

# EVOLUTION OF INSECT SOCIETIES : SOME INSIGHTS FROM STUDYING TROPICAL WASPS

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## 1. INTRODUCTION

With the exception of one species of a sphecid wasp all social wasps belong to the family Vespidae. This family is traditionally divided into three sub-families namely, the Stenogastrinae, the Polistinae and the Vespinae (Richards, 1962; 1978a,b). It is our good fortune that India is blessed with representatives of each of the three sub-families and also each of the three tribes of Polistinae (Gupta and Das, 1977). But it is also a great misfortune that, barring a few scattered reports (Davis, 1966a,b; Gadgil and Mahabal, 1974; Gadagkar, 1980 ; Belavadi and Govindan, 1981), there is virtually no information on the Indian fauna of social wasps. Although the Indian groups are in dire need of taxonomic revision, it must be acknowledged that the excellent, if somewhat outdated, works of the Dutch taxonomist, J. Van der Vecht (1941, 1957, 1962, 1966) have rescued those intent on studying the biology of Indian social wasps from despair. Das (1982) has recently attempted a heroic revision of the entire family but taxonomic works are of little value as long as they remain as unpublished Ph.D. theses.

It is in this background that we at the Indian Institute of Science, have initiated a long term study of two species of common social wasps namely *Ropalidia marginata* and *R. cyathiformis*. Primitive eusocial insects such as these are of great topical interest because of the insight they are expected to provide into the forces moulding the evolution of sociality in animals. Tropical species of wasps and bees live in colonies that may be initiated at any time of the year and their colonies may also be perennial, providing opportunities for queen replacements, colony fissions and emigration of potential queens to initiate new colonies. The increased scope for reproductive competition in such situations makes them excellent model systems in insect sociobiology. Tropical wasps have been extensively investigated from this point of view and these studies have also been repeatedly reviewed (West-Eberhard, 1969; Jeanne, 1972, 1980; Litte, 1977, 1979, 1981; Gadagkar, *in press*). In the limited time available, I shall briefly summarise some aspects of our work at the Indian Institute of Science.

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*Social Insects : An Indian Perspective* (eds.) G.K. Veeresh, A.R.V. Kumar and T. Shivashankar, Published by IUSSI-Indian Chapter, Bangalore (1990).

Insect sociobiology is characterised by an unusually large body of theoretical work. Without going into the myriad details of the many theoretical models, I may just mention that there are three main theories or hypotheses which attempt to explain the origin and evolution of insect societies. These are often called the theories of kin selection (more specifically, the haplodiploidy hypothesis) (Hamilton, 1964a,b), parental manipulation (Alexander, 1974), or sub-fertility (West-Eberhard, 1975) and mutualism (Lin and Michener, 1972). The kin-selection or haplodiploidy hypothesis states that workers may gain more inclusive fitness than solitary foundresses because of the genetic asymmetries created by haplodiploidy such that fullsisters are more closely related to each other (coefficient of genetic relatedness,  $r = 0.75$ ) than a mother is to her daughters ( $r = 0.5$ ). The parental manipulation hypothesis states that parents may leave behind more grand-children by manipulating some of their offspring into being sterile and helping their fertile sisters to produce more offspring. The sub-fertility hypothesis, a variant of this, argues that sub-fertile individuals would be more easily selected to take up worker roles. The theory of mutualism states that in many primitively social insects, sterility is not complete, altruism is probably absent and joint nesting is for mutual benefit. There are numerous other papers dealing with particular theoretical aspects of the evolution of eusociality (West-Eberhard, 1978; Craig, 1980; Seger, 1983; Joshi and Gadagkar, 1985; Stufflefield and Charnov, 1986) and many reviews too (Wilson, 1971, 1975; Starr, 1979; Andersson, 1984; Brockmann, 1984; Gadagkar, 1985a,b). It has been our aim to provide an empirical data base which would ultimately help in evaluating the merits of these theoretical ideas.

## 2. SOCIAL ORGANISATION

*Ropalidia marginata* is a common social wasp in peninsular India that is excellently suited for such studies (Gadagkar, 1980; Gadagkar *et al.*, 1982a,b). Our first step, in an effort to use *Ropalidia marginata* to understand the forces that mould the evolution of insect societies, has been an investigation of social organisation. We argued that, for species where there are no morphological differences between the different individuals comprising a colony, the most useful approach would be to focus on inter-individual differences in behaviour patterns. Deviating somewhat from traditional approaches to this problem, we have chosen to construct time activity budgets for individually identified wasps using all the common activities they perform. The behaviours we documented therefore included seemingly trivial ones such as Sitting or Sitting with raised antennae, behaviours which have not been traditionally the focus of much attention. We then used multivariate statistical techniques such as principal components analysis and hierarchical cluster analysis to examine inter-individual differences in behaviour patterns. This analysis has shown that each colony has three distinct kinds of individuals or behavioural castes.

### 3. THE SIGNIFICANCE OF BEHAVIOURAL CASTE DIFFERENTIATION IN *R. MARGINATA* AND *R. CYATHIFORMIS*

Superimposing this cluster analysis on data on frequencies of several behaviours such as Fighting, Building, Bringing food etc., behaviours which are rather rare but have traditionally been the focus of much attention, was very revealing and permitted the naming of the behavioural castes as Sitters, Fighters and Foragers (Gadagkar and Joshi, 1982b, 1983). The Sitters are those that spend a large proportion of their time in sitting and grooming themselves. Fighters are those that spend a great deal of their time in sitting with raised antennae and also show a high frequency of dominance behaviours. Foragers are those that spend a great deal of their time being absent from their nest and show high frequencies of bringing food and building material back to the nest. Equally revealing was to note the position of the queen among these clusters. The fact that data on egg laying was not used in the cluster analysis made this an important question. In 13 out of 14 colonies of *R. marginata* studies so far, the queens have always been Sitters (K. Chandrashekara and R. Gadagkar, *this volume* and unpublished observations). Our interpretation of this is that, *R. marginata* queens are programmed to waste little time and energy in doing anything other than egg laying. But since there is normally only one queen per colony (K. Chandrashekara and R. Gadagkar, *this volume* and unpublished observations) there are many Sitters who are not queens. These other Sitters may be hypothesized as being hopeful queens. Fighters are probably performing the function of keeping the colony active and guarding it against parasites. That fighters may also be hopeful queens is suggested by the fact that they show the highest frequency of fighting amongst themselves. We also hypothesize that the Foragers who leave the nest to perform the most risky tasks of gathering food and building material have the least chance of becoming queens in the future. These hypotheses, as we will see in a little while, can be put to experimental tests.

The comparative method is often as powerful as the experimental method in behavioural and evolutionary analysis. This is illustrated to a considerable extent by our comparative studies of *R. cyathiformis* and another tropical social wasp *Polistes versicolor*. Social organization is very similar in *R. cyathiformis*. But an important difference is that in all the 10 colonies studied so far, the queens always belong to the Fighter caste (Gadagkar and Joshi, 1982b, 1984; Gadagkar, 1987). This means that the queen in this species does more than just laying eggs. She is one of the most active individuals in the colony and unlike an *R. marginata* queen, she takes part routinely in aggressive interactions with her nestmates. She is also the one who most often nibbles and nudges her nestmates, perhaps to keep them active and, again, she is the one who spends much of her time in sitting with raised antennae probably in an effort to be alert to the presence of parasites and other dangers to the colony.

Now, why this difference between a queen of *R. marginata* and that of *R. cyathiformis*? Our hypothesis is that an *R. marginata* queen is a Sitter because she faces relatively little reproductive competition from her nestmates. An *R. cyathiformis* queen, on the other hand, we believe is a Fighter because she faces relatively high levels of reproductive competition from her nestmates. This idea is supported by several facts: First, *R. cyathiformis* colonies sometimes have multiple egg-layers; animals which are foraging for food also occasionally lay eggs and males are sometimes produced throughout the colony cycle. *R. marginata* colonies always have a single egg-layer, the distinction between foragers and egg-layers is always clear and males are normally produced only late in the colony cycle. Secondly, in single-foundress colonies of *R. cyathiformis*, the queen or egg-layer belongs to the Sitter caste and not to the Fighter caste. In other words, an *R. cyathiformis* queen is also a Sitter when she faces no reproductive competition (by virtue of being alone). Notice that the delineation of a Fighter is not done on the basis of fighting but by the analysis of time activity budgets of other more common behaviours.

#### **4. THE NEOTROPICAL PRIMITIVELY EUSOCIAL WASP *POLISTES VERSICOLOR***

A third line of evidence comes from a similar study of yet another species namely, the neotropical primitively eusocial wasp, *Polistes versicolor*. At this point, I will briefly illustrate our methodology, our results and their interpretation with the help of some hitherto unpublished data on *Polistes versicolor*, a species on which I have conducted studies similar to those that have already been published on *R. marginata* (Gadagkar and Joshi, 1983) and *R. cyathiformis* (Gadagkar and Joshi, 1984). The *Polistes versicolor* study was conducted on three monogynous nests (two pre-emergence nests and one post-emergence nest) built on the caves of sheds on Barro Colorado Island in Panama, (9°09'N, 79° 51'W) (see Leigh *et al.*, 1982 for a detailed treatment of the ecology of this island) between November 1980 and February 1981. In all, 240 h of observation were conducted. All the adults of each colony were individually identified by marking with spots of coloured paint, without removing them from the nest. New animals were marked upon their emergence and a census of all the animals present on each nest was taken at about 0600 hrs or after 2000 hrs, a time when all the animals were expected to be on the nest. I found no evidence of animals shifting from one nest to another, as reported by Ito (1984) for the same population. In all, sufficient data were available for 28 animals which are included in this analysis. Details regarding the nests and the animals associated with them are in Table 1.

Table 1.  
Nests Studied, Animals associated with them and Periods of observation

Nest Code	Pre/Post Emergence	Code nos. of Assoc-iated animals	Identity of Queen	Duration of Observation	No. of hours of Observation
J	Pre-Emergence	1-12	1	27 Nov 1980- 3 Jan 1981	100
L	Pre-Emergence	13-18	13	22 Dec 1980-22 Jan 1981	80
M	Post-Emergence	19-28	19	12 Jan 1981-31 Jan 1981	60

## 5. SAMPLING METHODS

The sampling methods used in this study were adapted from Altmann (1974) as described earlier (Gadagkar and Joshi, 1983, 1984). Briefly, *ad libitum* sampling was used for obtaining preliminary information regarding the behaviour of the species and for constructing an ethogram. *Instantaneous scanning* of the behavioural states of all animals in a colony was repeated at a large number of randomly chosen times. Rare behaviours such as dominance and subordinate behaviour, bringing and exchange of food and building material etc., were quantified in separate 5 minute sessions, begun at randomly chosen times, during which every act of each of the chosen set of rare behaviours, by every animal, was recorded. All observations were made between 0800 and 1800 hours. I recorded data in a pre-coded form on coding sheets in an 80 column format, ready to be punched on computer cards and analysed them using the DEC 1090 computer facility at the Indian Institute of Science, Bangalore

## 6. ANALYSIS OF DATA

Analysis of data was performed as previously described (Gadagkar and Joshi, 1983, 1984). Briefly, data from the instantaneous scans were used to construct time activity budgets for each animal. Proportions of times spent in the six most common behaviours namely Sit and Groom, Raise antennae, Raise Wings, Walk, In cells and Absent from Nests were used for subsequent analysis. These behaviours accounted for 93.4% of an average wasp's time from 0600 to 1800 hours. Frequencies per hour of rare behaviours were computed from "*all occurrences of rare behaviours*". The most frequent of these rare behaviours were Dominance display, Bring food, Snatch food, Lose food, Feed larva, Bring building material, Snatch building material and Lose building material. Apart from these 16 behaviours, all instances of egg laying seen during the observations were recorded and the queen was identified on the basis of this. The proportions of time spent by each animal in each of the six common behaviours were

subjected to principal components analysis (Anderberg, 1973; Frey and Pimentel, 1978) as well as hierarchical cluster analysis using a single linkage algorithm (DeGhett, 1978).

The time activity budgets in Figure 1 show clearly that the 28 animals showed wide variation in the manner in which they allocated their time between the six behaviours, Sit and Groom, Raise antennae, Raise wings, Walk, In cells and Absent from Nest, although all of them spent a very similar

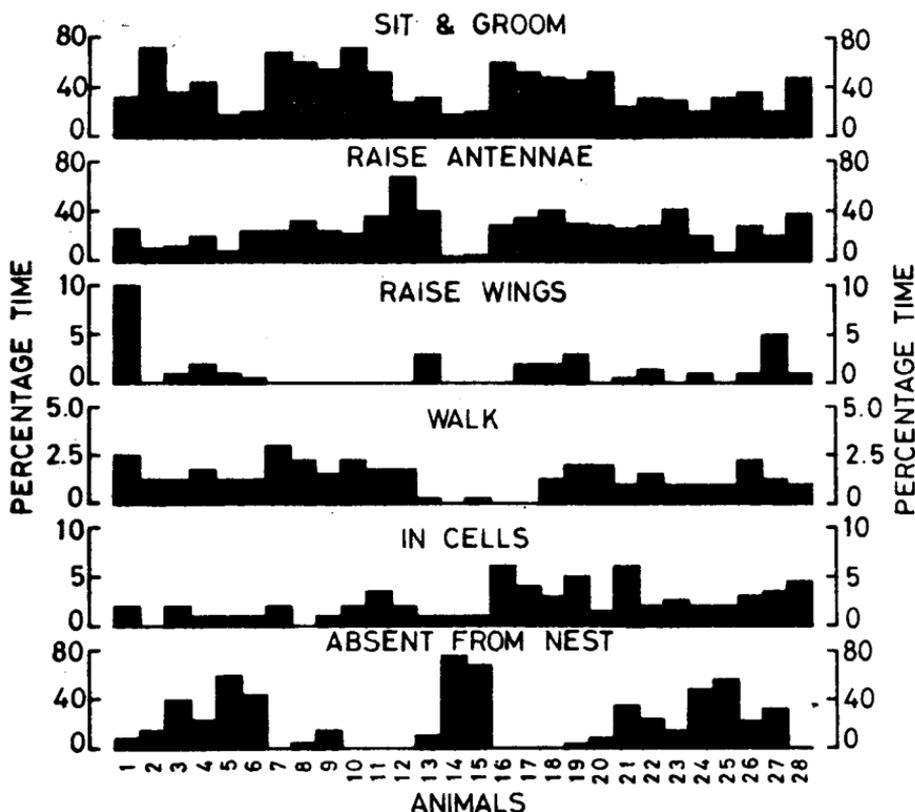


Fig. 1. Time activity budgets of 28 individually identified wasps from two pre-emergence and one post-emergence colony for six behaviours. The animals are arranged according to their colony affiliations beginning with the queen of each colony. Thus animals 1-12 belong to colony J with 1 as their queen, animals 13-18 belong to colony L with 13 as their queen and animals 19-28 belong to colony M with 19 as their queen.

amount of time in these six behaviours put together. I used the method of principal components analysis to see if there are clusters of wasps showing particular types of behavioural patterns. The results of this analysis (Table

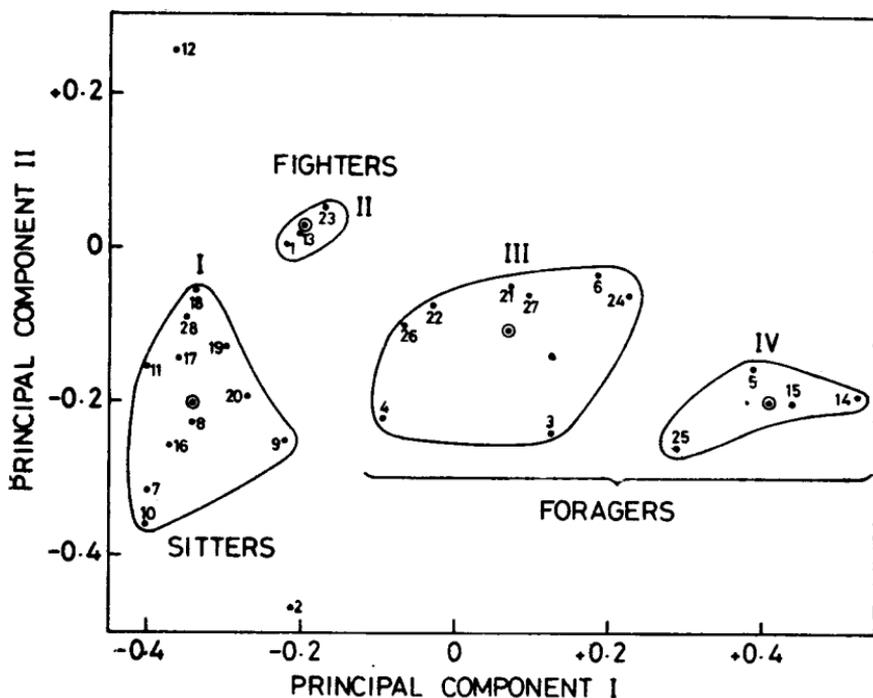


Fig. 2. Behavioural castes of *P. versicolor*. 28 wasps from two pre-emergence colonies and one post-emergence colony are shown as points in the co-ordinate space of the first two principal components. The cluster boundaries are confirmed by the criteria of nearest centroid. Centroids of each cluster are shown as circled dots. Wasps 1 and 13 are the queens of the two pre-emergence colonies and wasp 19 is the queen of the post-emergence colony. Animals 2 and 12 are examples of occasional eccentrics that we find in most colonies in all species whose significance we do not still understand.

2) show that the first principal component accounts for 78.37% of the variance with Absent from Nest as its dominant term (Weightage = 0.8183). The second principal component accounts for 19.80% of the variance and has Sit and Groom as its dominant term (Weightage = -0.7254). Thus the first two principal components together account for 98.17% of the variance and we are therefore justified in considering only the first two principal components. Other advantages of using the method of principal components analysis are discussed in Gadagkar and Joshi (1984). A two-dimensional plot employing the first two principal components shows clearly that there are four clusters of wasps (Figure 2). A very similar (although not completely identical) set of clusters is obtained using the method of hierarchical cluster analysis (Figure 3).

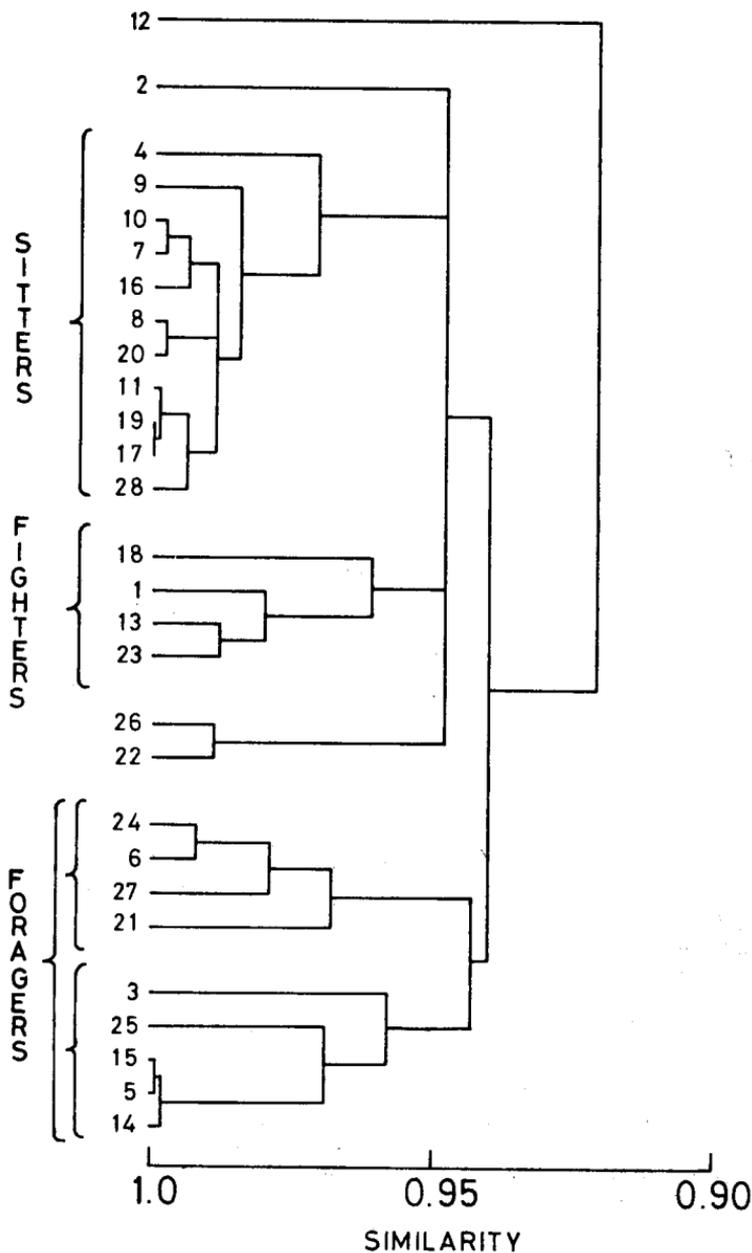


Fig.3. Hierarchical cluster analysis. Time activity budgets as shown in Fig.1 are used to calculate the pearson product moment correlation as an index of similarity between all pairs of wasps. The dendrogram is constructed using the single linkage algorithm.

Table 2.  
Eigen vectors of principal components, Eigen values, percentage of variance, and cumulative percentage of variance

Behaviour	Principal components		
	1	2	3
Sit and Groom	-0.4602	-0.7254	0.4386
Raise Antennae	-3.3437	0.6749	-0.5974
Raise Wings	-0.0035	0.0471	-0.4255
Walk	-0.1115	-0.0055	-0.0275
In cells	-0.0158	0.0253	-0.1633
Absent from Nest	0.8183	-0.1239	0.4923
Eigen value	0.0774	0.0196	0.0014
Percentage of variance	78.37	19.80	1.43
Cumulative percentage of variance	78.37	98.17	99.60

## 7. INTERPRETATION OF THE CLUSTERS

I shall now illustrate the interpretation of such clusters based on their mean behavioural profiles as well as by comparison of the patterns seen in *R. marginata* and *R. cyathiformis* (Gadagkar and Joshi, 1983, 1984). The results of principal components analysis suggest that clusters I and II are characterized by a low proportion of time in being Absent from Nest and conversely clusters III and IV are characterized by high proportions of time in being Absent from Nest. This is because Absent from Nest has the highest positive weightage in the first principal component and clusters I and II have negative amplitudes while clusters III and IV have higher amplitudes in this. Similarly cluster I is expected to be characterized by a high proportion of time spent in Sit and Groom. This pattern is confirmed by a computation of mean behavioural profiles of the clusters (Figure 4). Cluster I, which is characterized by the highest proportion of time in Sit and Groom is termed Sitters. Cluster II is characterized by high proportions of time in Raise Antennae and Raise Wings. In other species that we have studied, this is strongly correlated with a high frequency of dominance behaviour. In these colonies of *P. versicolor*, there was hardly any dominance behaviour except in colony J where the queen (who belongs to cluster II) exhibited all the 12 cases of dominance behaviour seen (frequency = 0.97 per h). For this reason and in conformity with other

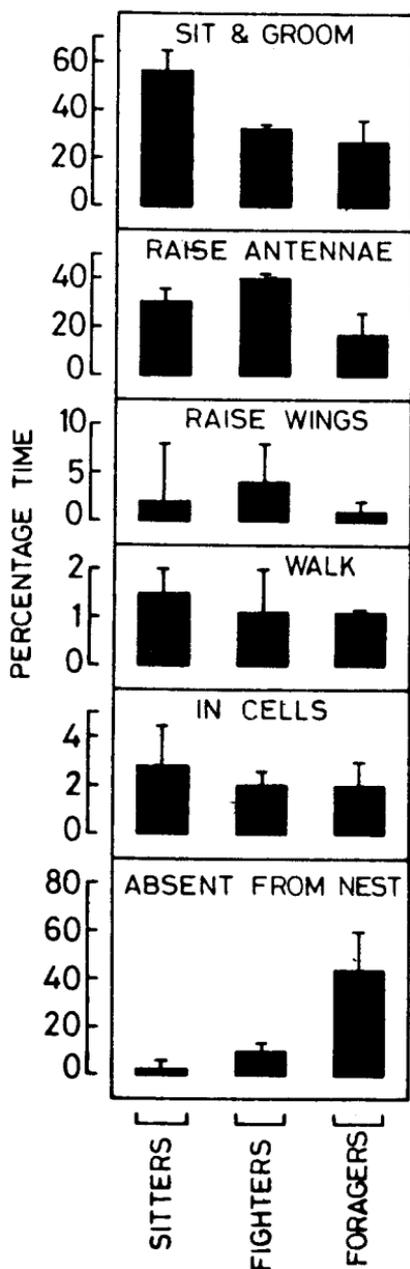


Fig. 4. Mean behavioural profiles (time activity budgets) of Sitters, Fighters and Foragers.

studies, we term this cluster, Fighters. Clusters III and IV are characterized by high proportions of time in being Absent from Nest and contain the only animals who were ever seen bringing food to the nest. These clusters are thus termed Foragers.

A number of behaviours which are sufficiently rare so as not to be included in the time activity budget analysis have been studied by computing the frequencies with which these behaviours were performed per hour by each animal. Such data for the three behavioural castes (Figure 5) show a number of interesting patterns. Fighters, Sitters and Foragers show interesting frequencies of dominance display, in that order. Sitters and Fighters were never seen bringing food but were certainly involved in snatching food from Foragers. Such food snatched from Foragers was never seen being lost to other animals. Every act of losing food to another animal was only by a Forager. Both Fighters and Foragers were involved in feeding larvae but the Sitters were not. Fighters were conspicuously involved in matters connected with building material. They showed the highest frequency of bringing building material, snatching building material as well as losing building material.

Some interesting conclusions can be drawn by looking at the position of the queens in the *P. versicolor* behavioural caste system. Of the three colonies studied, two were pre-emergence colonies and the queens of both these colonies are Fighters (Animal 1 of colony J and Animal 13

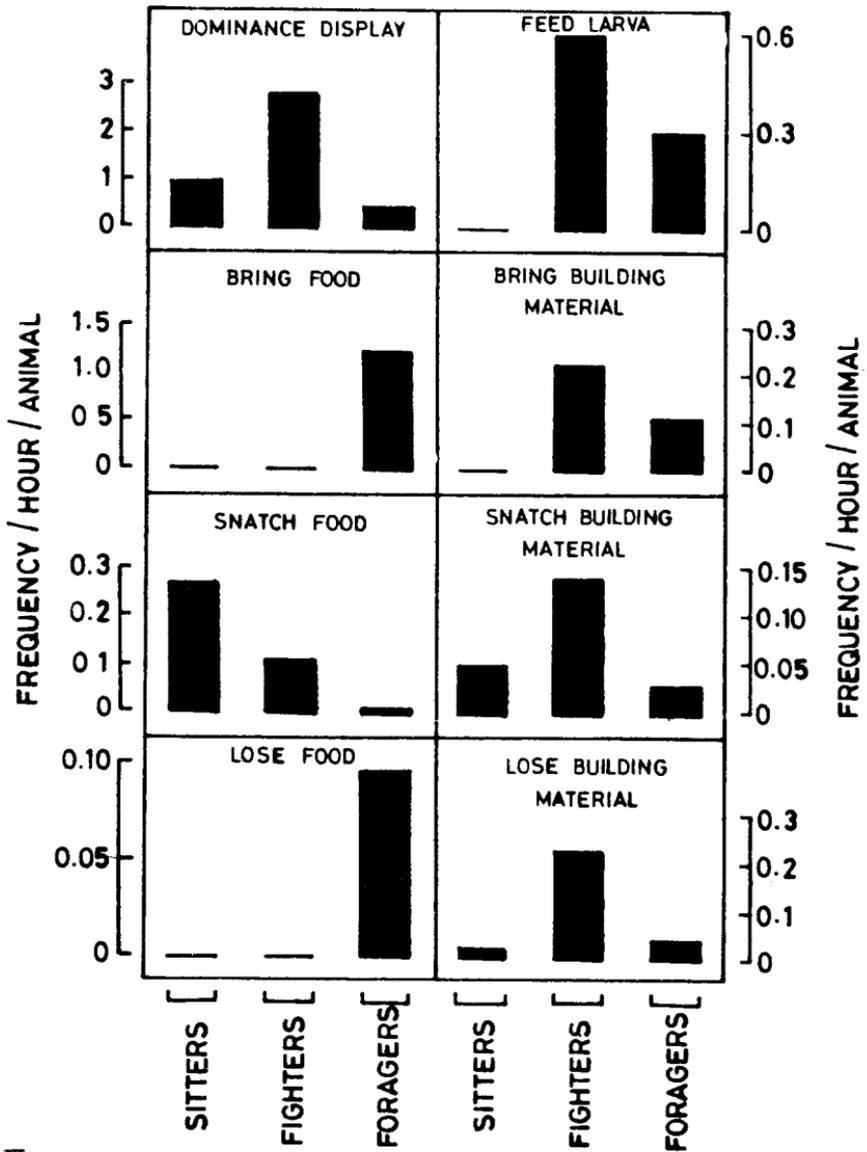


Fig.5. Frequencies per hour per animal are shown for the three behavioural castes, Sitters, Fighters and Foragers for eight rare behaviours.

of colony L). The third colony was a post-emergence colony and the queen of this is not a Fighter but a Sitter (Animal 19 of colony M). It is important to note that this third colony however did have a fighter (Animal 23 of colony M) although the queen herself was not one. While it is true that I have so far studied only two pre-emergence colonies and one post-emergence colony, the difference in the position of the queen in the behavioural caste system begins to provide further evidence, linking low reproductive competition with Sitter queens and high reproductive competition with Fighter queens. My reasoning is as follows: pre-emergence colonies are expected to consist of a group of foundress females who are most likely to be Sisters (see for eg. Jeanne, 1972; Litte, 1977). One of these foundresses assumes the role of the queen and the others become workers. If the workers (co-foundresses) are full-sisters of the queen then they rear brood consisting of nieces and nephews (average relatedness is equal to 0.375). If the workers (co-foundresses) are less closely related to the queen then of course they will rear brood that is even less related. In a post-emergence colony, on the other hand, subordinate co-foundresses begin to die out and the worker force is progressively replaced by the queen's daughters. When such replacement is complete, the workers consists entirely of daughters and thus rear a brood of brothers and sisters (average genetic relatedness is equal to 0.5, if brothers and sisters are reared in equal numbers). If the replacement is incomplete then the average genetic relatedness between workers and brood will lie somewhere between 0.375 and 0.5. We do not expect multiple matings by the queen to differentially effect worker-brood genetic relatedness in pre- and post-emergence colonies. In general, therefore, worker-brood genetic relatedness will be higher in post-emergence colonies when compared to that in pre-emergence colonies. Other things being equal, workers in pre-emergence colonies should hence, be much less altruistic and should be selected to seek reproductive options of their own. Workers in post-emergence colonies, on the other hand, should be relatively more altruistic and less likely to seek reproductive options of their own. In short, I suggest that queens face a greater threat of reproductive competition from their nestmates in pre-emergence colonies than in post-emergence colonies.

While interpreting the Sitter queens of *R. marginata* and the Fighter queens of *R. cyathiformis*, I have argued that queens would tend to be Sitters if they face relatively little reproductive competition from their nestmates while they tend to be Fighters if they face relatively high levels of reproductive competition. The result that the queens of the two pre-emergence *Polistes versicolor* colonies were Fighters and that the queen of the post-emergence colony was a Sitter clearly buttresses my speculation.

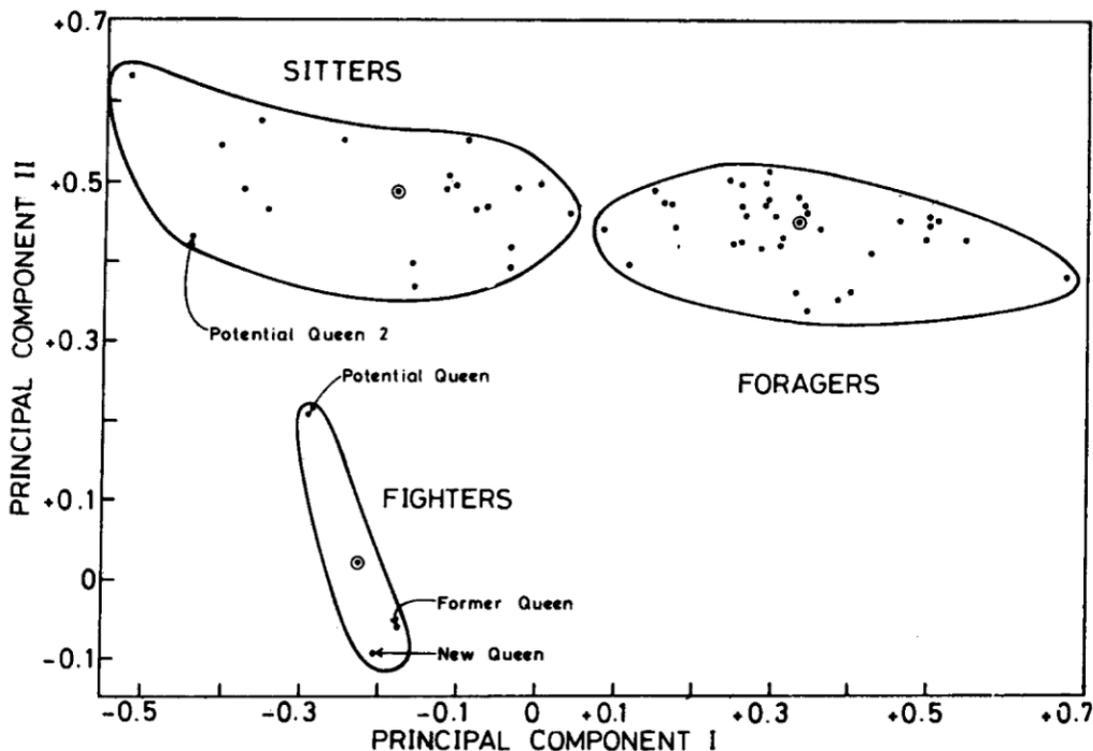


Fig. 6. Behavioural profiles of individually identified wasps in an *R. cyathiformis* colony analysed by principal components analysis. Each point represents one wasp (either before or after the queen was removed) plotted in the co-ordinate space of the first two principal components. When the wasp labelled as Former Queen was removed, that labelled as Potential Queen became the new queen and when she in turn was removed, the wasp labelled as Potential Queen 2 became the next queen (from Gadagkar, 1987). O denotes centroid

## 8. EXPERIMENTAL TESTS OF EVOLUTIONARY HYPOTHESES

As mentioned earlier, hypothesis regarding the biological significance of the behavioural castes are amenable to experimental verification. I have now conducted several experiments to test these hypothesis. My approach here has been to study the prevailing behavioural caste differentiation in a colony, experimentally remove the queen and study the subsequent changes in social organisation. In every *R. cyathiformis* colony studied so far, there appear to be one or two wasps who are very different from all other individuals in the colony but rather similar to the queen (see for eg. Figure 6). These are potential queens because they take over the role of the queen when she is removed. These potential queens always belong to the Fighter caste as the queen herself does in this species. Upon removal of the queen, one of the other Fighters becomes the queen. In addition, one of the Sitters changes her behavioural profile rather drastically and becomes a Fighter and, possibly, the next potential queen, ready to take over the role of the queen when the chance to do so, arises. In short, every colony has a potential queen already differentiated even when the original queen is around. This may be adaptive in ensuring rapid takeover in case of death of a queen, forestalling decline or usurpation of colonies. The significance of the behavioural caste differentiation is therefore quite clear in this species. The Fighters are potential queens but the Sitters also have a certain chance of becoming queens in future. These results make our present experiment with *R. marginata* even more exciting because here the queen herself is a Sitter. Another interesting fact regarding *R. cyathiformis* is that potential queens are often young and aggressive individuals who seldom forage for food or do other work. This is in complete contrast to North American *Polistes exclamans* where older foragers tend to become replacement queens (Strassmann and Meyer, 1983).

## 9. COLONY FISSION

Yet another line of work has provided additional insights into social organisation in *R. cyathiformis*. During one of our long term studies, a colony split, so that, about half its members left to form a new colony, just a few feet away. An analysis of the quantitative ethological data on these animals before and after colony fission lead to several interesting conclusions (Gadagkar and Joshi, 1985). The first of these conclusions was that the fitness (or inclusive fitness) of both, the individuals which left the colony (which we have called the Rebels) and those that stayed back in the old colony (which we have called the Loyalists) increased as a result of the fission. This is because of the prevailing low efficiency of rearing brood which was correlated with high levels of aggression before colony fission. Similarly, a high efficiency of rearing brood prevailed after colony fission and was correlated with low levels of aggression. These facts were true of both the original as well as the new colony. The second conclusion

was that the most dominant individual prior to colony fission became the queen on the new colony. Here therefore was an instance where a wasp managed to establish herself as a queen by offsetting the cost of having to challenge the original queen as well as the risk of brood parasitism that lone foundresses incur. A third conclusion was that the Loyalists and the Rebels each behaved as a co-ordinated group synchronising among themselves, their times of being on or away from the nest and avoiding members of the other group even before the actual fission (Gadagkar and Joshi, 1985).

