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QUANTITATIVE ETHOLOGY OF SOCIAL WASPS: TIME–ACTIVITY BUDGETS AND CASTE DIFFERENTIATION IN *ROPALIDIA MARGINATA* (LEP.) (HYMENOPTERA: VESPIDAE)

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Abstract. Time–activity budgets of several individually identified members of *Ropalidia marginata* colonies have been constructed with the aim of studying caste differentiation in social wasps that show no morphological differences between individuals. Analysis of these data by multivariate statistical techniques including principal components analysis and hierarchical cluster analysis has demonstrated the presence of three different behavioural castes which we have named Sitters, Fighters and Foragers. The Sitters in a colony consist of the queen and also some non-egg-laying individuals. The Fighters are non-egg-layers that show alarm reactions in response to disturbances and also fight with other individuals on the nest to a very large extent. The Foragers are also non-egg-layers and they spend a large proportion of their time making trips to places away from the nest to collect food, building material etc.

The social insects comprising ants, bees, wasps and termites are remarkable in that they exhibit extreme degrees of social organization and division of labour within members of a social group or colony. The present day species of bees and wasps exemplify a series of stages in the spectrum of social organization and caste differentiation from solitary to eusocial. In insect societies, a caste is defined as a set of individuals that are both morphologically distinct and specialised in behaviour (Wilson 1971). By this definition, the caste systems of social wasps (Vespidae) are often not as highly developed as those of the other social insects (West-Eberhard 1969; Jeanne 1972; Litte 1977). Morphological differentiation is not common and when present has a simple nutritional basis (Wilson 1971).

However, even in the complete absence of morphological differentiation, there could be behavioural differences between the members of a colony. At a very primitive level of division of labour, it is probable that the behavioural repertoire of all the individuals would be similar and the differences quantitative rather than qualitative. Such differences could be best detected by studying how each individual allocates its time between the different possible behaviour patterns. Hence, we have constructed time-activity budgets for individually identified members of *R. marginata* colonies and looked for evidence of behavioural caste differentiation.

Ropalidia marginata is a common paper wasp in India that builds small open nests on the eaves of undisturbed buildings or on the twigs of cypress bushes (*Cupressus sempervirens*) (Gadagkar 1980). The nests contain up to 100 adults, although smaller nests (< 10 adults) are more common. Small nests have a single egg-layer whereas large nests tend to have several egg-layers. No morphological differences are noticeable between egg-layers and non-egg-layers or between any of the adults on a nest, though individuals with well-developed ovaries tend to be among the heavier individuals (Gadgil & Mahabal 1974; Gadagkar et al., unpublished observation).

In this paper we show that analysis of the time–activity budgets of the adults of *R. marginata* colonies yields three distinct behavioural castes which we have named Sitters, Fighters and Foragers after their distinguishing attributes.

Subjects and Methods

A. Study Animal

The study was conducted on two small post-emergence nests of *R. marginata* (the total number of adults per nest varied between four and 40 during the period of study) built on cypress bushes (*Cupressus sempervirens*) in Cubbon Park in the city of Bangalore (13°00′ N and 77°32′ E), India, between November 1979 and April 1980. All the adults in the colonies were individually labelled by marking them with one or more spots of aeroplane paint of different colours on different parts of the body. The marking was done immediately after the emergence of an adult. A census of all the wasps present at the nest was taken at about 0700 hours since none of them was ever

seen to leave the colony before this time during preliminary ad libitum observations. Both the colonies had a single egg-layer (queen) each during the entire period of study. Males disappeared from the colony within a few days after emergence. Thus all the data in this paper pertain to the females.

B. Sampling Methods

Four kinds of sampling methods were used in the study (Altmann 1974).

(1) **Ad libitum sampling** was employed for constructing an ethogram, i.e. we compiled a descriptive catalogue of the behavioural repertoire of the species to get preliminary information on the basis of which subsequent sampling methods were chosen.

(2) **Instantaneous scanning** of the behavioural states of all animals in a colony was performed at 134 randomly chosen times during the period of study.

(3) **All occurrences of rare behaviours** were noted. Some behaviours that appeared to be relatively rare on the basis of ad libitum sampling were recorded in 100 separate 5-min sessions during which all occurrences of each such behaviour by every animal in the colony were recorded. