the nest at night.

The Analysis

We used Monte-Carlo simulations to compare ratios. The numerators of these ratios are the number of occurrences of events such as behaviours, interactions or number of attempts to get on the nest. The denominators are quantities such as number of hours spent in observing wasps of certain classes, number of individuals of certain classes or total number of interactions.

In one type of analysis for instance, we wished to find out whether individuals less than or equal to 8 days in age made more attempts to get on the nest compared to individuals older than 8 days. The number of wasps in each category varied and we therefore compared the number of attempts per wasp of one age class ($\lambda \leq 8$ days) with the number of attempts per wasp of the second age class (> 8 days). The null hypothesis here was that the number of attempts per wasp of both age classes were not different from one another. An experimental deviation was calculated by subtracting the attempts per individual of the second class from the first. N random numbers were generated, (where N was equal to the sum of the number of attempts made by individuals of both classes observed in the experiment), assigned to the two classes in proportion to the number of wasps present in each class and divided by the number of wasps in the respective class to yield the expected number of attempts per wasp in each class. This was repeated a 100 times to obtain 100 expected deviations. The proportion of times this expected deviation was equal to or greater than the experimental deviation, gave the p -value, i.e., the probability that the experimentally observed result could have been obtained by chance alone. We then repeated the simulation a 100 times to give a 100 p values and computed the mean and standard deviation of these p values.

A similar procedure was used to compare young (less than or equal to 8 days) and old (greater than 8 days) individual on the basis of the proportion of successful attempts to join the nest out of the total number of attempts made by that class of wasps. Finally (using a similar procedure), we tested the null hypothesis that certain behaviours occurred on different days of the experiment, in proportion to the number of hours of observations on the respective day classes.

Results and Discussion

Acceptance of Foreign Wasps

The main aim of this study was to determine the effect of the age of foreign conspecifics on their acceptance or otherwise onto unrelated colonies of the primitively eusocial wasp *Ropalidia marginata*. We have tested 44 wasps belonging to 18 different age categories ranging from 0 to 29 days by introducing them into laboratory cages containing apparently healthy colonies, in their early post emergence phase. Our main result is that wasps which are 8 days old or younger have a finite probability of being accepted onto unrelated colonies but those greater than 8 days of age have virtually no chance of doing so. Out of 29 wasps, 8 days old or younger that were introduced, 17 were accepted. Of 15 wasps older than 8 days that were introduced, none was accepted. These two ratios are significantly different from each other and could not have been obtained from chance alone (Monte-Carlo test, $p < 0.001$, see Methods) (table 1).

The probability of acceptance onto unrelated colonies is negatively correlated and falls sharply with age (figure 1). Since wasps are not necessarily accepted immediately upon introduction or at some fixed time after, we examined the probability of acceptance as a function of age on the day of acceptance by computing the ratio of the number of accepted individuals in each age class to the number of wasps of that age class encountered by the colony throughout the experiments. The latter quantity is the sum of the number of wasps of any age category introduced plus the total number of wasps attaining this age before being accepted. The general relationship of the probability of acceptance with age at acceptance is similar to that with age at introduction (figure 2). However, two differences deserve mention. The first is that wasps were not accepted on the day of their eclosion. This is because they were inactive and stayed in a corner of the cage,

Table 1 Summary of Nests, Introductions and Acceptances

	Expt.1	Expt.2	Expt.3	Expt.4	Total
Source nest	A & B	C	D	E	5
Recipient nest	F	G	H	I	4
No. of cells on recipient nest	28	60	112	31	-
No. of eggs on recipient nest	11	14	67	12	-
No. of larvae on recipient nest	9	13	25	12	-
No. of pupae on recipient nest	8	4	20	3	-
No. of adults on recipient nest	40	31	19	14	-
Number introduced	6	14	7	17	44
Ages introduced	0-6	0-13	11-29	0-12	0-29
Number accepted	1	4	0	12	17
Number introduced 8 days old or younger	6	9	0	14	29
Number accepted 8 days old or younger	1	3	0	13	17
Number introduced over 8 days old	0	5	7	3	15
Number accepted over 8 days old	0	0	0	0	0

Table 2 Logistic Regression Analysis: Determinants of probability of acceptance of unrelated conspecifics on nests of *Ropalidia marginata*

Variable	Estimated coefficient (β)	Standard error	Z
Intercept	687.861	1327.177	0.5183
Colony 1	-683.939	1327.178	-0.5154
Colony 2	-689.395	1332.777	-0.5173
Colony 3	-681.817	1327.179	-0.5137
Colony 4	-688.209	1327.177	-0.5186
Age at introduction	-0.857	0.314	-2.7321*

* $p < 0.01$

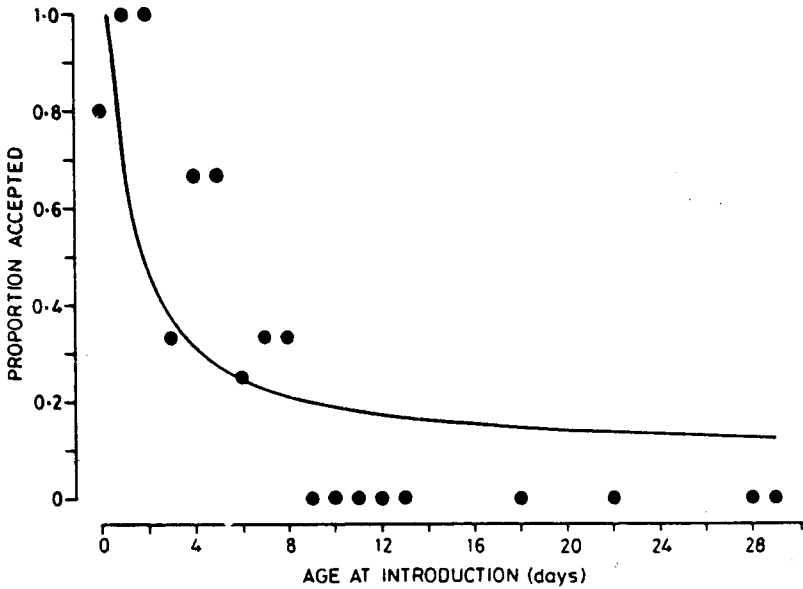


Figure 1 Acceptance onto *R. marginata* colonies, of foreign conspecifics as a function of their age at the time of introduction. The fitted line is given by the equation $Y=1.1275 (1/x+1)+0.0851$ where the slope is significantly greater than zero, $p < 0.001$

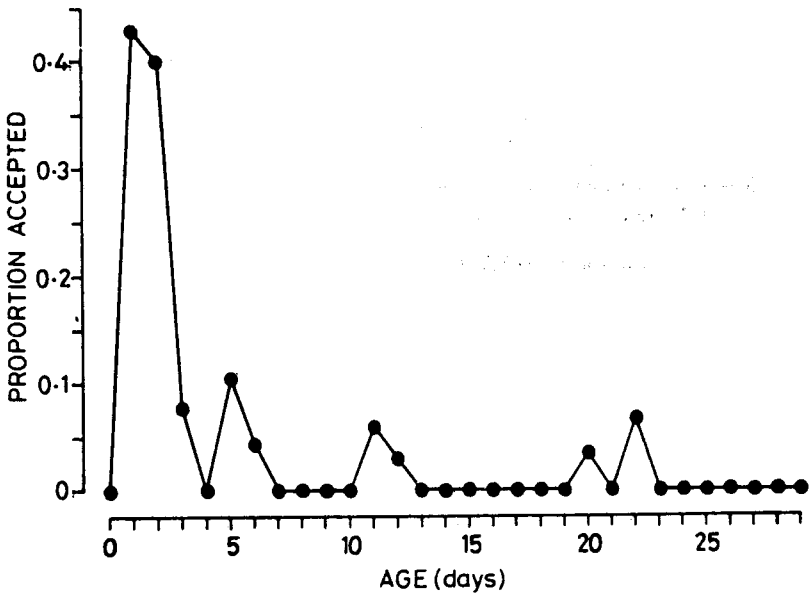


Figure 2 Proportion of foreign conspecifics accepted out of the total number available of each age. Numbers available for each age are the sums of the numbers of wasps introduced at that age and the numbers introduced at a younger age but attain that age during the experiment