## 10. PRE-IMAGINAL CASTE BIAS

We have just seen how tropical wasps such as *Ropalidia* provide rich model systems for studying the role of interactions between adult wasps in social organization. But are interactions between adult wasps sufficient to tell us all there is to know about social organization? A review of the literature shows that it has, by and large, been assumed to be so. There has never been a direct test, for instance, of whether all female wasps at eclosion are potentially capable of becoming queens. An experiment to test the null hypothesis that "all eclosing females are potentially capable of laying eggs" has just been completed in my laboratory (Gadagkar *et al.*, *in press*). Our strategy in testing this hypothesis has been to collect a large number of natural nests, clear them of adults and isolate the females eclosing after the nest was brought to the laboratory, into individual cages. These wasps were never allowed to come into contact with any conspecific but were provided the set of conditions under which this species readily initiates nests and produces large numbers of apparently healthy offspring.

Of the 197 animals from 22 nests so tested, only 97 built nest and laid eggs while the remaining 100 died without doing so, in spite of living, on the average, longer than the time taken by the average egg-layer to lay its first egg. These data suggest that female *R. marginata* eclose with varying reproductive potentials and that perhaps all eclosing females are not potentially capable of becoming egg-layers. What factors make some females egg-layers and others, non-egg-layers? We found that the number of empty cells from which an animal ecloses and its own rate of feeding are both positively correlated with an animal's probability of becoming an egg-layer (Table 3). Our interpretation of this is that, because the number of empty cells is an indicator of the queen's influence, she has a role in biasing the future caste of her brood. Our model for pre-imaginal biasing of caste in primitively eusocial insects visualizes that in a colony with a young and healthy queen or with a queen that is in the phase of worker production, the queen's influence is high. As a consequence of this, empty cells are not allowed to accumulate in the nest and the brood are channelled into a developmental pathway leading to the production of "poor eaters" and "poor egg-layers". Alternatively, in a colony with an old or unhealthy

queen or in one that is in the phase of queen production, the queen's influence would be low and as a consequence empty cells would begin to accumulate in the nest and the queen would fail to prevent the brood from entering a developmental pathway leading to the production of "good eaters" and "good egg-layers". Clearly, these results provide support for the parental manipulation and subfertility hypothesis. In other words, the sub-fertile females eclosing with low probabilities of egg laying will be selected to become workers and help their more fertile sisters to reproduce. However, this level of pre-imaginal caste bias is obviously insufficient to explain the fact that most adult wasps become workers while only a few become queens.

Table 3.  
Logistic Regression Analysis : Determinants of Probability of Egg laying  
by Females of *R. marginata*.

Variable	Estimated coefficient ( $\beta$ )	Standard error	z	p
model 1: nest properties as determinants of the probability of egg laying by eclosing females				
no. of empty cells	0.0519	0.0218	2.3845	< 0.02
model 2: feeding rate and body size as determinants of the probability of egg laying				
feeding rate	3.4993	1.2871	2.7188	< 0.007

## 11. MULTIPLE MATING AND LEVELS OF GENETIC RELATEDNESS

As is well known, the haplodiploidy hypothesis rests on a high level of worker-brood genetic relatedness made possible by the asymmetries in genetic relatedness created by the haplodiploid genetic system. It is also well known, however, that this genetic asymmetry is destroyed if queens mate multiply and simultaneously use sperms from different males and produce different patriline of daughters who would be half-sisters of each other. It is therefore, crucial to understand mating patterns of queens for evaluating the haplodiploidy hypothesis. On reviewing the literature, we found that multiple mating and the consequent low levels of worker-brood or intra-colony relatedness to be very widespread (Gadagkar, 1985b). However, most such information in the literature concerns the highly eusocial ants and honeybees. Since one could always argue that polyandry

may be secondarily derived after the attainment of eusociality and after workers lose all reproductive options, we were more interested in information regarding polyandry and sperm usage in primitively eusocial species. Such information did not exist barring a study of *Polistes metricus* (Metcalf and Whitt, 1977), in which there was evidence of the queens mating with at least 2 males and using sperm in the ratio 9:1. We therefore set out to study mating patterns and the patterns of sperm usage in *R. marginata* which is both primitively eusocial and tropical and therefore of crucial interest in this regard. We have now clear evidence of multiple mating and of considerable mixing of sperms from different males in *R. marginata*. (Muralidharan *et al.*, 1986). This should lower intra-colony relatedness (Table 4) and reduce the efficiency of haplodiploidy in driving social evolution.

Table 4.  
Multiple mating and levels of relatedness in *Ropalidia marginata*

Colony	No. of Patriline	No. of daughters tested	Genetic relatedness between daughters
M11	3	10	0.48
M13	2	6	0.61
M15	3	12	0.46
M23	1	3	0.75
Weighted Mean :			0.53

There is another important factor which further reduces genetic relatedness between workers and the brood they rear. This is the phenomenon of polygyny or the presence of more than one egg-layer in a colony. There is clear evidence for this in *R. cyathiformis* (Gadagkar and Joshi, 1982a, 1984). Although, we have never seen the presence of two egg-layers simultaneously in *R. marginata*, queens are frequently replaced by one of their daughters or sisters so that this form of serial polygyny would also result in decreased intra-colony genetic relatedness.

## 12. KIN RECOGNITION

Gadagkar (1985b) reviews various ways that have been suggested for rendering the low levels of intracolony relatedness consistent with the haplodiploidy hypothesis. The possibility that workers discriminate between full- and half-sisters and care only for their full-sisters, thus effectively

raising worker- brood relatedness, was the most appealing of these. There is much evidence for kin recognition now, but only in the honeybees there appears to be evidence that full- and half-sisters can be distinguished inspite of habituation to both classes of sisters. While reviewing this literature, I (Gadagkar, 1985b) was also struck by the futility of the debate regarding the possibility of genetic alleles for innate ability to recognize kin. I, therefore, suggested that instead of trying in vain to discriminate between recognition templates produced by recognition alleles and those produced by learning, one should try to experimentally discriminate between templates which are self-based and non-self-based. I also outlined an experimental protocol to achieve this by comparing the behaviour of normal animals towards animals deprived of external cues, with the behaviour of the latter towards normal animals. Once again, I thought it is more important from the point of view of kin selection, to investigate this phenomenon in a primitively eusocial insect.

We have now performed such experiments using a triplet assay similar to the one used by Shellman and Gamboa, (1982). We find that adult female wasps present on the nest at the time of collection but subsequently isolated for several days, discriminate nestmates from non-nestmates. Similarly, female wasps emerging in the laboratory but who are allowed to remain with their nests and nestmates for several days ('Exposed wasps'), also discriminate nestmates from non-nestmates (Venkataraman *et al.*, *this volume*). Female wasps which are isolated from their nests and nestmates either within one or two minutes of their natural eclosion or those who are experimentally removed from their pupal cases 24-48 hours prior to their expected time of natural eclosion and allowed to complete their development in an incubator ('Isolated' wasps), i.e., those wasps who have been deprived of an opportunity to stay with their nests and nestmates, fail to discriminate between nestmates and non-nestmates (Table 5) (Venkataraman *et al.*, *this volume*). Visualizing nestmate discrimination as a process of comparing a template in the brain of the discriminating animal with a label possessed by the encountered animal, we concluded at this stage that either the label or the template, or both, must be missing at the time of eclosion. Experiments designed to test the ability of Exposed wasps to discriminate between Isolated nestmates and non-nestmates and the converse experiments designed to test the ability of Isolated wasps to discriminate between Exposed nestmates and non-nestmates show that both the discriminating and the discriminated animals have to be exposed (unpublished observations). We conclude, therefore, that both labels and templates are missing at the time of eclosion and that the nest and nestmates are somehow necessary for acquiring both labels and templates. If all wasps acquire labels and templates from such common external sources, it is highly unlikely that full-and half-sisters within a colony will be discriminated.

