Division of Labour and Organization of Work in the Primitively Eusocial Wasp *Ropalidia marginata*

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Ropalidia marginata is a primitively eusocial polistine wasp with the expected lack of morphological caste differentiation between queens and workers. The lack of morphological caste differentiation appears to be compensated by a system of behavioural caste differentiation. The wasps in a colony may be classified into three behavioural castes which we have called Sitters, Fighters and Foragers and the queens are almost always in the Sitters caste. Consistent with this and unlike in most other primitively eusocial species studied, *R. marginata* queens are relatively inactive, behaviourally subordinate individuals. There is no evidence that they regulate activities of their workers. The workers continue to remain active, bring food and feed the larvae, even if the queen is removed. Worker activity appears to be regulated by the workers themselves through the use of dominance behaviours which are hypothesized to have come to represent larval and adult hunger signals, to the foragers. In undisturbed colonies, intranidal workers who also unload food and pulp bearing foragers, appear to regulate foraging rates. In the absence of unloaders, the foragers themselves feed the larvae and apparently obtain first-hand information about larval hunger levels. In spite of its primitively eusocial status, *R. marginata* has a well developed age polyethism. Workers show strong preferences to feed larvae, build the nest, bring pulp and bring food, in that order, as they age. However, the relative position of a wasp in the age distribution of the colony, rather than her absolute age, is a stronger predictor of her task performance. Soliciting behaviour (a form of trophallaxis) provides a plausible mechanism for the wasps to assess their relative ages. A computer simulation model, adapting the verbal activator-inhibitor model proposed for honey bees, demonstrates that a relative-age based rule for division of labour provides the necessary flexibility for colonies to respond adaptively to changing colony demography or varying demands for food. Thus, morphologically identical individuals, and in spite of retaining some reproductive options, have access to a variety of mechanisms to efficiently divide labour and organize work.

Key Words: *Ropalidia marginata*, Division of labour, Primitively eusocial wasp, Caste differentiation, Age polyethism, Work organization, Time-activity budgets

Introduction

Insects exhibit a bewildering variety of social organization ranging from the non social or solitary to the large societies of honey bees and army ants that have sometimes attracted the label, super-organism. To cope with such social diversity, a class of "truly" social species are distinguished from all others that exhibit "lower" degrees of social development. Such "truly" social insects are called eusocial (a term coined by Batra 1966), and are defined as those that exhibit three properties namely, cooperative brood care, differentiation of colony members into fertile reproductive castes (queens or kings) and sterile non-reproductive castes (workers) and an overlap of generations such that offspring assist their parents in brood care and other tasks involved in colony maintenance. (Michener 1969, Wilson 1971). It is customary to recognize two further subdivisions of the eusocial - the primitively eusocial and the highly eusocial. The most widely accepted criterion for separating the primitively and highly eusocial stages is the presence

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of morphologically differentiated reproductive and non reproductive castes in the highly eusocial species and their absence in the primitively eusocial ones.

In highly eusocial species, workers are expected to have little or no opportunities to gain direct fitness and are thus largely dependent on indirect fitness gained through contributing to the fitness of their colonies. Hence they may be relatively easily moulded by natural selection to sacrifice what might be good for their direct fitness and adopt behaviours that might be good for their colonies. In primitively eusocial species on the other hand, workers often retain opportunities for gaining direct fitness so that natural selection may not so easily mould them to sacrifice what is good for their personal reproduction and adopt behavioural strategies that maximize colony fitness. To the extent that efficient division of labour and efficient organization of work requires participating individuals to sacrifice any personal fitness in favour of colony fitness, primitively eusocial species face a dilemma. The study of division of labour and organization of work in primitively eusocial insect species is therefore of special interest.

Ropalidia marginata is a tropical, primitively eusocial polistine wasp, widely distributed in peninsular India. Colonies are strictly monogynous, consisting of a single queen and several workers, the latter seldom exceeding hundred in number. As expected for a primitively eusocial species, queens and workers are not morphologically differentiated; indeed most individuals in a colony appear to be capable of adopting both queen and worker roles. We have employed this species to investigate division of labour and organization of work using three different approaches. The first approach involves identification of patterns in the way in which different individuals in the colony allocate their time to different tasks. The second approach involves understanding the role of dominance-subordinate interactions in regulating task allocation. The third approach involves an explicit investigation of the role of age of the wasps in task performance.

Behavioural Caste Differentiation

If the wasps that constitute a colony are all morphologically undifferentiated, how does the colony function as a social unit? How is labour divided between the members of a colony so that all required work elements are efficiently organized? How do workers achieve an appropriate balance between what is good for the colony as a whole and what might be best from their individual fitness point of view? These questions have been repeatedly asked, and answered with a great deal of success in the highly eusocial insects such as honeybees (Winston 1987, Seeley 1995) and ants (Oster & Wilson 1978). But highly eusocial species (Hölldobler & Wilson 1990, Bourke & Franks 1995) are quite different – they have pre-imaginal caste determination and morphological caste differentiation between queens and workers and often between sub-groups of workers specializing in different tasks as well. A primitively eusocial species such as *R. marginata* has none of these features. However, morphologically similar individuals can be behaviourally diverse and, given that they have multiple options open to them (Gadagkar 2001), we should expect the wasps in a colony to pursue diverse behavioural strategies.

The key to answering the questions raised above must therefore lie in understanding inter-individual differences in behaviour. In any effort to objectively investigate quantitative behavioural variability, an important question concerns the behaviours one should focus on. A perfectly reasonable approach would be to focus on those behaviours that appear to the human observer as being crucial for the well being of the colony and efficient rearing of brood – and this is what most studies generally do. Such an approach would lead one to focus on behaviours involved in building the nest, bringing food, feeding other adult wasps and the larvae, dominance/subordinate behaviours and so on. But, can one also make an objective selection of an appropriate subset of behaviours without using criteria that seem "important" from our point of view?

Such a selection of behaviours was attempted by Gadagkar and Joshi (1983). In *R. marginata*, it turns out that an average wasp spends about 95% of her time in performing one the following six behaviours: (1) Sit and Groom, (2) Raise antennae, (3) Raise wings, (4) Inspect cells, (5) Walk, and (6) Absent from the nest (these names are more or less self explanatory but, for precise definitions, see Gadagkar 2001). Some of these behaviours may

appear to be trivial, compared especially to behaviours mentioned earlier, such as building the nest, feeding the larvae, dominance behaviour and so on. However, if the wasps spend such an overwhelming proportion of their time in these behaviours, there must be a clue in that. The time-activity budgets of twenty wasps drawn from two nests with respect to the above 6 behaviours are shown in figure 1. Notice that although each wasp spends about 95% of her time in the 6 behaviours put together, the manner in which different wasps allocate their time between these six behaviours is highly variable. For example wasp 1 spent a large proportion of her time in sit and groom and no time in being absent from the nest. Wasp 2 spent a high proportion of her time in sitting with raised antennae, wasp 10 in sitting with raised wings, wasps 11 and 12 in walking and wasps 15 and 16 in being absent from the nest.

What, if any, is the significance of such variability? Is the variability random or is there some underlying pattern? Is the variation continuous or are there sub-groups of wasps that show less intra-group variability and high inter-group variability – if so, these sub-groups could be thought of as forerunners of the morphologically different-iated sub-castes seen among the workers of highly social species. In an attempt to detect any underlying pattern in such variability, the time-activity budgets were subjected to principal components analysis (Gadagkar & Joshi 1983). As is usually the case, the first two principal components account for an overwhelming proportion of the variance in the data. The first principal component accounts for 72.3% of the total variance with Absence from Nest as its dominant eigenvector. The second principal component, whose dominant term is Raise Antennae, accounts for 20.2% of the total variance. Since the first two principal components together account for 92.5% of the total variance, it is entirely appropriate to represent each wasp as a point in the coordinate space of the associated amplitudes of the first two principal components. When plotted in this fashion, the points (wasps) fall into three apparently distinct clusters (figure 2) (Gadagkar & Joshi 1983). Individual 13 alone does not fall into any of the three clusters. This could be because most of the data on this individual were collected when the nest was in

the process of being abandoned due to predation by *V. tropica*. It must be emphasized here that the three clusters emerged as a result of an objective analysis of the data in as much as no *a priori* assumptions were made regarding the criteria to be used for classification or the number of clusters required.

In another approach to discerning patterns in the inter-individual variability in time-activity budgets, the dimensionality of the data was reduced from six to just one, by computing the Pearson product moment correlation between all pairs of wasps using the time spent in each of the six behaviours. Such a correlation coefficient can be used as an index of similarity between individuals because, wasps that are highly correlated in the manner in which they allocate their time between different behaviours, can be legitimately said to be similar to each other. The
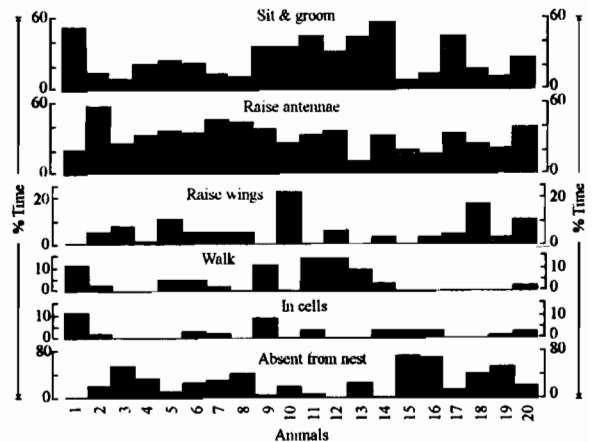

Figure 1 Time-activity budgets of 20 individually identified wasps drawn from two colonies of *R. marginata*. (Redrawn from Gadagkar 1985)
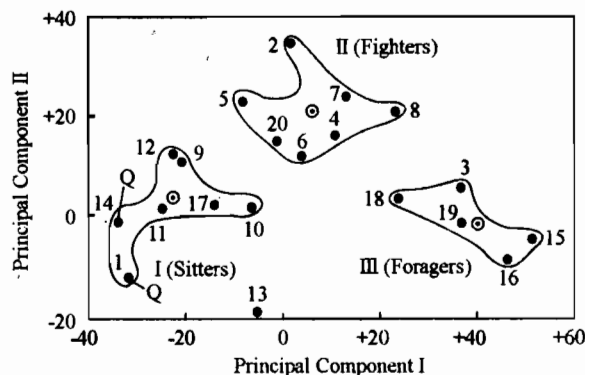

Figure 2 Behavioural castes of *R.marginata*. Twenty wasps are shown as points in the coordinate space of the amplitudes associated with the first two principal components. Circled dot = Centroid. Q = queen. (Redrawn from Gadagkar & Joshi 1983)

question again is whether there is a pattern in the levels of similarity and dissimilarity between different pairs of wasps. A one dimensional index of similarity such as the Pearson product moment correlation can be conveniently subjected to cluster analysis by any one of a number of algorithms of hierarchical cluster analysis (De Ghett 1978) (figure 3). Even in this method of cluster analysis, individual 13 was well separated from all the others and, among the remaining 19 individuals, one can recognize three clusters with exactly the same composition as the clusters obtained from the principal components analysis. This complete concurrence of the two methods is clearly an indication of the robustness of the clusters (Gadagkar & Joshi 1983).

(a) Sitters, Fighters and Forgers

What is the biological significance of these clusters? This question, can be answered at two levels, one, concerning its immediate consequence to division of labour in the colony (which may be called the "ergonomic significance of behavioural caste differentiation) and, two, concerning the selective forces that mould the behaviour of wasps in a manner so as to yield these clusters (which may be called the "evolutionary significance of behavioural caste differentiation)? Let us approach this question at both levels, the ergonomic and the evolutionary. A closer look at the results of the principal components analysis provides some insights. The dominant eigenvector in the first principal component is associated with Absent from Nest, and clusters I and III thus represent two extremes for this behaviour, while cluster II is intermediate. Similarly, the dominant eigenvector in the second principal component is associated with Raise Antennae and the members of cluster II are thus different from those of clusters I and III in the time spent with Raised Antennae. This line of reasoning suggests that we should go back to the raw data and examine the behavioural profiles of the three clusters (figure 4A). Although it is not necessary for any one behaviour
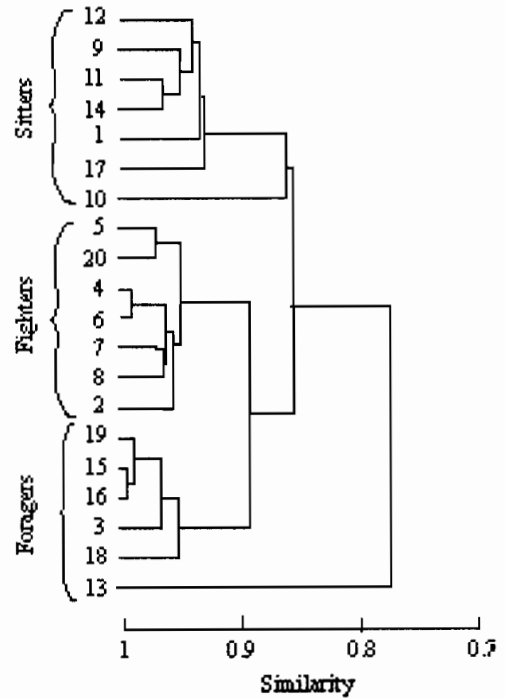

Figure 3 Hierarchical cluster analysis of same 20 adults of *R.marginata* that were used in the principal components analysis in figure 2. The similarity between individuals shown is the Pearson product moment correlation calculated using the percentage of time spent by the 20 wasps in the same six behaviours used for the principal components analysis in figure 2. The method of single linkage algorithm has been used for clustering. (Redrawn from Gadagkar & Joshi 1983).
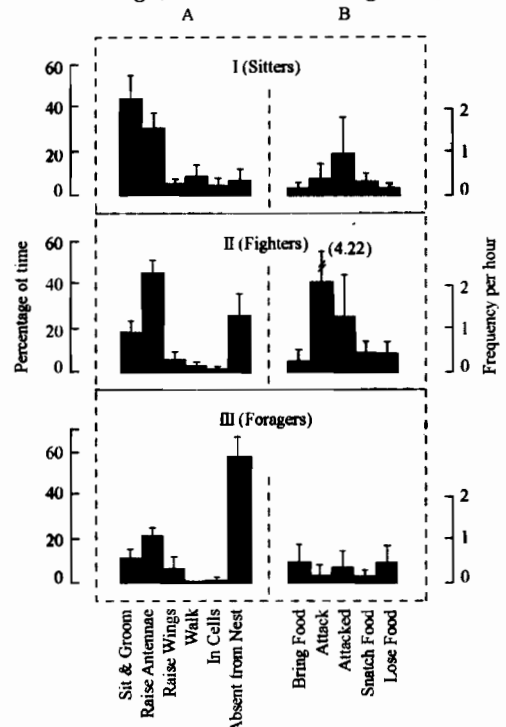

Figure 4 Mean behavioural profiles of the clusters obtained by principal components analysis and hierarchical cluster analysis, in figures 2 and 3. (A) Mean percentages of time spent in each of the six activities that were used in obtaining the clusters. (B) Mean frequencies per hour of the five behaviours that were not used to obtain the clusters. (Redrawn from Gadagkar & Joshi 1983)

alone to show significant differences between the clusters (as the clusters have been obtained by the consideration of six behaviours simultaneously), it is obvious from figure 4A that the time spent in Sit and Groom, Raise Antennae and Absent from the Nest are the most distinguishing attributes of clusters I, II and III respectively. Thus there was no difficulty in naming cluster I as Sitters but naming clusters II and III was not as straightforward. However, there are also clues in the data on other behaviours, which appear important from our point of view but in which the wasps themselves do not spend large amounts of time. Some behaviours of this kind for which a reasonable amount of data existed were: bring food, dominance behaviour, subordinate behaviour, snatch food, and lose food.

These five, relatively rare behaviours can be used to construct alternative behavioural profiles of the three clusters obtained through principal components analysis of the proportion of time spent by the wasps in the six more common behaviours (figure 4B). A high frequency of Dominance behaviour now emerges as a very conspicuous attribute of cluster II. Moreover, there is a significant positive correlation between the time spent by an individual with Raised Antennae (the conspicuous feature of cluster II in figure 4A) and her frequency of attacking other individuals ($P < 0.01$). Cluster II was therefore labelled as Fighters. The most distinguishing feature of cluster III in figure 4B is the frequency with which they brought food to the nest. Taken together with the fact that Absent from the nest is the most distinguishing feature of this cluster in figure 4A, cluster III was thus labelled as Foragers. Thus the adult wasps in *R. marginata* colonies could be classified into three behavioural groups which can be called Sitters, Fighters, and Foragers. Such behavioural differentiation into Sitters, Fighters and Forgers is perhaps a rudimentary form of caste differentiation and is therefore referred to as behavioural caste differentiation.

It is important to note that the queens of both colonies (wasps 1 and 14) are Sitters. Data on queens and workers were not analysed separately, nor was the rate of egg laying used as one of the behaviours while delineating the behavioural castes. This was done deliberately to avoid making any a priori assumptions about queen-worker dichotomy in

behaviour. Instead, the strategy was to establish the existence of behavioural caste differentiation, identify the Sitters, Fighters and Foragers and then locate and attempt to interpret the position of the queen in this system of behavioural caste differentiation.

Sitters are those that spend much more time Sitting and Grooming, compared to other wasps. They do little or no foraging and seldom indulge in dominance behaviour, either with other Sitters or with any others (see below). While both queens belong to this group there are other non egg laying members of this group. The queens may be Sitters because that may be the best strategy to conserve energy and maximize egg laying. The non egg laying Sitters may have some chance of reproducing in the future and, who therefore emulate the behaviour of the queens and thus maximize their chances of ascending to the status of the queen, in this or in another colony. Non-egg-laying Sitters may thus be an example of what West-Eberhard (1978a) has called "hopeful queens". If this interpretation is correct, the Sitters may contribute little towards the division of labour in the colony. On the other hand, at least some of the non-egg-laying Sitters may be young individuals, yet to be recruited into the work force of the colony.

For understanding division of non-reproductive labour, we must perhaps turn to the Fighters and Foragers. Fighters are individuals that spend a large proportion of their time with Raised Antennae, and show high levels of dominance behaviour. Sitting with Raised Antennae probably serves the function of guarding the nest and its brood against parasites and predators. This is supported by the fact that wasps remain in this position for extended periods of time if the nest is disturbed either by the observer or by tachinid flies that parasitise their brood. Thus Fighters may be akin to soldiers of the highly eusocial species. What then is the function of Dominance behaviour, especially that is shown by workers rather than by the queens? One way to begin to understand the function of fighting by the Fighters is to see who they fight with. Fighters show the highest frequency of Dominance behaviour towards other Fighters and a lower frequency towards Sitters and Foragers (figure 5). It is conceivable that dominance shown by the Fighters
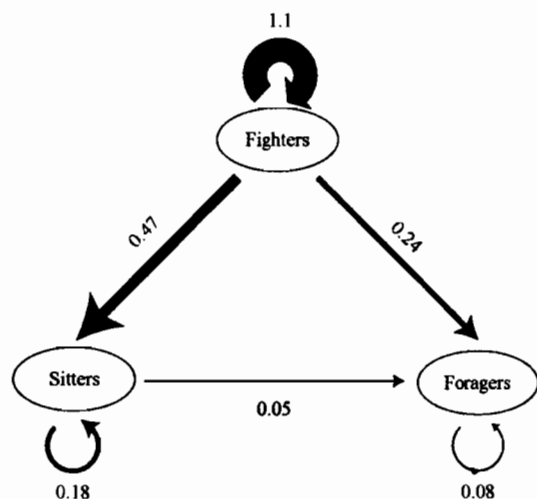
Figure 5 Frequencies of Dominance behaviour (computed as frequencies/individual/hour) within and between the behavioural castes. (Redrawn from Gadagkar & Joshi 1983)

induces the other members of the colony to work. Individuals often leave the nest as a result of repeated attacks from nestmates and later may return with food or pulp. Further support for this idea comes from the observation that Fighters also snatch food from other individuals to a large extent; there is a significant positive correlation between the frequency of Dominance behaviour and that of Snatching Food ($P < 0.01$). In other words, Fighters may also function as "policemen", coercing other wasps to go out and forage (more on this below). But why should Fighters fight more often with other Fighters. This suggests that Fighters may also have some chance of becoming future queens. Using high levels of aggression, especially towards other Fighters to stay on top of the social hierarchy, may be another way of maximizing their chances of becoming queens in the future (see below).

Foragers appear to constitute the principal worker force of a colony. They show the lowest frequencies of Sitting and Grooming, Dominance behaviour and the highest frequencies of being Absent from the nest and of bringing food. Therefore, they do not seem to be much involved in reproductive competition with their nestmates and may have the least chance of becoming future queens. In summary, Sitters and Fighters may both be potential queens and may be pursuing alternative strategies of maximizing their chances of becoming future queens while Foragers may be those that have

little or no chance of becoming future queens and may thus be selected to work for the welfare of the colony and acquire indirect fitness.

(b) Other Correlates of the Behavioural Castes

Chandrashekara and Gadagkar (1992) examined 12 additional colonies by the same methods of observation and statistical analysis and confirmed the existence of behavioural caste differentiation into Sitters, Fighters and Foragers in all the colonies (West-Eberhard 1978b). This study also confirmed that the queens in 11 out of the 12 colonies were Sitters and only in one case the queen was a Fighter. This large body of data from 12 colonies permitted better exploration of the biological significance of behavioural caste differentiation. In what way are Sitters, Fighters and Foragers different from each other?

Twelve variables namely, Sit and groom, Raise antennae, Absent from nest, Dominance behaviour, Bring food, Snatch food, Feed larvae, Extend walls of cells, Index of ovarian condition, Index of body size, Dry weight (mg), and Fat content, were tested for potential differences between Sitters, Fighters and Foragers. Each of these variables was separately modelled to test its influence on the probability of an individual being a Sitter, Fighter and Forager, by using the method of logistic regression analysis (see West-Eberhard 1978b for details).

The coefficient of regression associated with Sit and Groom was significantly greater than zero ($p < 0.001$) in a comparison of Sitters, either with Fighters or Foragers. This can be interpreted to mean that Sitters spend significantly more time in Sit and Groom than either Fighters or Foragers do. Interpreting other coefficients similarly, one finds that Fighters spent significantly more time in Raise antennae than either Sitters or Foragers did ($p < 0.001$) and Foragers spent significantly more time in being Absent from nest compared to either Sitters or Fighters ($p < 0.001$). These results were of course expected on the basis of the mean behavioural profiles of the castes which were used to name them in the first place. However, these variables were only included as internal controls to establish the correct interpretation of the results of logistic regression analysis and to justify the use of this method to identify other correlates of the behavioural castes.

Other variables which were not used in deriving the clusters, were then used to establish the patterns of task allocation between the castes. Foragers brought food significantly more often than either Sitters or Fighters did ($p < 0.05$) and Fighters did so significantly more often than did Sitters ($p < 0.01$). Fighters showed Dominance behaviour significantly more often than either Sitters or Foragers ($p < 0.05$) and Sitters did so significantly more often than Foragers ($p < 0.01$). Sitters and Fighters snatched food, fed larva and extended walls of cells significantly more often than did Foragers ($p < 0.05$) and were indistinguishable from each other by any of these three variables. Sitters had significantly better developed ovaries than either Fighters or Foragers ($p < 0.05$) and Fighters had significantly better developed ovaries than Foragers ($p < 0.05$). This result could have been due to the inclusion of queens in the Sitter caste in 11 out of 12 colonies. When queens of all 12 colonies were excluded from the data set, we found that Sitters and Fighters still had significantly better developed ovaries than Foragers ($p < 0.05$) but Sitters and Fighters were now indistinguishable from each other on the basis of their ovaries. The Index of body size, dry weight and fat content did not differ significantly between the behavioural castes. We also have more direct but preliminary evidence suggesting that body size is unlikely to be a determinant of dominance or egg laying ability (Nair et al. 1990).

These results suggest that division of labour and social organization are closely linked to behavioural caste differentiation. The extranidal task of foraging is performed primarily by the Foragers while the intranidal tasks of feeding larvae and nest building are shared between Sitters and Fighters. That both Sitters and Fighters have better developed ovaries compared to Foragers, but are indistinguishable from each other by their ovaries, leads to the prediction that new queens to replace old queens should be more likely to be drawn from amongst either Sitters or Fighters, rather than from among the Foragers. As mentioned before, primitively eusocial species are characterized by the lack of morphological differentiation between queens and workers and the consequent flexibility in the social roles that the adult insects may adopt. The lack of any systematic differences between the behavioural castes namely, Sitters, Fighters and Foragers in body size and fat reserves suggests that *R. marginata* gains the benefits of behavioural specialization among the workers without paying the cost of the inflexibility associated with morphological or physiological specialization.

(c) An Experimental Approach

Correlation studies can only take us thus far and further progress in understanding the evolutionary significance of behavioural caste differentiation must come from different approaches. A powerful approach, especially to test the predictions made from correlational studies, is of course the experimental approach. While not always feasible in evolutionary studies, the experimental approach is however quite feasible in the present context. It is tedious but relatively simple to study several colonies, identify the Sitters, Fighters and Foragers and then remove the existing queen. Fortunately, in *R. marginata* such experimental removal of the queen results in one of the workers taking over the role of the queen and this often happens within a day or two of the removal of the original queen (the identity of the new queen is often evident within minutes of removing the previous queen). One can therefore experimentally determine the probabilities with which Sitters, Fighters and Foragers become queens and put to test, the prediction we have made merely by examining the correlates of the behavioural castes. Chandrashekara and Gadagkar (1992) undertook an independent study of an additional 12 colonies for this purpose. As before, the colonies were observed over a two week period, time-activity budgets of individually marked wasps were constructed, these data were subjected to principal components analysis to identify the Sitters, Fighters and Foragers by the same criteria described before. Having done that, the queens of each of these colonies were removed and after 24 to 48 hr, the same colonies were observed again for another two weeks. Removal of the queen, however, resulted in a disruption in the normal behaviour patterns so that such a behavioural differentiation could not be clearly discerned in the post-queen-removal periods and even in some pre-queen-removal periods when the same colony was subjected to repeated queen-removal experiments.

In 9 out of 12 pre-queen removal periods, however, the pattern was similar to that seen in undisturbed colonies. In all of these, the queen, as expected, was in the Sitter group. After identifying the individual who takes over as the new queen during the post-queen removal observations, pre-queen removal principal components plots were used to locate the "wasp who would be queen". These individuals, during the period when they are not yet queens, are referred to as potential queens. What then is the behavioural caste of the potential queens? Of the nine potential queens we could thus identify (one in each colony), six were Sitters, two were Fighters and one was a Forager. Roughly speaking, the number of potential queens which were Sitters is higher than that expected by chance (there were 77 Sitters out of a total of 184 wasps in the nine colonies) but the numbers of potential queens that were derived from the Fighter and Forager groups were less than expected by chance (there were 72 Fighters and 35 Foragers respectively, out of the total of 184 individuals in the nine colonies). Unfortunately the sample size of nine potential queens is obviously too meagre to permit any rigorous statistical analysis. Nevertheless, the fact that it was usually the Sitters and not the Foragers that become future queens is consistent with the interpretation made so far. But the significance of the Fighters is not so clear and perhaps will not be, until we look more closely at the significance of fighting itself (see below).

We argue that this system of behavioural caste differentiation has evolved in response to the predicament that the wasps find themselves in – most individuals have a finite probability of becoming queens in their life time and gaining direct fitness but in fact, most individuals end up dying as sterile workers, with only indirect fitness to their credit. Wasps must then be selected to attempt to maximize their chances of becoming queens and gain direct fitness. This may however, work to the detriment of the colony as a whole and thus reduce their indirect fitness. Hence they must also be selected to work towards the welfare of their colonies and maximize their share of indirect fitness, just in case they die without any direct fitness - net inclusive fitness (direct component + indirect component) is what is finally reckoned by natural selection. It is not surprising therefore that queens do whatever it takes to maximize their rates of egg laying by being Sitters and thus paying more attention to gaining direct fitness and somewhat neglect indirect fitness. On the other hand, the non egg laying Sitters and the Fighters have relatively lower probabilities of becoming future queens, but that probability is not zero. Not surprisingly, they perform mainly the less risky intranidal tasks. Finally, Foragers have the least chance of becoming future queens and, not surprisingly, they perform most of the risky extranidal tasks. Behavioural caste differentiation thus appears to permit the wasps to strike a fine balance between cooperation and conflict (Chandrashekara & Gadagkar 1990, Gadagkar 1997). But these results raise other questions, especially about the significance of the dominance/subordinate behaviours shown by the wasps: What is the position of the queen in the dominance hierarchy of a colony? What is the significance of the dominance/subordinate behaviours, shown largely by the workers? Who, if anybody, regulates worker activity in R. marginata colonies, with their docile queens and aggressive workers? These questions prompted a direct exploration of the nature and significance of dominance behaviour in R. marginata.

Dominance Behaviour and Regulation of Worker Activity

A variety of dominance behaviours are indeed shown by the wasps in a colony. One wasp, dominant by definition, may attack, peck, chase or nibble another, subordinate by definition. The frequencies of all these behaviours are pooled to obtain the frequency of dominance behaviour, for each individual. Similarly, the sum of the rates at which each individual is attacked, pecked, chased or nibbled is her frequency of subordinate behaviour. One member of a pair of individuals was nearly always dominant over the other, in all interactions between them and thus their dominance-subordinate status was unambiguous. The network of dominance/subordinate interactions in a typical colony is shown in figure 6. Using an index of dominance, which is a modified form of the index of fighting success developed for Red Deer by Clutton-Brock et al. (1979), wasps in a colony can be arranged
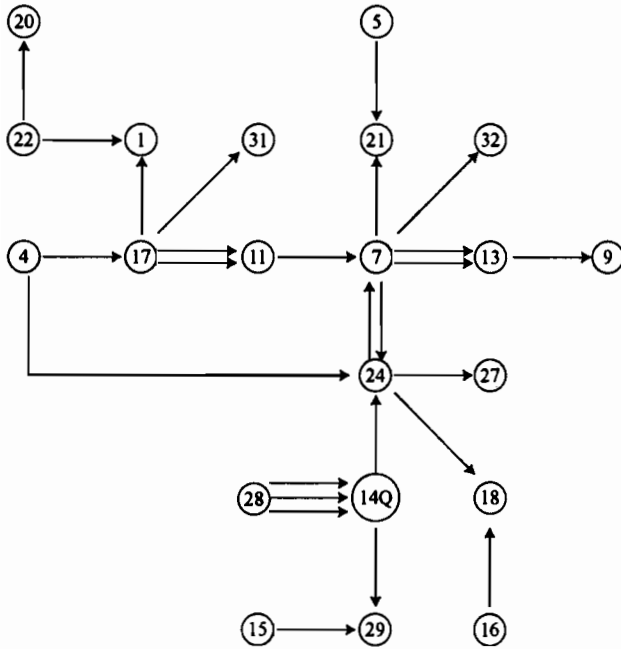
Figure 6 The dominance-subordinate network in a typical colony as an example of intermediate complexity. Arrows connect dominant animals to their subordinate partners. Each arrow represents one instance of Dominance behaviour. Since all animals were observed for the same duration, these numbers are directly comparable. Each circle represents a wasp and the number inside, its code. (Redrawn from West-Eberhard 1978b)

in a dominance hierarchy (Premnath et al. 1990). Thus the network in figure 6 gives rise to the hierarchy in figure 7 (for details see Gadagkar 2001).

(a) The Position of the Queen

We have seen above that the queens of *R. marginata* are relatively docile Sitters in comparison to many of their nestmates who may be described as aggressive Fighters. Using the frequencies of dominance behaviour and also the dominance index, it is instructive to examine the position of the queen in the system of dominance relationships prevailing in *R. marginata* colonies. Chandrashekara and Gadagkar (1992) found that in 3 out of 12 colonies, the queen did not participate in dominance interactions at all, although several such interactions were recorded among her nestmates. This could not have been merely because of insufficient sampling effort. In one of these 3 colonies 75 instances of dominance behaviours were recorded and 14 of them by a single worker - nevertheless, none were shown by the queen. In another 3 of the 12 colonies, the queen participated in dominance interactions in her colony but was dominated by one or more of her nestmates. Even in the remaining 6 colonies where none dominated the queen and where she herself
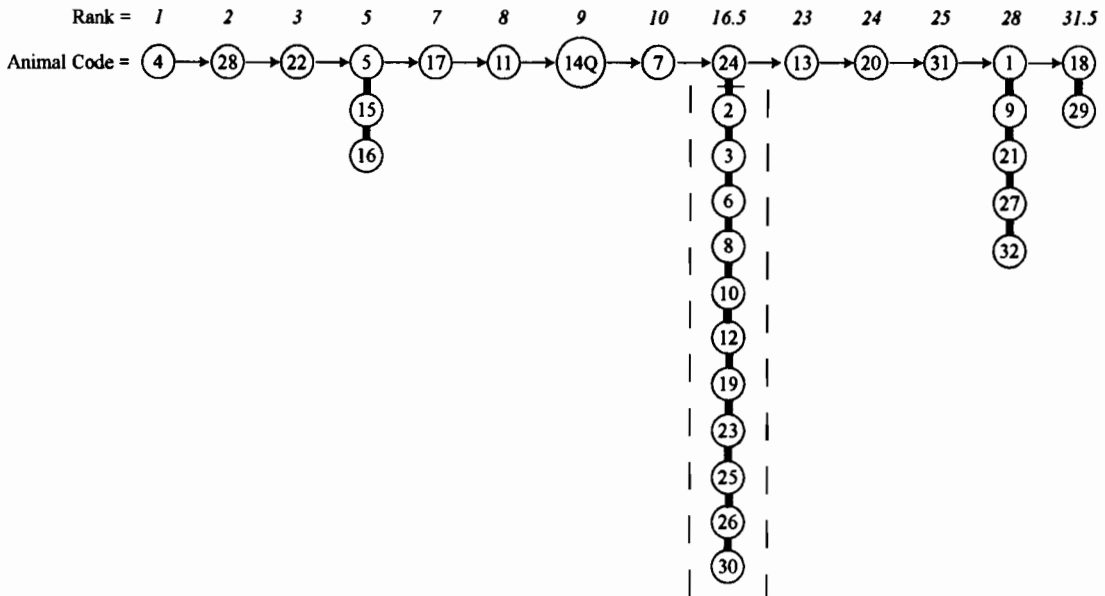

Figure 7 The dominance hierarchy in *R. marginata*. The network of dominance-subordinate relationships shown in figure 6 are converted here into a dominance hierarchy using the index of dominance described in the text and arranging the dominance index scores in descending order. Tied individuals are ranked one below the other. The dotted rectangle encloses those individuals who obtained a dominance index value of 1.0 by virtue of not interacting with any one in the colony. (Data from West-Eberhard 1978b)

participated in dominance interactions, the queen was never the one to show the highest frequency of dominance behaviours in her colony. Out of the total number of dominance behaviours shown in a colony, the proportion shown by the queen was indistinguishable from that shown by one or more of her nestmates in 3 of these 6 colonies (test of proportions, $p < 0.05$) and significantly less than that shown by at least one of her nestmates in the remaining three colonies (test of proportions, $p < 0.05$). When a dominance hierarchy was constructed by computing the index of dominance for all wasps in each of the 12 colonies, the queen was ranked number 1 (with the highest value of D) only in two colonies. She was tied at rank 1.5, with one of her workers in one colony and she was ranked lower than at least one to eight of her workers in the remaining nine colonies. These results clearly indicate that queens of *R. marginata* colonies are not usually at the top of the behavioural dominance hierarchies prevailing in their colonies.

(b) Queen Succession

How and when is the queen's successor chosen? Is the choice made after loss of the original queen or when her replacement becomes imminent? Alternatively, is the next successor identifiable even in the presence of a healthy queen? What are the characteristics of the successor – is she the oldest, youngest, fattest, most hard-working, or most aggressive individual, for example? In addition to the obvious human interest in these issues, their implications for the evolution of division of labour and of sociality itself are profound. Contrast the following two scenarios. In *Polistes exclamans*, old and active foragers have the highest chance of becoming replacement queens, – a system dubbed "gerontocracy" by Strassmann and Meyer (1983, see also Hughes & Strassmann 1988, Hughes et al. 1987). In *Mischocyttarus drewseni* a relatively young non-forager has the highest chance of becoming a replacement queen (Jeanne 1972). The former scenario must make it relatively easy for selection on workers to encourage working for the welfare of their colonies without necessarily jeopardizing their opportunities to gain direct fitness. The latter scenario, in contrast, must lead to a relatively greater conflict between individual interests and

colony interests and hence between avenues for maximizing the direct and indirect fitness components.

Chandrashekara and Gadagkar (1992) analysed behavioural data obtained during the pre-queen removal periods in the experiments described above, to ask how the potential queen was different from all other individuals that did not become queens. During the pre-queen removal period, even the observer was quite unaware of which wasp would become the successor queen. Compared to the average value for individuals that did not become queens after the original queen was removed, potential queens spent significantly less time being absent from the nest ($p = 0.023$) and showed significantly higher rates of dominance behaviour ($p = 0.034$). It may appear therefore that the time spent in being absent from the nest and the rates of dominance behaviour may be good diagnostic features of potential queens. However, comparison of a potential queen with an average worker may be inappropriate as there is considerable inter-individual variability among workers. To address this problem, the values for potential queens were also compared with the values for those individuals in their colonies that showed the lowest (labelled "Min." in figure 8) and the highest (labelled "Max." in figure 8) value, for a given variable. Although potential queens spent less time being absent from their nests than the average worker, they spent significantly more time being absent than "Min" workers. Similarly, although potential queens showed a significantly greater frequency of dominance behaviour than the average worker, their rates of dominance behaviour was indistinguishable from that of Max workers in their colonies. Indeed, for all variables studied, potential queens either had significantly higher values than, or were statistically indistinguishable from, "Min" workers in their colony. Similarly, potential queens either had significantly lower values than or were indistinguishable from "Max" workers in their colony (figure 8).

Potential queens were the highest-ranking individuals in the dominance hierarchies of their colonies among the workers in only 3 of 12 cases. In the remaining 9 cases, there were 2 to 22 individuals with higher dominance ranks than the potential
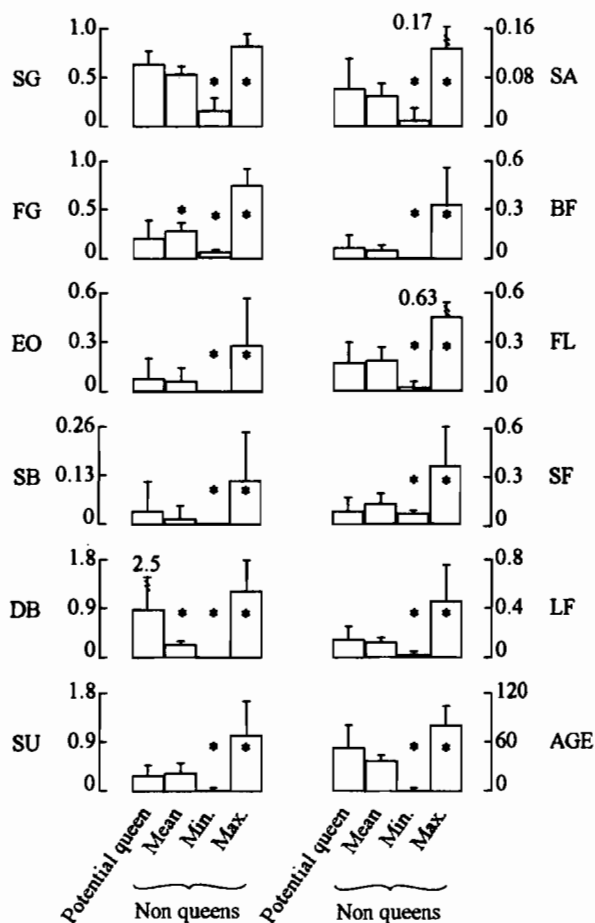
Figure 8 Behavioural profiles and ages of potential queens, mean, min. and max. workers (see text for definitions). Mean and one standard deviation from 12 experiments are shown. Statistical comparison is always between potential queens and workers, using a two-tailed Wilcoxon matched-pairs signed-ranks test. An asterisk in or above a bar denotes that, that value is significantly different from that of the potential queen ($n = 12$; $0.002 < p < 0.043$). SG = sit and groom, FG = forage, EO = extend walls of cells, SB = snatch building material from nestmate, DB = dominance behaviour, SU = subordinate behaviour, SA = raise antennae, BF = bring food, FL = feed larva, SF = snatch food from nestmate, LF = lose food to nestmate, AGE = number of days since eclosion. (Redrawn from Chandrashekara & Gadagkar 1992)

queen. The ages of the potential queens were statistically indistinguishable from the average age of the workers of their colonies. Potential queens were significantly older than the youngest workers of their colonies and significantly younger than the oldest workers (figure 8). The potential queen was the oldest worker (1 colony), was one of the 2 similar-aged oldest workers (2 colonies), younger than 1 to 12 workers and equal in age to 1 to 5 workers (8 colonies). In the last 8 cases, the oldest worker was 1

to 112 days (mean $\pm$ S.D.= 32.12 ± 37.73) older than the potential queen. Thus it is clear that potential queens do not have any consistent characteristics that may permit us to identify them even in the presence of their predecessors. It may therefore be most appropriate to think of the potential queens in *R. marginata* as "unspecialised intermediates". While interpreting similar results they obtained for the primitively eusocial sweat bee *Lasioglossum zephyrum,* Brothers and Michener (1974) speculated that such unspecialised intermediates might be able to respond quickly to the loss of a queen and succeed her.

(c) The Significance of Worker Aggression

In mature colonies with a well established queen, if she herself does not need overt physical dominance to retain her reproductive monopoly in the colony, why do the workers indulge in such frequent dominance behaviour? An obvious possibility is that workers need to indulge in dominance–subordinate interactions among themselves so as to compete effectively with each other in becoming replacement queens. However we saw above that Fighters do not necessarily have a very high chance of becoming replacement queens. Similarly we know that wasps that become replacement queens (referred to as potential queens) are not necessarily more aggressive or of higher rank compared to some of their nestmates who do not become replacement queens. What then is the possible significance of worker dominance behaviour? One way to ask this question is to ask, what kinds of individuals show high frequencies of dominance behaviour? And one way to answer this question is to compute the correlation between the frequency per hour of dominance behaviour on the one hand and other behaviours and anatomical and morphological variables on the other. It turns out that the frequency of dominance behaviour is significantly positively correlated with the frequency of subordinate behaviour, feed larva, extend walls of cells, build new cells, the index of ovarian condition, index of body size, dry weight, and fat content (West-Eberhard 1978b). We also find that as the frequency of dominance behaviour increases, the number of individuals receiving that dominance also increases (West-Eberhard 1978b). This suggests that dominance behaviour is not necessarily directed towards one or a small number

of individuals (perceived as a potential threat), but is distributed over a large number of individuals with more or less the same rate of dominance shown towards each opponent.

The question of the significance of dominance behaviour was also raised in the section on behavioural caste differentiation in the context of the role of Fighters. It was speculated that Fighters could be "policemen" whose job is to coerce other individuals to work, especially outside the nest. The data examined in this section also lend some support to such a policing hypothesis. Individuals who show high levels of dominance behaviour also show high levels of subordinate behaviour. This is more consistent with the idea that dominance-subordinate behaviours constitute a signalling system, by which the workers inform each other of the colony's needs rather a mechanism by which one individual attempts to suppress reproduction by all others. The correlation of dominance behaviour with body size is satisfying – policing is a job better done by large bodied individuals. That individuals showing high levels of dominance behaviour distribute their interactions among a larger number of recipients also makes sense if the function of dominance is to coerce or signal several nestmates to work for the colony (Premnath et al. 1996b). How such a docile Sitter queen establishes and maintains her reproductive monopoly is a question which we will not go into here. Suffice to say here that a queen establishes her reproductive monopoly by extreme overt aggression (recall the behaviour of the potential queen, upon removal of the queen) but later maintains that monopoly with a more subtle, pheromonal mechanism (Premnath et al. 1996a, Sumana & Gadagkar, Unpublished observations). The answer to the question of how a docile, Sitter queen regulates foraging and other activities of her workers is the relevant question here. And the answer might well be that, she doesn't. But somebody must and perhaps the workers themselves do so by means of dominance behaviours.

(d) Regulation of Worker Activity

The mechanism of regulation of worker activity in the primitively eusocial wasp, *Polistes fuscatus* has been well studied (Reeve & Gamboa 1983, 1987, Gamboa et al. 1990). They made observations on intact colonies with their queens, colonies deprived of their queens, after re-introduction of a removed queen. In undisturbed colonies, the queen was the most active and most dominant individual, initiating high levels of behavioural interactions with her nestmates and thereby regulating their activity. The workers synchronized their states of activity and inactivity with those of the queen. In the absence of the queen, the workers went into a lull and the colony virtually came to a stand-still. Once the queen was re-introduced, things sprang back to normal. Quite rightly they called the queen, the colony's "central pace-maker" and concluded that, the mechanisms underlying queen control of worker reproduction may be the same as or, intimately linked to the mechanism of regulation of worker activity (see also Breed & Gamboa 1977, Dew 1983). It would be hard to believe that such a situation would hold for colonies of *R.marginata*, whose queens are not behaviourally the most dominant or active individuals and who do not regulate worker reproduction through overt behavioural means. Premnath et al. (1995) conducted experiments involving observations (for 10 hr each day) of undisturbed nests on day one, observation after experimental removal of the queen on day 2 and observation after replacing the queen on day 3.