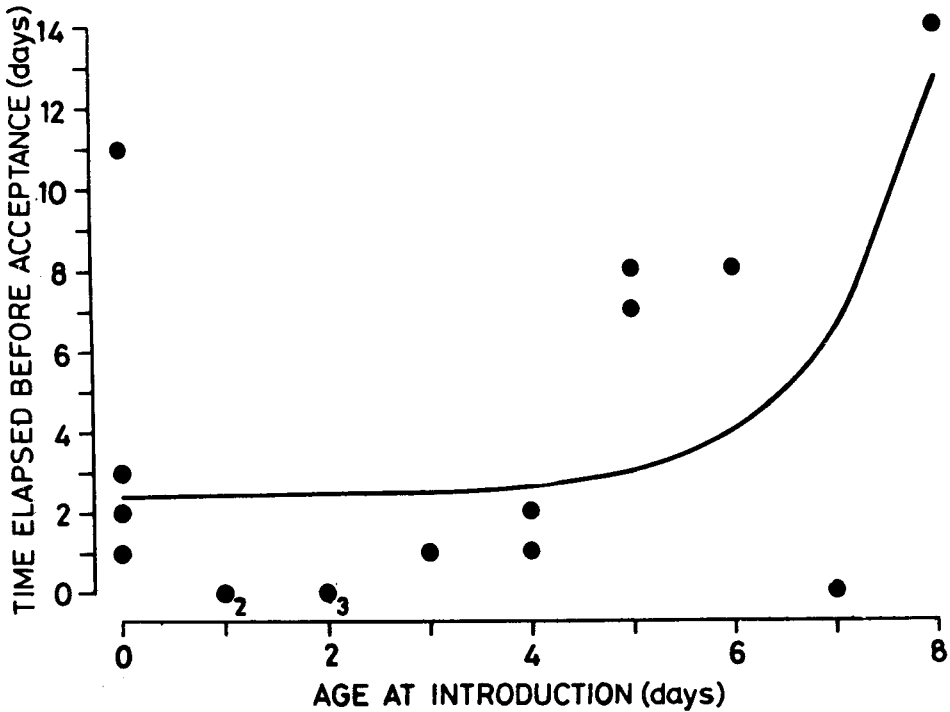


Figure 3 Time required for acceptance of foreign conspecifics onto *R. marginata* colonies as a function of the age of the introduced wasps. The fitted line is given by the equation $Y=0.0034e^x+2.4112$ where the slope is significantly greater than zero, $p < 0.01$