Table 5.  
Comparison of tolerance indices by the Wilcoxon matched-pairs signed-ranks and Mann-Whitney U-tests. (Data summarised from Venkataraman *et al.*, this volume.

Treatment	No. of Expts	Tolerance among nestmates	Tolerance among non-nest mates	T <sup>a</sup>	p <sup>b</sup>
Wasps present on the nest at the time of collection and subsequently isolated for several days (Treatment I)	14	17.23 ± 3.92	12.80 ± 2.97	12	0.006
Wasps eclosed in the laboratory but exposed to half of their nest and a subset of nestmates (Treatment II)	16	17.44 ± 2.67	15.28 ± 3.82	34	0.039
Wasps isolated from nest and nestmates immediately after eclosion (Treatment III)	16	13.86 ± 4.41	11.96 ± 3.44	44	0.107
Wasps isolated from their nest and nestmates prior to their natural eclosion (Treatment IV)	17	17.87 ± 2.6	17.06 ± 2.72	63	0.261
Tolerance of Isolated wasps by Exposed wasps	16	7.84 ± 3.56	8.32 ± 2.17	64	0.418
Tolerance of Exposed wasps by Isolated wasps	15 (13 <sup>c</sup> )	8.94 ± 2.31	8.19 ± 2.76	30	0.139

a Statistic for the Wilcoxon matched-pairs signed-ranks test

b p value for Wilcoxon matched-pairs signed-ranks test

c as sample size is only 13 for Wilcoxon matched-pairs signed-ranks test

It thus appears that kin recognition is unlikely to restore the prominent role that might be ascribed to haplodiploidy in the absence of multiple mating. We have speculated that the system of nestmate discrimination which is based on acquiring labels and templates from a common external source has not evolved for discrimination of different levels of genetic relatedness within a colony but has evolved for discriminating nestmates from non-nestmates so that aliens who may be potential usurpers or social parasites can be kept away. In addition, I wish to suggest that this mechanism also leaves room for the possibility that aliens who are not perceived as a potential threat, may sometimes be accepted because, once an unrelated individual becomes part of the group, it can acquire the complete status of a nestmate and its genetic dissimilarity can be obliterated. Since mixed colonies with genetically unrelated individuals can be readily established in the laboratory and since there is anecdotal evidence of the occasional acceptance of aliens (many instances reviewed by Lin and Michener, 1972; Ito, 1984; Chandrashekara and Gadagkar, unpublished observations), I would like to speculate that nestmate discrimination may not be all too rigid. In tropical species such as *R. marginata* where colonies are perennial and where queen turnover and colony fission are not uncommon, one might expect the workers to pursue complicated strategies to enhance their chances of direct reproduction at some future time. We suspect therefore that the decision to accept a non-nestmate in such a situation would depend, not merely on genetic relatedness but on a number of complex factors. The observed mechanism of nestmate discrimination potentially permits such decisions to be made and for the aliens to be quickly integrated into the recipient group. In short, this is just the sort of mechanism one would expect if mutualism (Lin and Michener, 1972) has a prominent role in the evolution of Hymenopteran societies.

### 13. THE THEORY OF MUTUALISM AND THE GAMBLING HYPOTHESIS

This brings me to the theory of mutualism proposed by Lin and Michener (1972), who drew attention to a large number of primitively social insect species where genetic relatedness is very low and individual members probably get together for mutual benefit accrued on account of protection from parasites and predators. At first sight, it may appear that such a force cannot, however, lead to the stage of eusociality with sterile castes. West-Eberhard (1978) and Gadagkar (1985a) have suggested a related kind of hypothesis which can lead to the eusocial state. Consider a situation where two wasps nesting separately can produce 10 offspring each. If they nested jointly instead, let us say that they would, together, be able to rear not merely  $10 + 10 = 20$ , but 21 offspring - a slight advantage of group nesting. Now even if only one of them produced all of the 21 offspring but

the roles of egg-layer and non-egg-layer (worker) were decided by the toss of a coin (by chance), each wasp would on the average produce  $21/2 = 10.5$  offspring. A mutant genotype which is programmed to take the risk of joint nesting will thus do better on the average (by producing 10.5 offspring) than its wild type counterpart which will only produce 10 offspring. I call this the "Gambling" hypothesis. The consequence of this would be that we may keep seeing 50% of the individuals as sterile workers apparently programmed to be sterile by an allele and wonder how such an allele has spread in the population. In reality, the allele may only make its bearer gamble so that, on the average, half the gamblers will be queens and half will be workers. Since each gambler will, on the average, do better than a wild type risk-averse non gambling individual, the gambler allele will spread in the population. Notice that this hypothesis does not need close genetic relatedness between joint-nesters nor does it need an ability to discriminate between different levels of genetic relatedness.

#### 14. CONCLUDING REMARKS

I would like to argue that simultaneous polygyny in *R. cyathiformis* (Gadagkar and Joshi, 1982a), serial polygyny due to queen supercedure in *R. marginata* (Gadgkar, R. and Chandrashekara, K., unpublished observations), the causes and consequences of colony fission in *R. cyathiformis* (Gadagkar and Joshi, 1955), the complex behavioural caste differentiation in *R. marginata*, *R. cyathiformis* (Gadagkar and Joshi, 1982b, 1983, 1984), and in *P. veriscolor* (this paper) all add upto make mutualism and Gambling plausible models for the origin of sociality in primitively eusocial species such as *R. marginata* and *R. cyathiformis*. Parental manipulation or other factors leading to subfertility, play some role but haplodiploidy probably is less important than has been thought so far. I would also like to suggest that insect sociality, in general, originates because of the benefits of mutualism and gambling rather than due to haplodiploidy but that haplodiploidy is probably important in reaching and maintaining the highly eusocial state represented by honeybees and most ants.

#### 15. SUMMARY

The last two decades have seen a tremendous interest in primitively eusocial insect societies. Much of this interest can be attributed to a large body of theory which attempts to explain the evolution of social behaviour through the process of natural selection. Tropical social wasps are perhaps ideal model systems for this purpose. The Indian sub-continent is blessed with representatives of all the three sub-families of social wasps viz., Stenogastrinae, Polistinae and Vespinae and also representatives of all the three tribes of Polistinae viz., Ropalidini, Polistini and Polybini. It is rather unfortunate, however, that our social wasp fauna is one of the most poorly

studied in the world. Modern taxonomic work is represented almost only by the work of van der Vecht. As far as biology and behaviour are concerned, there is little more than a few scattered notes and incidental observations: A detailed programme of investigations of social organisation in two species of the genus *Ropalidia* was initiated at the Indian Institute of Science, Bangalore some years ago. The emphasis of this programme has been on quantitative ethology although some biochemical and other kinds of techniques have also been used. An attempt to provide empirical data bearing on different theories for the evolution of social behaviour has been the explicit objective of this programme. Studies on multiple mating and kin recognition suggest that the form of kin selection which depends on the genetic asymmetries created by haplodiploidy is unlikely to be important. An investigation of reproductive potentials has shown the existence of some pre-imaginal caste bias suggesting that parental manipulation may have a role. Behavioural studies have revealed a rich array of complex behavioural strategies and sufficient behavioural diversity so that mutualism and reciprocity suggest themselves as possible avenues for future exploration.