On day 1, as expected, the queen was not the most dominant individual in her colony. Nor did she initiate any behavioural interaction with her nestmates. The potential queen, max worker (that worker with the highest value for that variable) and mean worker all had values significantly higher than the queen for these two variables (figures 9 and 10). Instead the queen was a quiet individual spending significantly more time on the nest compared to the max and mean workers. The queen's synchronization of activity with her nestmates was indistinguishable from that of an average worker and was significantly lower than that of the potential queen as well as that of the max worker. The queen was not particularly active in unloading food nor in feeding larvae. Her values for these variables were significantly lower than that of both max workers and mean workers. These findings made it clear that an *R. marginata* queen cannot possibly be described as a central pace maker of her colony. But the results of the second day of the experiment (without the queen) provided even more striking confirmation of the growing impression
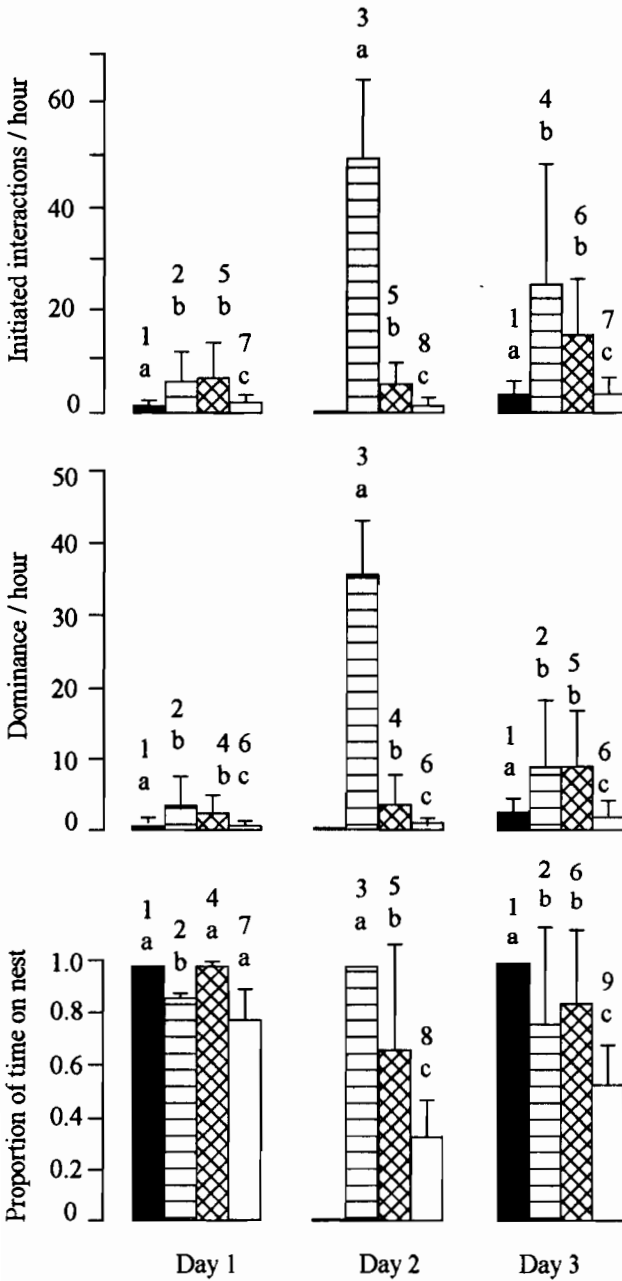
Figure 9 Frequencies per hour of initiated interactions (A), dominance; (B), and proportion of time present on nest; (C), shown by the queen (black bars), potential queen (horizontally marked bars), max workers (cross bars), mean worker (open bars) on days 1, 2 and 3 (see text for a description of the experiment). Bars that carry different letters are significantly different from each other ($p < 0.05$ or less) within each day; bars that carry different numbers are significantly different from each other ($p < 0.05$ or less) among the 3 days. Comparisons are by two-tailed Wilcoxon Matched-pairs signed-rank test. (Redrawn from Premnath et al. 1995).
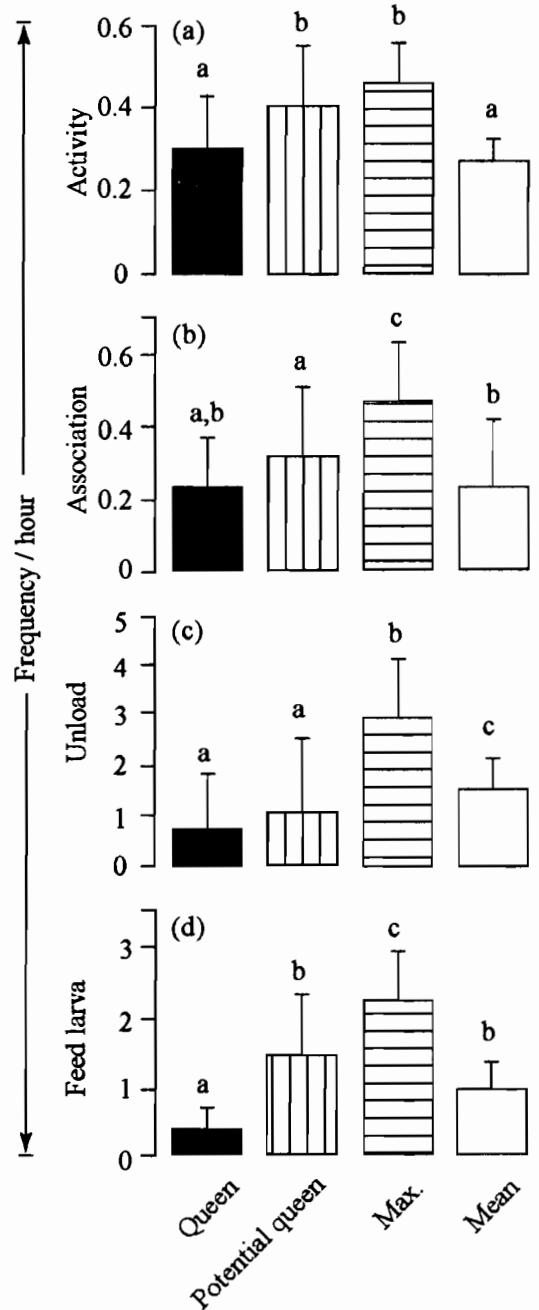
Figure 10 Activity level (A), Yule's association coefficient (B), frequencies per hour of unloading (C), and frequencies of feeding larvae (D), by the queen (black bars), potential queens (vertical marked bars), max worker (horizontally marked bars), and mean worker (open bars) on day 1 (see text for a description of the experiment). For each variable bars that carry different letters are significantly different from each other ($p < 0.05$ or less). Comparisons are by the two-tailed Wilcoxon Matched-pairs signed-rank test. (Redrawn from Premnath et al. 1995).

that in the matter of regulation of worker activity, *R. marginata* and *P. fuscatus* are in complete contrast.

How did *R. marginata* workers respond to the absence of their queen, in the matter of regulating their activities? As expected from previous experiments, the most striking response to the absence of the queen was that one individual (the potential queen) became extremely aggressive and literally drove all her nestmates away and kept dominating them, whenever they alighted on the nest, during the whole day. As a result the proportion of time spent on the nest went down for everybody except the potential queen. But what about other behaviours? Rather surprisingly, the frequencies with which food was brought to the nest and fed to the larvae showed no difference between the three days - in the undisturbed colony with its queen, in the absence of the queen and after queen replacement (figure 11).

Remarkably enough, approximately the same number of foragers were active in the presence and absence of the queen and indeed, active at about the same rates. The proportional contribution to the colony's foraging effort by different individuals was positively correlated between days 1 and 2 thus demonstrating even less effect of the absence of the queen. However, the foragers did have a serious problem on day 2. Because the potential queen aggressively drove out almost all the wasps and permitted them to land briefly, only to be dominated by her, the foragers had much less help in unloading their loads of food or pulp. The frequency with which incoming foragers were unloaded by wasps sitting on the nests was similar on days 1 and 3 but significantly less on day 2(figure 12). How did the foragers respond to this? Contrary to their normal practice, they themselves fed the larvae. The contribution of the foragers to the colony's task of feeding the larvae was indistinguishable on days 1 &3 but was significantly higher on day 2 (figure 12).

When the queen was returned on day 3, she was accepted without any overt aggressive behaviour either on her part, on the part of the potential queen, or any body's part. And the potential queen dramatically reverted to her original self. She lowered her levels of dominance behaviour and initiated interactions and began leaving the nest – her values for these variables on day 3 were
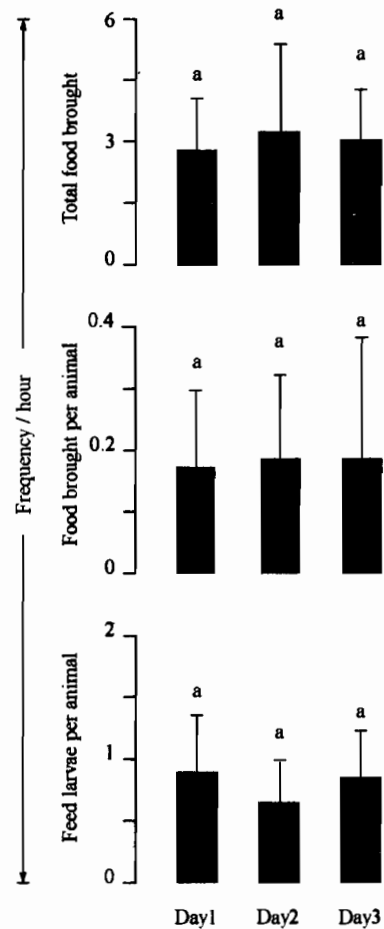

Figure 11 Frequencies per hour of total food brought to the nest, food brought per individual per hour and feed larvae per individual per hour are all not significantly different among days 1, 2 and 3 (see text for a description of the experiment). Comparisons are by two-tailed Wilcoxon matched-pairs signed-rank test. (Redrawn from Premnath et al. 1995).

statistically indistinguishable from her own values on day 1, except dominance behaviour which was still in the process of coming down to her value on day 1. Equally remarkable is the fact that the queen's behaviour on day 3 was indistinguishable from her behaviour on day 1.

There are several hints in this study that point once again to the possibility that dominance-subordinate interactions among workers may constitute the mechanism of regulation of worker activity. On day 1, the frequency of dominance received by the foragers (2.06 ± 3.99) was significantly greater than that received by non foragers (0.85 ± 0.49; $p < 0.05$). Besides, the frequency of dominance received by a forager was significantly correlated
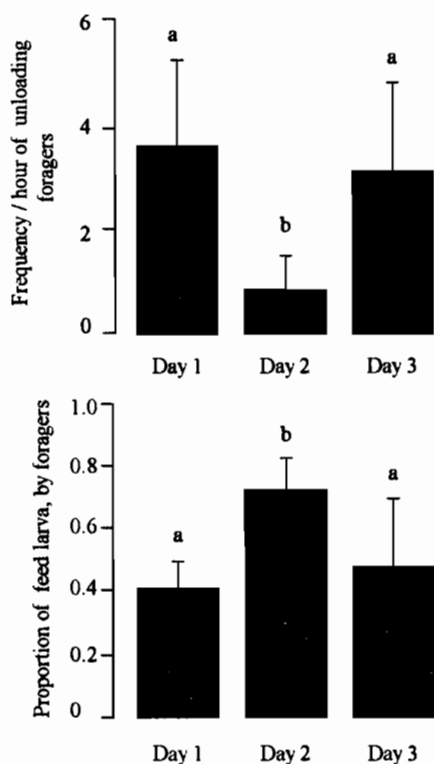
Figure 12 Frequencies at which foragers were unloaded (upper panel) and proportion contributed by foragers to feeding of larvae (lower panel) on days 1, 2 and 3. For each behaviour, bars with different numbers are significantly different from each other. Comparisons are by the two-tailed Wilcoxon matched-pairs signed ranks test (Redrawn from Premnath et al. 1995).

with her foraging rate ($r = 0.20$, $p = 0.02$, n = 67). Most telling however is the result that the fraction of the total dominance received by a forager is positively correlated with her fractional contribution to the colony's foraging efforts. When these results are viewed in conjunction with the finding that, foragers are not unloaded efficiently on day 2 and that they respond to this by feeding larvae themselves, we have a plausible theory for the regulation of worker activity (Premnath et al. 1995). The dominance-subordinate interactions exhibited by the workers should perhaps be viewed as a system of signals, informing the extranidal workers of the hunger levels of the colony's adults and larvae. Fighters who perform most of the dominance behaviours also specialize in intranidal tasks including feeding the larvae and hence they should possess maximum information about larval hunger levels. When foragers are not unloaded efficiently, they themselves feed the larvae and thereby obtain first hand

information about larval hunger levels and the colony's need for food. That dominance behaviours in established colonies are rather mild and ritualized makes them not unsuitable for serving as signals. It has been well established in honey bee colonies that the efficiency with which a nectar forager is unloaded gives her a signal of whether or not the colony is in need of more nectar. If foragers returning with nectar are ignored by unloader bees and they have to wait around with nectar in their crops, they are less likely to bring nectar again for some time. On the other hand, if they are immediately attended to and their nectar load is received with eagerness they will continue to forage for nectar (Seeley 1995). In a similar way one can imagine that if a returning *R. marginata* forager is attacked, pecked, chased or nibbled upon her return from a foraging trip or even when she is idling on the nest, she would consider this a signal that the colony members (larvae and adults) are hungry.