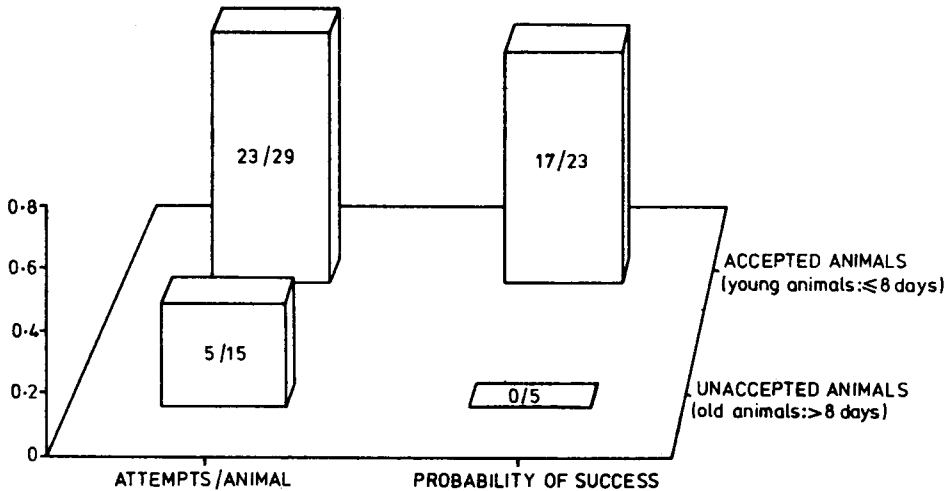


Figure 4 The bars on the left represent the number of attempts/wasp for wasps less than and equal to 8 days and for wasps greater than 8 days. The bars on the right represent the number of successful attempts/total attempts for wasps less than or equal to 8 days and wasps greater than 8 days. Wasps less than or equal to 8 days make significantly more attempts/ individual than wasps greater than 8 days (Monte-carlo test, $p < 0.02$) wasps less than or equal to 8 days also have a higher success rate per attempt than wasps greater than 8 days (Monte Carlo test, $p < 0.001$)

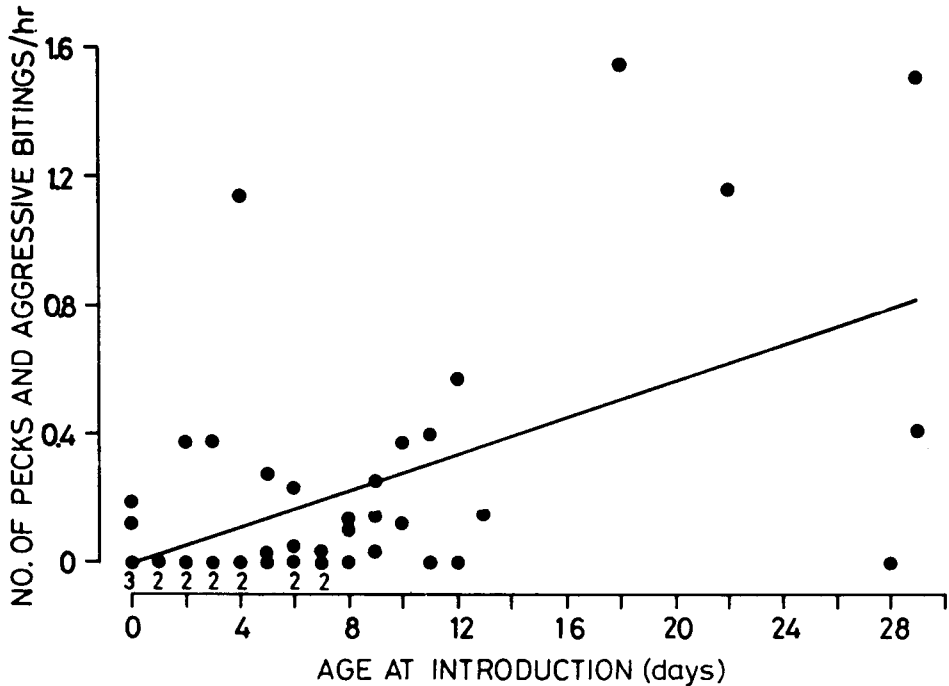


Figure 5 Aggressive behaviour received by foreign conspecifics as a function of their age at the time of introduction into a cage containing colonies of *R. marginata*. The fitted line is given by the equation $Y=0.0283X-0.0003$ where the slope is significantly greater than zero $p < 0.001$. Each data point corresponds to one wasp: Numbers adjacent to points indicate the numbers of overlapping points

close to the location of their introduction and made no attempt to join the resident colony. The second difference is that individuals older than 8 days had a small probability of being accepted. The instances of 11, 20 and 22 day old wasps being accepted as seen from figure 2 however, invariably corresponded to wasps that were 8 days old or younger at the time of their introduction. One possible explanation for this is that the age at introduction is more important than the age at acceptance. The introduced wasps may be identified by the resident wasps away from the nest soon after their introduction and recognized as "acceptable" or "unacceptable" depending on their age. The actual acceptance, however, may take place a few days later depending on the

attempts made by the introduced wasps to join the colonies. An alternate explanation may simply be that sample sizes for age of acceptance greater than 8 days are substantially larger in figure 2 compared to figure 1. This is because all introduced wasps less than 8 days which were not immediately accepted attained various ages and thus contributed to increased sample sizes of older wasps from among which some acceptances were observed.