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## REFERENCES

- Alexander, R.D., 1974. *Annu. Rev. Ecol. Syst.*, 5 : 325-383.  
Altmann, J., 1974. *Behaviour* 49 : 227-267.  
Anderberg, M.R., 1973. Cluster analysis for applications, New York Acad. Press.  
Andersson, M., 1984. *Annu. Rev. Ecol. Syst.* 15 : 165- 189.  
Belavadi, V.V. and Govindan, R., 1981. *Colemania*, 1 : 95-102.  
Brockmann, H.J., 1984. The evolution of social behaviour in insects; in *Behavioral Ecology* (eds) J.R. Kerbs and N.B. Davies (Oxford, London: Blackwell Scientific Publications) Chap.13 : 340-361.  
Chandrashekara, K. and Gadagkar, R., 1990. *this volume*.

- Craig, R., 1980. *J. theor. Biol.*, **87** : 55-70.
- Das, B.P., 1982. Studies on Indian wasps belonging to the families Vespidae and Stenogastridae (Hymenoptera : Vespoidea); *Ph.D. Thesis, Delhi University*.
- Davis, T.A., 1966a. *Nature*, **210** : 966-967.
- Davis, T.A., 1966b. *Entomol. News* **77** : 271-277.
- De Gheff, V.J., 1978. Hierarchical cluster analysis; in *Quantitative Ethology* (ed) P.W. Colgan (New York : John Wiley and Sons) : 115-144.
- Frey, D.F. and Pimentel, R.A., 1978. Principal component analysis and factor analysis; in *Quantitative Ethology* (ed) P.W. Colgan (New York : John Wiley and Sons) : 219-245.
- Gadagkar, R., 1980. *Curr. Sci.*, **49** : 772-775.
- Gadagkar, R., 1985a. *Proc. Indian Acad. Sci. (Anim. Sci.)* **94** : 309-324.
- Gadagkar, R., 1985b. *Proc. Indian Acad. Sci. (Anim. Sci.)* **94** : 587-621.
- Gadagkar, R., 1987. Social structure and the determinants of queen status in the primitively eusocial wasp *Ropalidia cyathiformis*; in *Chemistry and Biology of Social Insects Proc. X Internatl. Congr. IUSSI, Munich* (Eds) J. Eder and H. Rembold *Verlag J. Peperny* : 377-378.
- Gadagkar, R., Gadgil, M. and Mahabal, A.S., 1982a. Observations on population ecology and sociobiology of the paper wasp *Ropalidia marginata* (Lep.) (Family : Vespidae); *Proc. Symp. Ecol. Anim. Popul. Zool. Surv. India Pt. 4* : 49- 61.
- Gadagkar, R., Gadgil, M., Joshi, N.V. and Mahabal, A.S., 1982b. *Proc. Indian. Acad. Sci. (Anim. Sci.)* **91** : 539-552.
- Gadagkar, R. and Joshi, N.V., 1982a. *J. Zool. Lond.* **98** : 27-37.
- Gadagkar, R. and Joshi, N.V., 1982b. A comparative study of social structure in colonies of *Ropalidia*; in *The Biology of Social Insects, Proc. of the Ninth Congress of the International Union for the Study of Social Insects.* (Eds.) M.D. Breed, C.D. Michener and H.E. Evans (Boulder, Colorado, August 1982, Westview Press) : 187-191.
- Gadagkar, R. and Joshi, N.V., 1983. *Anim. Behav.*, **31** : 26-31.
- Gadagkar, R. and Joshi, N.V., 1984. *Z. Tierpsychol.*, **64** : 15-32.
- Gadagkar, R. and Joshi, N.V., 1985. *Curr. Sci.*, **54** : 57-62.
- Gadagkar, R., Vinutha, C., Shanubhogue, A. and Gore, A.P., 1988. Pre-imaginal biasing of caste in a primitively eusocial insect; *in press*.
- Gadgil, M. and Mahabal, A., 1974. *Curr. Sci.*, **43** : 482.
- Gupta, V.K. and Das, B.P., 1977. *Entomol.* **2** : 209- 213.
- Hamilton, W.D., 1964a. *J. theor. Biol.*, **7** : 1-16.
- Hamilton, W.D., 1964b. *J. theor. Biol.*, **7** : 17-52.
- Ito, Y., 1984. *Insectes Soc.*, **31** : 103-111.
- Jeanne, R.L., 1972. Social biology of the neotropical wasp *Mischocyttarus drewseni*; *Bull. Mus. Comp. Zool. Harv. Univ. Cambridge, Massachusetts, USA* **144** : 63-150.
- Jeanne, R.L., 1980. *Annu. Rev. Entomol.*, **25** : 371- 396.
- Joshi, N.V. and Gadagkar, R., 1985. *J. Genet.*, **64** : 41-58.

- Leigh, E.G. Jr., Rand, A.S. and Windsor, D.M., 1982. The ecology of a tropical forest, seasonal rhythms and long-term changes; *Washington D.C. Smithsonian Institution Press*.
- Lin, N. and Michener, C.D., 1972. *Q. Rev. Biol.*, **47** : 131-159.
- Litte, M., 1977. *Behav. Ecol. Sociobiol.*, **2** : 229- 246.
- Litte, M., 1979. *Z. Tierpsychol.*, **50** : 282-312.
- Litte, M., 1981. *Smithson. Contrib. Zool.*, **327** : 1- 28.
- Metcalf, R.A. and Whitt, G.S., 1977. *Behav. Ecol. Sociobiol.*, **2** : 339-351.
- Muralidharan, K., Shaila, M.S. and Gadagkar, R., 1986. *J. Genet.* **65** : 153-158.
- Richards, O.W., 1962. A revisional study of the Masarid wasps (Hymenoptera : Vespoidea); *British Museum, London*, : 1-294.
- Richards, O.W., 1978a. The Australian social wasps (Hymenoptera : Vespidae); *Aust. J. Zool. Suppl. Ser.* **61** : 1-132.
- Richards, O.W., 1978b. The social wasps of the Americas excluding the vespinae; *British Museum (Natural History) London*.
- Seger, J., 1983. *Nature*, **301** : 59-62.
- Shellman, J.S. and Gamboa, G.J., 1982. *Behav. Ecol. Sociobiol.*, **11** : 51-53.
- Starr, C.K., 1979. Origin and evolution of insect sociality : a review of modern theory; in *Social Insects. Vol.1* (ed) H.R. Hermin (New York: Academic Press) : 35-79.
- Strassmann, J.E. and Meyer, D.C., 1983. *Anim. Behav.* **34** : 431-438.
- Stubblefield, J.W. and Charnov, E.L., 1986. *Heredity*, **55** : 181-187.
- Vecht, J. van der, 1941. *Treubia*, **18** : 103-190.
- Vecht, J. van der, 1957. *Zool. Verh., Leiden*, **34** : 1- 83.
- Vecht, J. van der, 1962. *Zool. Verh., Leiden*, **57** : 1- 72.
- Vecht, J. van der, 1966. *Zool. Verh., Leiden*, **82** : 1- 42.
- Venkataraman, A.B., Swarnalatha, V.B., Nair, P., Vinutha, C. and Gadagkar, R., 1990. *this volume*.
- West-Eberhard, M.J., 1969. The social biology of Polistine wasps; *Univ. Mich. Mus. Zool. Misc. Publ.* **140** : 1-101.
- West-Eberhard, M.J., 1975. *Q. Rev. Biol.*, **50** : 1-33.
- West-Eberhard, M.J., 1978. *J. Kans. Entomol. Soc.*, **51** : 832-856.
- Wilson, E.O., 1971. The insect societies; *Cambridge Massachusetts : Harvard University Press*.
- Wilson, E.O., 1975. Sociobiology; *Cambridge, Massachusetts : Harvard University Press*.