Conspecific aggression is so widespread in all solitary species that it may be the perfect pre-adaptation needed to signal hunger levels to foragers in incipient societies. An early step in social evolution might thus be the use of dominance behaviour to suppress worker reproduction as well as to regulate worker activity. This is the case for example in such species as *P. fuscatus* (Reeve & Gamboa 1983, 1987) and *Ropalidia cyathiformis* (Gadagkar 2001, Kardile & Gadagkar, unpublished observations). *R. marginata* appears to be a step beyond this as the dominance/subordinate interactions (in addition to being used by the queens to initially establish their status), seem also to be used to signal colony hunger levels to the foragers.

In summary, queens of *R. marginata* are not at the top of their colony's behavioural dominance hierarchies; not only are they Sitters, but they may be described as meek Sitters. They are by no means pacemakers of worker activity, indeed they appear to play no role in regulating worker foraging. The workers frequently indulge in dominance-subordinate interactions, at least one of whose functions seems to be to convey larval and adult hunger signals to foragers. Thus it may be said that workers themselves regulate each other's activities. In contrast to a system such as *Polistes fuscatus* where the queen functions as a central pacemaker, foraging by *R. marginata* workers appears to be self

organized. In the following section we shall focus more explicitly on such self organization and consider another important variable not considered so far namely the age of the wasps (Jeanne 1996, Jeanne et al. 1988, O'Donnell & Jeanne 1990, O'Donnell 1996, 1998).

Age and Division of Labour

In their life span of about six weeks, honeybee workers are known to sequentially perform four principal tasks – cleaning, feeding the larvae, storing food (including building the comb, if necessary), and foraging (Seeley 1982, 1995, Winston 1987). This system of age polyethism provides honey bees with a flexible mechanism for division of labour, compared with the polyethism based on morphologically differentiated worker castes seen in many ants and termites (Wilson 1968, Oster & Wilson 1978, Seeley 1985, 1995, Bourke & Franks 1995). Because primitively eusocial species lack morphological caste differentiation, even between queens and workers, let alone between workers specializing in different tasks, age polyethism would appear to be a convenient way for them to organize work in their colonies. However, the empirical evidence for age polyethism in primitively eusocial species has been weak (Naug & Gadagkar 1998a,b, 1999, but see O'Donnell & Jeanne 1995a,b for arguments about how age polyethism can evolve without seriously compromising workers' interests). Naug and Gadagkar (1998a,b, 1999) have investigated the role of age in division of labour and work organization in *R. marginata*.
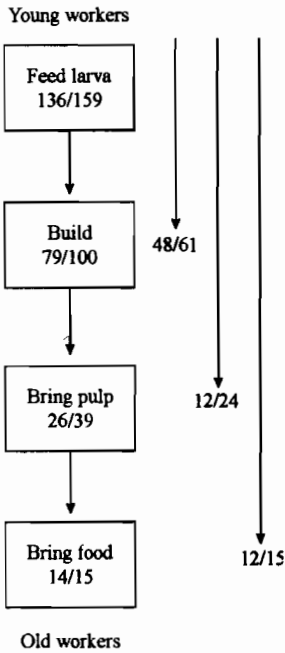
(a) Age Polyethism

The data come from 6 hours of observations per day on four colonies for periods ranging from 2 to 3 months each. The tasks performed by the wasps can be unambiguously classified into two major categories: tasks performed on the nest (intranidal tasks) and tasks performed outside the nest (extranidal tasks). Feeding larvae and building (the nest) constitute two major intranidal tasks, and bringing pulp (for building) and bringing food constitute two major extranidal tasks. There is clear evidence of a significant effect of age on task performance. Of 39 wasps that were seen to perform both intranidal and extranidal tasks, none performed any extranidal tasks (bringing pulp or bringing food)

before performing at least some intranidal tasks (feeding larvae and building). A clear effect of age on the choice of tasks by wasps is evident even when a finer classification of tasks is used. Feeding larvae, building, bringing pulp, and bringing food, in that order, was the most preferred sequence in which the four tasks were performed as the wasps advanced in age (figure 13). Wasps performing their first act of feeding larvae were significantly younger than those performing their first act of building. Similarly, the age of first performance of building was significantly lower than the age of first performance of bringing pulp, and the age of first performance of bringing pulp was significantly less than the age of first performance of bringing food (figure 14).
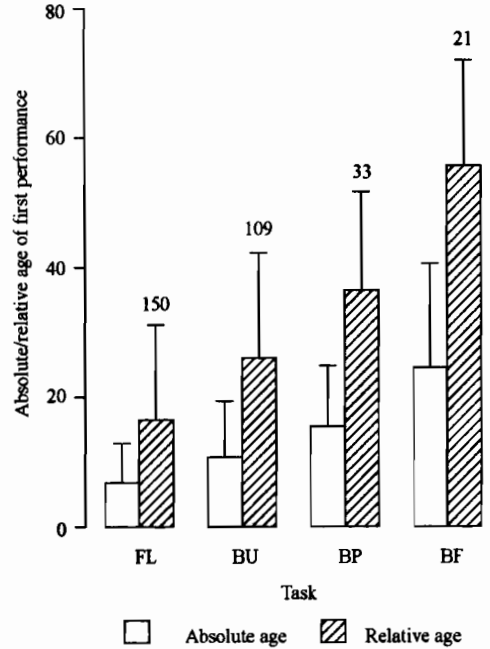
For a more detailed analysis of the effect of age on task performance two measures of task performance and two measures of age were used: Probability of task performance (PTP) is defined as the probability that a worker of a given age will perform a given task relative to other tasks she performs (Seeley 1982). Frequency of task performance (FTP) is the rate (number of times per hour) at which a worker of a given age performs a task. Absolute age is simply the number of days since the eclosion of a given wasp. Relative age is the ranked age of a wasp in her colony and is a measure of her position in the age distribution of the colony.

PTP and FTP for intranidal and extranidal tasks and also separately for feeding larvae, building, bringing pulp, and bringing food were regressed against the absolute and relative ages of the wasps (Naug & Gadagkar 1998b). Figure 15 is an example of the kind of results obtained, complete with all data points and the regression statistics. In addition, figure 16 provides a quick, bird's-eye view of all the 32 fitted polynomial regression lines without the data points. The reader may refer to Naug and Gadagkar (1998b) for details. In every case a significant influence of age was observed (both absolute and relative), on both PTP and FTP. In general the probability and frequency of performing intranidal tasks declined with age and those of extranidal tasks increased with age. Among the two intranidal tasks, the pattern for feeding larvae was qualitatively similar to the pattern seen for intranidal tasks – it declined with age. The other intranidal task, building, peaked in middle age. Extranidal tasks increased with age. Again the pattern
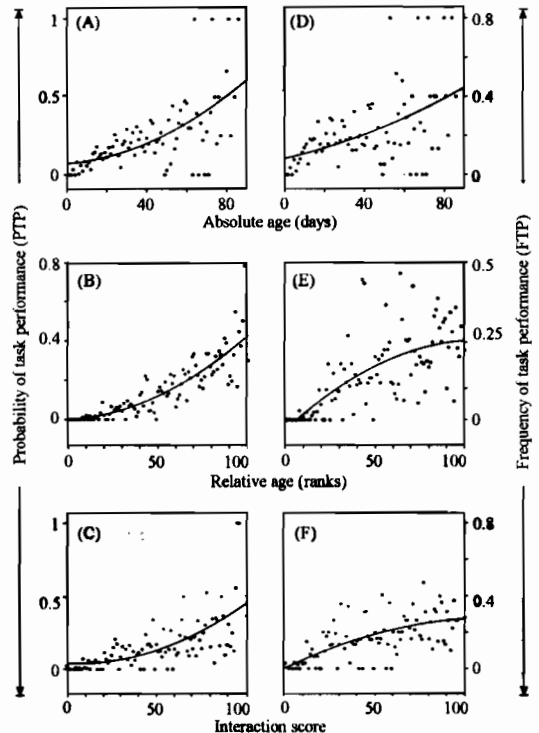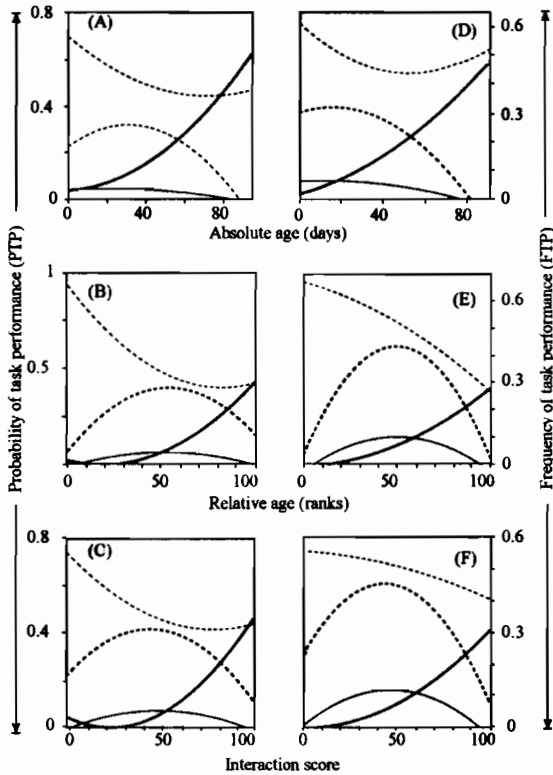
Young workers

Feed larva
136/159

Build
79/100   48/61

Bring pulp
26/39   12/24

Bring food
14/15   12/15

Old workers

Figure 13 Temporal polyethism in *R. marginata*: the most common sequence in which the four tasks feeding larvae, building, bringing pulp, and bringing food are performed. The numbers within each box represent the observed proportions of wasps that performed the task in that position, independent of what they did at any other position. Out of 159 wasps that did at least one task, 136 did feed larvae as their first task. Of 100 wasps that did at least two tasks, 79 did build as their second task, and so on. Each of these proportions is significantly different from the proportion expected (1/4) if wasps were taking up tasks at random ($G = 258.86, 128.32, 29.91$, and 32.04 respectively, $p < 0.001$). The numbers at the heads of the arrows, represent the observed proportions of wasps that followed the particular sequence corresponding to the arrow. Out of 61 wasps that performed only two tasks, 48 followed the sequence feed larvae and build; of 24 wasps that performed only three tasks in their lives, 12 followed the sequence feed larva, build, and bring pulp, and so on. Since there are four tasks to choose from, the expected probability of a sequence with two tasks is 1/12 and that of a sequence with three or four tasks is 1/24. The observed proportions of sequences with two, three, and four tasks were significantly different from those expected at $p < 0.001$ ($G = 177.61$, $G = 44.02$, and $G = 61.51$, respectively) (Redrawn from Naug & Gadagkar 1998b)

Figure 15 Probability of task performance (PTP) and frequency of task performance (FTP) as a function of absolute age, relative age, and interaction scores fitted with second-order polynomial regression lines. Data points represents the mean value for all individuals in that age class in five colonies. A: Extranidal tasks with respect to absolute age and PTP; $y = 0.07 + 0.001x + (5.47 \times 10^{-5})x^2$, $r^2 = 0.38$, $P < 0.0001$. B: Extranidal tasks with respect to relative age and PTP; $y = (3.84 \times 10^{-4}) + (5.21 \times 10^{-4})x + (3.73 \times 10^{-5})x^2$, $r^2 = 0.75$, $P < 0.0001$. C: Extranidal tasks with respect to interaction score and PTP; $y = 0.04 - (5.76 \times 10^{-4})x + (4.70 \times 10^{-5})x^2$, $r^2 = 0.43$, $p < 0.0001$. D: Extranidal tasks with respect to FTP and absolute age; $y = 0.08 + 0.002x + (1.92 \times 10^{-5})x^2$, $r^2 = 0.24$, $p < 0.0001$. E: Extranidal tasks with respect to FTP and relative age; $y = -0.03 + 0.005x - (2.44 \times 10^{-5})x^2$, $r^2 = 0.48$, $p < 0.0001$. F: Extranidal tasks with respect to FTP and interaction score; $y = 0.003 + 0.004x - (2.008 \times 10^{-5})x^2$, $r^2 = 0.34$, $p < 0.0001$. (Redrawn from Naug & Gadagkar 1998b)