Not all the accepted wasps were accepted immediately. The time required for acceptance ranged from 0 to 14 days after introduction. There was a significant increase in the time re-

quired for acceptance for older wasps even within the 0 to 8 day categories (figure 3).

Why are Young Wasps Accepted and Older Wasps Rejected? - Proximate Causes

We consider two hypotheses. The first is that young wasps made more attempts to join colonies, than did older wasps. As mentioned earlier, introduced individuals occasionally landed on the nest and were often aggressively repelled and forced to leave. Each such sighting of an introduced wasp on the nest including the first time it was seen on the nest on the day it was accepted is defined as an 'attempt' to join the nest. Twenty three such attempts by a total of 29 accepted (young, ≤ 8 days) wasps were recorded while only 5 such attempts by a total of 15 unaccepted (old, 8 days) wasps were recorded (figure 4). The ratio 23/29 is significantly greater than 5/15 (Monte-Carlo test, $p < 0.02$). The second hypothesis we consider is that older wasps were not accepted despite their attempts to join the colonies. Out of 23 attempts by young (accepted wasps), 17 were successful in that 17 wasps were accepted. Out of 5 attempts, by old (unaccepted) wasps none was successful (figure 4). The ratio 17/23 is significantly greater than 0/5 (Monte Carlo test, $p < 0.001$). We conclude therefore that young wasps are accepted and old wasps are rejected both because young wasps made significantly more attempts to join the colony and because they had a significantly higher rate of success per attempt compared to old wasps. Old wasps had a lower rate of success per attempt presumably because they were perceived as 'undesirable' by the resident wasps.

But why should old introduced wasps make fewer attempts to join? One possibility is that they also perceive the resident wasps as undesirable. Another possibility is that interactions between resident and introduced wasps outside the nest were such that old introduced wasps were deterred from attempts to join. The latter possibility is supported by the observation that the

rates of aggressive behaviour shown towards introduced wasps (number of pecks and aggressive bitings per hour) outside the nest is significantly positively correlated with the age of the introduced wasps (figure 5). There is much scatter in this data but it is nevertheless quite clear that more aggression is shown towards old wasps. Eight days old or younger wasps showed significantly less aggression (0.105 ± 0.229 , $n=15$) than those older than 8 days (0.445 ± 0.527) (difference significant by a two-tailed Mann-Whitney U test, $Z = -3.0694$, $p = 0.0022$).

And why should old wasps be less successful per attempt, in being accepted? Previous work in our laboratory has shown that unfamiliar nestmates, isolated from their nests and nestmates, are not treated as tolerantly as unfamiliar nestmates who have been exposed to a fragment of their nests and a subset of their nestmates. Thus nestmate discrimination appears to be based on recognition labels and templates acquired and learned, after eclosion, from the nest and/or nestmates (Venkataraman et al. 1988). These results however cannot be interpreted to mean that the isolated wasps were treated less tolerantly merely because they lacked a familiar odour. The results of the present study show that young isolated wasps, even if they are non-nestmates, are treated tolerantly and accepted onto alien nests. These young isolated wasps must also lack the required familiar odour. Perhaps the old isolated wasps lack the required familiar odour and at the same time possess an endogenously produced undesirable odour, the latter being related to their better ovarian development (see below). Conversely, young isolated wasps, while lacking the colony-specific familiar odour, do not simultaneously possess the undesirable "developed ovary" dependent odour, characteristic of the old wasps. Nestmate discrimination is bound to be as complicated and more. We also find that in experiments similar to the present ones, when presented with genetically related and unrelated,

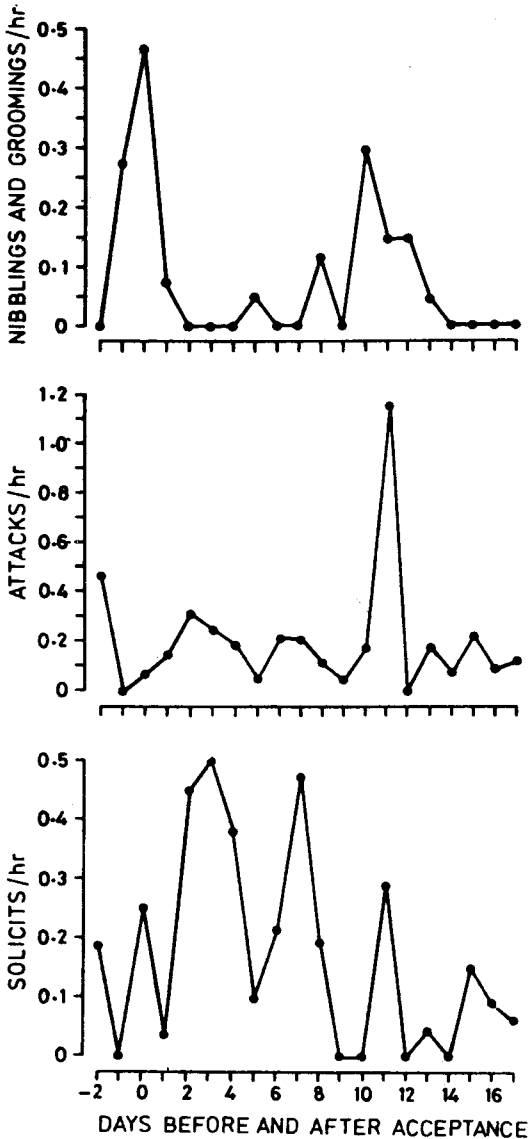


Figure 6 The freq/hr of the three classes of behaviours, Nibbling and Grooming, Attack and Solicit are shown for the days before wasps are accepted, the day on which a wasp is accepted and the days after wasps are accepted. Nibbling and Grooming occur at a higher frequency on day 0 than the overall frequency for all other days and also at a higher frequency on day 0 than day 10 which has the next highest frequency (Montecarlo test, $p < 0.001$). No such pattern is significant in the cases of Attack and Solicit

isolated, old, non-nestmate wasps, resident wasps treat genetically related intruders more tolerantly than unrelated intruders, although none are accepted onto the nests (Venkataraman & Gadagkar 1992). When presented with socially experienced non-nestmates rather than isolated, non-nestmates, tolerance depended on how much time the intruders had spent away from their natal nests; those that spent more time away from their natal nests are likely to have had poorly developed ovaries as well as less of their natal colony-specific odour (Venkataraman & Gadagkar 1993).

Are Nestmate Discrimination Odours Transferred to Accepted Individuals?

The mechanism of nestmate discrimination suggested by an earlier study (Venkataraman et al. 1988) permits the occasional acceptance of unrelated individuals. This is because discrimination odours appear to be acquired from the nest or from nestmates. Thus a foreign wasp may sneak onto a colony, acquire the colony's odour and may masquerade as a member of that colony. This appears to be even easier for young wasps who presumably do not have to lose any existing "foreign" odours but may only have to acquire new ones. Our observations suggest that young wasps find it easier to get accepted onto unrelated colonies for yet another reason: resident wasps on the unrelated nest may actively help them to acquire the new set of colony odours. We have often observed newly eclosed wasps on natural colonies being subjected to generous amounts of grooming and mild nibbling by older nestmates. This may well be a way of transferring odours onto the newly eclosed wasps. It was therefore most striking to watch newly accepted wasps being subjected to a similar process of grooming and nibbling by the resident wasps. If such grooming and nibbling constitute a mechanism of transfer of odours and not merely a way of treating foreign conspecific, then such behav-

behaviour should occur significantly more often immediately after acceptance as opposed to later days.

To test this assumption, we tested two null hypotheses: (i) The frequency/animal/hr of nibbling and grooming on the day an individual was accepted is not different from the corresponding frequency of all other days pooled; (ii) The frequency/animal/hr of nibbling and grooming on the day a wasp was accepted is not different from the corresponding frequency on that day after acceptance, having the next highest frequency among all other days (assuming that the day of acceptance had the highest frequency). Monte-carlo simulations show that the frequency/animal/hr of nibbling and grooming on day zero (i.e. on the day of acceptance) is significantly greater than the frequency for all other days pooled (figure 6; $p < 0.001$). Similarly, we find that the frequency/animal/hr of nibbling and grooming on day zero is significantly greater than the frequency/animal/hr on the tenth day after acceptance (which has the second highest frequency among all days).

To ensure that a high frequency of nibbling and grooming does not occur as a result of a general burst of behavioural activity associated with the acceptance of a wasp, we similarly examined rates of two other behaviours that are often directed towards foreign wasps, namely Attack and Solicit. Neither of them were however shown with a higher rate on the day of acceptance as compared to the corresponding rates on subsequent days (figure 6b, c).

To check whether rates of nibbling and grooming on the day a wasp was accepted as well as the immediate days before and after acceptance were significantly higher than other days, we also performed a G-test (Sokal & Rohlf 1981). The null hypothesis here was that behaviours occurred in proportion to the number of hours spent observing each day class (see below). Because the process of acceptance is likely to be gradual and not restricted to a single day,

we pooled the days into two classes. -2, -1, 0, 1 and 2 days were lumped into one class and days 3 to 17 in the second class. We calculated the expected frequencies by calculating the proportion of observation hours for each class out of the total and multiplying this by the total number of behaviours shown for all the days. We once again repeated this test for both Solicit (SC) and Attack (AT). The frequencies of nibbling and grooming were significantly higher in the first day class ($G = 12.748$, d.f. = 1, $p < 0.05$). For Solicit and Attack however, there was no significant difference in the frequencies between the two classes, ($G = 0.374$, d.f. = 1, $p > 0.05$, $G = 0.002$, d.f. = 1, $p > 0.050$). We therefore reject the null hypothesis for Nibble and Groom but accept it for both Solicit and Attack. This corroborates our earlier result with the Monte-Carlo simulations. It appears therefore that nibbling and grooming occurs predominantly on the day a wasp is accepted and is not a consequence of a burst of general behavioural activity.

What happens to Unrelated Individuals after They are Accepted?

In an earlier study (Venkataraman and Gadagkar, unpublished observations) we found that an individual accepted when it was 0 days old foraged for its foster colony after reaching an age of 13 days. A second individual accepted at 1 day of age foraged at 9 days of age. In the present study we once again found that a wasp accepted at age 5 days began foraging at age 16 days.

In only one case an accepted individual spent sometime on its foster colony and after a great deal of aggression, was directed towards it by the resident wasps, eventually left. We also saw accepted wasps often inspecting cells containing larvae and eggs, carrying building material on the nest and being involved in exchanges of building material, food, honey and water with nestmates. Thus introduced, unrelated individuals once accepted, appear to integrate well into their foster colonies to perform

intra-nidal as well as extra-nidal tasks. Preliminary analysis of a more extensive series of experiments to monitor the long-term fate of such accepted wasps suggests however that they can become foragers as well as future queens (Arathi and Gadagkar in press).

Why are Young Wasps Accepted and Older Wasps Rejected? - Ultimate Causes

Why has such a strategy of accepting young foreign wasps and rejecting older ones evolved? *R. marginata* females can discriminate nestmates from non nestmates if both the discriminating and discriminated individuals have been exposed to a fragment of their natal nests and a subset of their nestmates (Venkataraman et al. 1988). However individuals older than a month previously exposed to a fragment of their natal nest and a subset of their nestmates were not accepted onto nests occupied by an unfamiliar subset of their own nestmates. Isolated virgin wasps such as those used in these experiments gradually develop their ovaries; there is a significant positive correlation between age and degree of ovarian development (Gadagkar unpublished observations). We speculate therefore that foreign wasps with developed ovaries are not accepted onto nests while those with underdeveloped ovaries may be accepted. The adaptive significance of not accepting individuals with developed ovaries is obvious in a primitively eusocial wasp such as *R. marginata*. Not only do most individuals have reproductive options (see Gadagkar 1991a, for review) but frequent queen turnover leading to serial polygyny is the rule rather than the exception (Gadagkar et al. 1991, 1993). But why should any foreign wasp be accepted at all - young or old, with developed ovaries or otherwise? While the proximate reason for this may be the inability to distinguish foreign callows from natal callows, the ultimate advantage must lie in the utility of accepting foreign callows. For one thing foreign callows with as yet poorly developed ovaries may be