Figure 14 Age polyethism in *R. marginata*: mean (± S.D.) age of first performance for each task in terms of absolute age (open bars) and relative age (gray bars). FL = feed larvae; BU = build; BP = bring pulp; BF = bring food. The sample size for each task is given above each bar. Multiple comparisons of mean ages of first performance using the Tukey-Kramer method indicated significant differences across tasks ($p < 0.05$). The first performance of a task was significantly influenced by absolute age (one-way ANOVA; $F = 33.47, p < 0.0001$) and relative age ($F = 49.12$, $p < 0.0001$). Mean ages for successive tasks were also significantly different ($p < 0.05$) when subjected to a Mann-Whitney U test. (Redrawn from Naug & Gadagkar 1998b)

Figure 16 Fitted polynomial regression lines for PTP over (A) absolute age, (B) relative age, and (C) interaction score and FTP over (D) absolute age, (E) relative age, and (F) interaction score, for all four tasks. The fitted lines for all tasks are plotted together on the same scale to discern the overlap of performance of different tasks across age. Thin broken lines refer to feeding larvae, thick broken lines to building, thin solid lines to bringing pulp and thick solid lines to bringing food. Note that there is more overlap in the age of individuals engaged in building and bringing pulp, although this overlap lessens when the tasks feeding larvae and bringing food are considered. (Redrawn from Naug & Gadagkar 1998b)

of variation of one of its components-bringing food, mirrors the pattern seen for extranidal tasks as a whole. The other component, bringing pulp, like building, peaks at about middle age. The qualitative similarity of the patterns observed, irrespective of whether we used PTP or FTP, absolute age or relative age, was striking. However Relative age is a consistently better predictor of task performance than absolute age.

Relative to what we know of other primitively eusocial species, *R.marginata* thus seems to exhibit rather strong age polyethism. Indeed, the pattern of age polyethism we found in *R.marginata* is strikingly similar to the pattern of age polyethism seen in honey bees. The observed sequence of task performance bears a logical biological interpretation (Naug & Gadagkar 1998b). The postponement of the

inevitably more risky, extranidal tasks for later ages and the devotion of the early ages to the less risky intranidal tasks is not surprising. That colony-level selection will favour such a pattern has often been recognized (see, for example, Jeanne 1986). In *R. marginata* both individual-level and colony-level selection are expected to operate, but this pattern will be equally favoured by both. That feeding larvae precedes building among intranidal tasks and bringing pulp precedes bringing food among extranidal tasks may have to do with the relatively lower levels of skill and experience required for feeding larvae as compared with those required for building the nest and for scraping twigs for cellulose fibres as opposed to hunting for live prey.

(b) The Flexibility of Age Polyethism

In recent times the phenomenon of age polyethism has been criticized on the grounds that age dependent task allocation is an inflexible mechanism, making it difficult for social insect colonies to adaptively respond to internal contingencies (skewed age distribution, altered demands, and so on) and external contingencies (bad weather, unexpected abundance of food, and so on). However, if the workers indeed used their relative age in their colonies, rather than their absolute ages, to choose their tasks, colonies should be able to respond to situations of skewed age distributions by reallocating tasks. It is well known that honey bees can do so. In a colony consisting of only young individuals, honey bee workers begin to forage well below the typical age for foraging in normal colonies - these are called precocious foragers. Conversely, in a colony consisting of only old workers when no bee that normally does nursing is available, some foragers revert to nursing duties – these are called overaged nurses. In the language of Huang and Robinson (1992) workers go through a certain rate of behavioural development in normal colonies, progressing from one task to another. In young worker colonies behavioural development is accelerated to yield precocious foragers, and in old-worker colonies behavioural development is retarded to yield overaged nurses.

Naug and Gadagkar (1998a) conducted experiments to see if *R. marginata* can behave in the same way. Colonies consisting of a 7-day cohort of individuals whose absolute ages ranged from 1 to 24

days during the period of observations, were studied. The results were as expected for honey bees – precocious foragers were found in the young-worker colonies. The mean age of foragers in the young-cohort colonies (12.04 ± 4.88 days) not only was significantly lower than the mean age of foragers in the normal colonies (31.54 ± 12.01 days) but also was significantly lower than the mean age of foragers from among the individuals 1 - 24 day old in normal colonies (14.94 ± 0.18 days) (figure 17). The proportion of foragers in the young-worker colonies was significantly higher than the proportion of foragers among the individuals 1-24 days old in the normal colonies and statistically indistinguishable from the proportion of foragers in the entire normal colonies (figure 17). The same results hold for PTP and FTP also. PTP and FTP for foraging were significantly higher in the young-worker colonies than in the group aged 1-24 days in the normal colonies and not different from the PTP and FTP in entire normal colonies (Figure 17). Thus young-worker colonies of *R. marginata*, like honey bees, can readjust their work allocation and respond adaptively to skewed age distributions. Our finding of precocious foragers in particular and the general pattern of the readjustment of task allocation in young colonies strengthen the conclusion that work organization in *R. marginata* is based on relative age.

(c) The Assessment of Relative Age

But how does a wasp know its relative position in the age distribution in the colony? What ever the mechanism, it must involve some form of interaction with an individual's nestmates during which information about the relative age of the interactants may potentially be gathered. There are three major forms of adult-adult interactions seen in *R. marginata:* – dominance–subordinate interactions, food exchange, and a behaviour we call soliciting. The former two have well-defined, specific functions not explicitly connected with age. We therefore consider them unlikely to be involved in the assessment of age. Soliciting involves mouth-to-mouth contact between two individuals, without any obvious dominance or exchange of food. Soliciting is the most frequent form of interaction between adult wasps and occurs more or less randomly across different age classes and behavioural castes. It is comparable to the
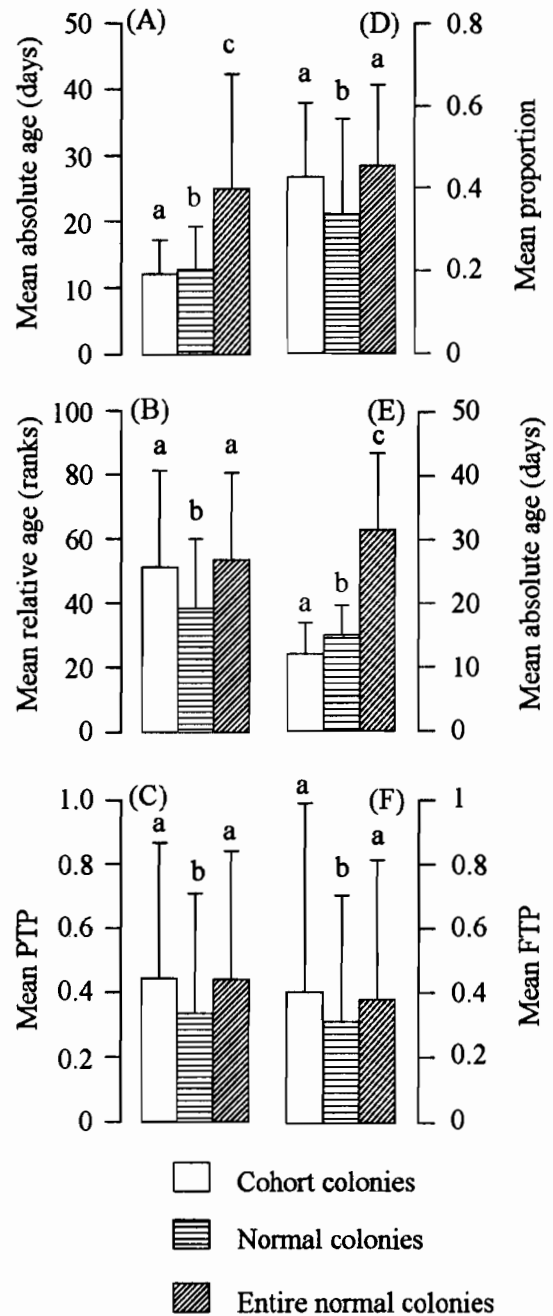

Figure 17 Mean (± S.D.)(A) absolute ages of all individuals, (B) relative ages of all individuals, (C) absolute age of foragers, (D) proportion of foragers, (E) PTP of all individuals, and (F) FTP of all individuals, in the young-cohort colonies (open bars), the corresponding age group in normal colonies (horizontally hatched bars), and the entire normal colonies (gray bars). One-way ANOVA followed by multiple comparisons of means by the Tukey-Kramer method was used to test the effect of age distribution on the parameters given above. Within each graph, bars with different letters are significantly different from each other ($p < 0.05$), while those with the same letters are not significantly different ($p > 0.05$). (Redrawn from Naug & Gadagkar 1998a)

trophallaxis seen among honey bee workers. Presumably some exchange of saliva takes place that may result in a transfer of information, as is well known in honey bee trophallaxis (Winston 1987).

The ratio between the number of soliciting interactions that an individual has with wasps younger than herself and the total number of interaction with all wasps on that day was computed as an interaction score for each individual. Such an interaction score is strongly correlated with relative age, and thus it is possible for the wasps to assess their relative ages by means of such inter-individual interactions. The interaction score it turns out is about as good a predictor of task performance as absolute or relative age. Thus we postulate that the wasps in a colony participate in inter-individual interactions, assess their relative position in the age distribution of the colony and appropriately adjust their choice of task.

(d) The Activator – Inhibitor Model

Huang & Robinson (1992) have proposed the so-called activator-inhibitor model for age polyethism in honey bees. This model also assumes that worker-worker interactions modulate age-correlated behavioural changes and hence it is attractive to consider for *R. marginata*. Huang & Robinson (1992) postulated an interplay between an intrinsic activator (A) that promotes behavioural development and an inhibitor (I) that is transferred among workers during behavioural interactions and retards behavioural development. The production of both activator and inhibitor are assumed to increase with the age of the worker. Naug & Gadagkar (1999) translated this verbal model of Huang and Robinson (1992) into a numerical computer-simulation model to see if it could suggest a plausible proximate mechanism for age polyethism in *R. marginata*.

The assumptions employed in the simulation model are described in figure 18. The following empirical information was derived from the colonies used for demonstrating the influence of age on task performance described at the beginning of this paper. The number of individuals per colony was 22.12 ± 10.82. The wasps ranged from 1 to 80 days in age. The age distribution was best described by the negative exponential function $0.55e^{-0.48age}$ ($R^2 = 0.86$). The wasps
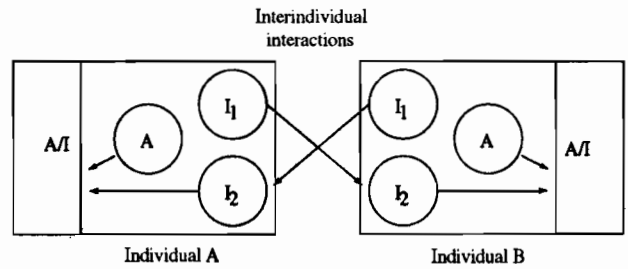
Figure 18 The activator-inhibitor model for age polyethism (Huang & Robinson 1992). Each individual has three pools, A, I_1 and I_2. The pools A and I_1 contain an activator and an inhibitor respectively, the synthesis of which is coupled with and increases with the age of the individual. Social interactions result in the transfer of the entire quantity of accumulated inhibitor from pool I_1 of one individual to the pool I_2 of the other and vice versa. The inhibitor so lost from pool I is replenished instantaneously. The resultant A/I ratio determines the task profile of the individual. The inhibitor in pool I_1 does not interact with the activator in pool A and hence an individual cannot inhibit itself. (Redrawn from Naug & Gadagkar 1999)

participated in 9.25 ± 4.17 solicits per day. For the simulations, we therefore generated 1000 colonies with 25 wasps per colony, by randomly picking individuals between 1 to 80 days of age using the above mentioned negative exponential function. We assumed that 250 inter-individual interactions (25 individuals x 10 interactions/individual) took place randomly between the members of a colony, as per the model described in figure 18. After all these interactions, the total accumulated quantity of inhibitor in the I_2 pool of each wasp was computed. In the empirical studies, individuals younger than 6.23 ± 5.30 days were found not to perform any tasks (defined as idlers). Those older than this but younger than 17.84 ± 12.89 days were found to perform only intranidal tasks, which consisted largely of feeding larvae; these were defined as nurses. Still older individuals performed extranidal tasks, principally foraging, although these individuals continued to perform intranidal tasks also. These older wasps were defined as foragers here (not to be confused with the behavioural castes of foragers in the principal component analysis). Thus the A/I ratio obtained from the simulation for 6-day-old individuals (0.018) and for 18-day-old individuals (0.056) were set as the threshold for nursing and foraging respectively. In other words, individuals with A/I ratios less than 0.018 were classified as idlers, those with values between 0.018 and 0.056 were classified as nurses, and those with values above

0.056 were classified as foragers. The simulation thus permitted us to determine the ages as well as the proportions of idlers, nurses, and foragers.

The first result from the simulation was the demonstration of age polyethism. The mean age of idlers, nurses, and foragers turned out to be 4, 14, and 37 days respectively. The proportion of these categories were 0.28, 0.36, and 0.36. A crucial aspect of this model is that it is expected to provide a mechanism by which age polyethism becomes sufficiently flexible so that the colony can respond to contingencies such as a skewed age distribution. To see if the model would indeed do so, we simulated not only colonies with individuals ranging in age from 1 to 80 (typical colonies) days but also colonies with individuals ranging in age from 1 to 10 days (young-worker colonies) as well as colonies with individuals ranging in age from 70 to 80 days (old-worker colonies)(for more details see Naug & Gadagkar 1999). The different age distributions in the three kinds of colonies (typical, young-worker, and old-worker) significantly influenced both the mean ages and the proportion of idlers, nurses, and foragers (figure 19). Nevertheless, the flexibility of age polyethism was very clear. Young-worker colonies had precocious foragers with a mean age of 8 days, and old-worker colonies had overaged nurses with a mean age of 75 days. These findings must be contrasted with the mean age of 14 days for nurses and 37 days for foragers in colonies with typical age distribution. Young-worker colonies had fewer idlers than typical colonies, and old-worker colonies had none – not surprising, since colonies with a skewed age distribution had a limited work force. Besides, young-worker colonies had a higher proportion of foragers and old-worker colonies had higher proportion of nurses than normal colonies – this too makes sense, since precocious foragers and overaged nurses cannot be expected to be as efficient as their counterparts in typical colonies.

The next step was to see if this model can also explain how colonies respond to other contingencies, such as an altered task demand. A higher than normal larva/adult ratio can produce a high demand on workers. But a high larva/adult ratio also means more interaction between larvae and adults, leaving that much less time for the adults to interact with each other. Thus we simulated changes in demand by altering the rate of adult-adult interactions. Colonies were simulated with 5 (corresponding to high demand situations), 10 (corresponding to intermediate demand situations) and 15 (corresponding to low demand situations) interactions per individual. These different rates of inter-individual interaction significantly influenced the mean age and proportion of idlers, nurses, and foragers (figure 20). Remarkably enough, the mean ages of idlers, nurses, and foragers decreased with increasing demand, meaning that workers worked harder to meet the increased demand. The proportion of idlers and nurses decreased with increasing demand but that of foragers increased. The decrease in the proportion of idlers and increase in the proportion of foragers is clearly useful to deal with a high-demand situation. But the decline in the proportion of nurses may seem perplexing because an increased larva/adult ratio must increase the demand not only for foraging but also for nursing. We think that the clue to this riddle is that while nurses seldom participate in foraging tasks, foragers routinely combine foraging and nursing duties. Indeed, there appears to be some evidence that *R. marginata* foragers increasingly combine foraging with nursing duties under conditions of high demand. In summary this simple activator-inhibitor model seems to account for all observed empirical results concerning age polyethism in normal colonies as well as in colonies with altered age distributions or altered task-demand levels.

What is the evidence for the presence of the proposed activator and inhibitor molecules? In *R. marginata* none as yet, but there is reasonable evidence for them in honey bees. The obvious candidate for the activator molecule is the Juvenile hormone (JH) (Fahrbach & Robinson 1996, Fahrbach 1997, Robinson & Vargo 1996). There is plenty of evidence that JH is involved in *Apis mellifera*. Young bees performing intranidal tasks have low titres of JH, while older bees performing extranidal tasks have high titres (Robinson 1987, 1992, Robinson et al. 1989). JH appears to play a causal role in modulating task performance in honey bees and also in the swarm-founding wasp *polybia occidentalis*. Injection or external
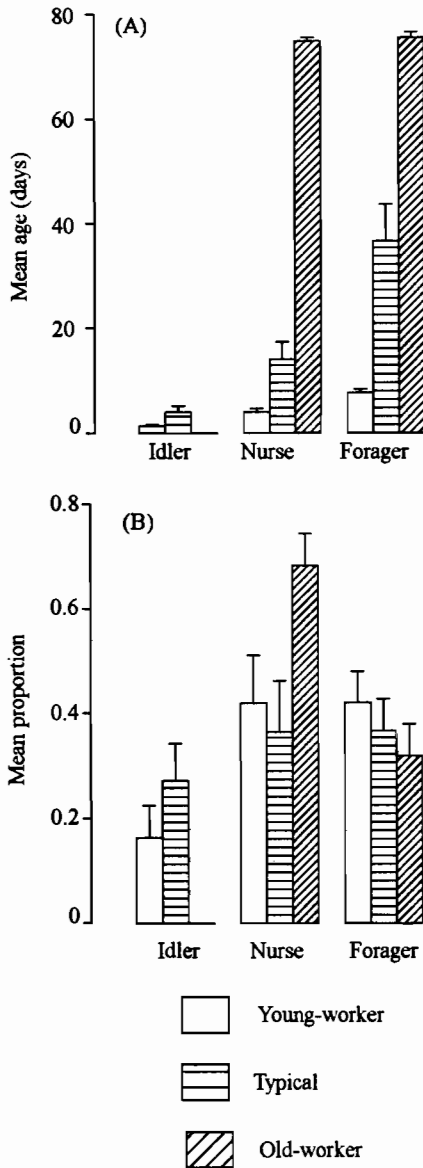
Figure 19 Above: Mean (± S.D.) ages of the three task groups, idlers, nurses, and foragers, in relation to colony age distributions. Age distribution of the colonies significantly influenced the mean age of idlers (one-way ANOVA; $F = 4821.53$, $p < 0.0001$), nurses ($F = 382412.27$, $p < 0.0001$), and foragers ($F = 67446.61$, $p < 0.0001$). Multiple comparisons of means within each task group with the Tukey-Kramer method indicated significant differences across age distributions ($p < 0.01$). Below: Mean (± S.D.) proportions of the three task groups, idlers, nurses, and foragers, in relation to colony age distributions. Age distribution of the colonies significantly influenced the mean proportion of idlers (one-way ANOVA; $F = 1289.54$, $p < 0.0001$), nurses ($F = 4050.52$, $p < 0.0001$), and foragers ($F = 718.55$, $p < 0.0001$). Multiple comparisons of means within each task group with the Tukey-Kramer method indicated significant differences across age distributions ($p < 0.01$). (Redrawn from Naug & Gadagkar 1999)
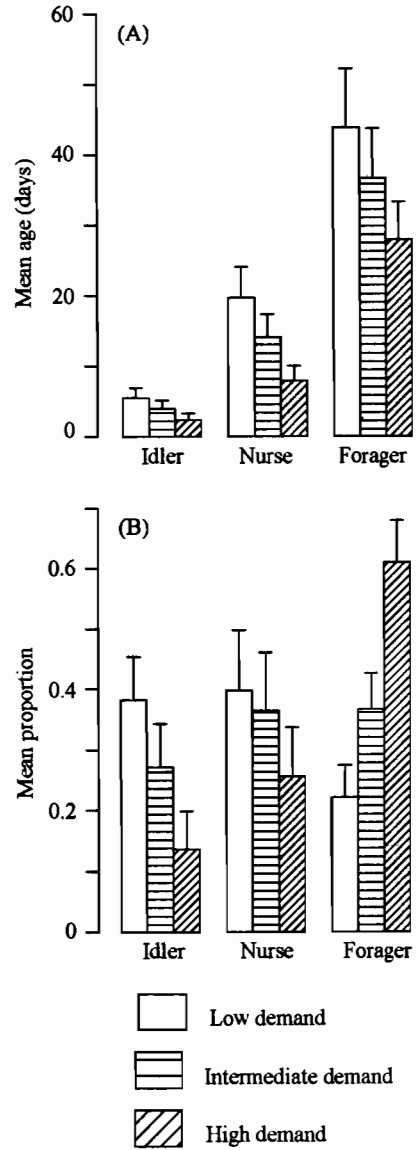
Figure 20 Above: Mean (± S.D.) ages of the three task groups, idlers, nurses, and foragers in relation to colonies with different demand levels (brood/:adult ratios). The demand levels of the colonies significantly influenced the mean age of idlers (one-way ANOVA; $F = 1677.86$, $p < 0.0001$), nurses ($F = 3101.14$, $p < 0.0001$) and foragers ($F = 1297.31$, $p < 0.0001$). Multiple comparisons of means within each task group with the Tukey-Kramer method indicated significant differences across demand levels ($p < 0.01$). Below: Mean (± S.D.) proportions of the three task groups, idlers, nurses, and foragers, in relation to colonies with different demand levels (brood/:adult ratios). The demand levels of the colonies significantly influenced the mean proportions of idlers (one-way ANOVA; $F = 3199.39$, $p < 0.0001$), nurses ($F = 643.20$, $p < 0.0001$), and foragers ($F = 10173.13$, $p < 0.0001$). Multiple comparisons of means within each task group with the Tukey-Kramer method indicated significant differences across demand levels ($p < 0.01$). (Redrawn from Naug & Gadagkar 1999).

application of JH can produce precocious foragers, for example. Precocious foragers and overaged nurses produced by the alteration of colony demography have JH titres appropriate for the task they are performing, though inappropriate for their age (Jaycox 1976, Rutz et al. 1976, Robinson 1987, Robinson et al. 1992, Huang & Robinson 1992, 1996, O'Donnell & Jeanne 1993). Although the inhibitor is not yet identified even in honey bees, there appears to be evidence for social inhibition of behavioural development. Worker bees kept in isolation precociously synthesize JH at high rates (Huang & Robinson 1992, 1996). Huang et al. (1998) have shown that physical contact with other bees is necessary for social inhibition of behavioural development. They have also shown that removing the mandibular gland of bees renders them less inhibitory or completely uninhibitory, which suggests mandibular glands as a possible source of the potential inhibitor.

Two major conclusions emerge from the results described in this section. One, a fairly sophisticated, almost honey bee like, age polyethism can evolve in a primitively eusocial species such as *R. marginata*, where workers still retain reproductive options. Two, the seemingly unbridgeable gap between the hypothesis of age as a causative agent in behavioural development and the criticism that age polyethism is potentially inflexible (Franks & Tofts 1994, Robinson et al. 1994, Franks et al. 1997, Robson & Beshers 1997, Traniello & Rosengaus 1997, Calderone 1998) can indeed be bridged by exploring relative age rather than absolute age as the agent of work organization.

Acknowledgements

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