suppressed and recruited into the colony worker force (see above). For another, cooperative nesting, even with unrelated individuals may be of great advantage during colony founding. Mutualistic interactions with related or unrelated individuals and other associated demographic factors rather than genetic asymmetries created by haplodiploidy appear to be responsible for the evolution of a worker caste in *R. marginata* (Gadagkar 1990a, b, 1991a, b, c). Evidence for the role of such acceptance/joining of young foreign conspecifics is however equivocal. In one colony of *Ropalidia marginata* that was monitored for over 600 days, 128 individuals accounting for 15.4% of all individuals that left the natal nest, disappeared at the age of 8 days or less. But no foreign conspecifics was seen to join this nest (Chandrashekara & Gadagkar, unpublished observations). However, young wasps join groups of foundresses who are about to begin or have just begun to found colonies. In a recent study where we monitored 145 naturally initiated pre-emergence nests and 676 foundress wasps, 32% of the foundresses were seen to drift from one nest to another and 48% of the nests received at least one drifter (Shakarad & Gadagkar, in press).

Are our conclusions influenced by the fact that we have used four different colonies and pooled the data?

Our data are not entirely statistically independent because we used four different colonies for introducing alien conspecifics and pooled data from all the four colonies for analysis. Ideally we would have liked to introduce all the 44 wasps into a single cage containing only one colony so that there would be no problem about variation from colony to colony in this regard. However, we were keen on introducing a somewhat limited number of wasps per colony so as not to swamp the colony with foreign wasps. For this reason we have used four different cages with a colony each to introduce the 44 wasps. We made some

attempt to use similar nests; for instance, all the four nests were in their early post emergence phase. However, because the experiment had to be done opportunistically depending on the availability of suitable wasps for introduction better control was impossible. For the same reason, it was also not possible to introduce the same number of wasps or individuals of very similar age distributions into each cage. Nevertheless, we suspect that our results are not significantly influenced by the colony onto which the foreign wasps were introduced. The fate of introduced wasps into each nest separately, showed that individuals 8 days old or younger have some chance of being accepted while those greater than 8 days old have no chance of acceptance (table 1). More importantly, we have verified that our main result that the probability of acceptance of an alien conspecific is a function of its age at introduction and not a function of the colony of introduction, by performing a logistic regression analysis where we explicitly consider the problem of having introduced the 44 wasps into four different nests. We did this by creating four variables, one for each nest and assigning a value of 1 or 0 for each introduced individual for each nest variable depending on whether that individual was or was not introduced into that nest. Using such values of four nest variables and the age at introduction as independent variables and acceptance or otherwise as the dependent variable, we find that only the logistic regression coefficient associated with age at introduction rather than any of the variables associated with the nest of introduction is significant (table 2).

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Were Young Wasps Accepted More often than Old Wasps because they had Acquired more of the Smell from the Plastic Boxes in which They were Held?

After these experiments were completed, it was brought to our notice that since we hold our wasps in plastic boxes, prior to introduction, the wasps may be acquiring some odour from the plastic boxes. This seems plausible and potentially weakens our conclusions because older wasps would have been held longer in the plastic boxes, acquired more of the odour and would be more likely to be rejected by the resident wasps compared to young wasps who on the contrary would have been held in plastic boxes for a shorter duration and would have acquired less of the smell. To check this possibility and to investigate other related questions, we have initiated another series of experiments where we hold the wasps before introduction in clean glass containers rather than plastic boxes. In three of these experiments completed so far, our main conclusion that acceptance of alien conspecifics is a function of age has been completely upheld; as before, wasps older than eight days of age were never accepted while wasps younger than eight days of age had a finite probability of acceptance (Arathi & Gadagkar, *unpublished observations*).

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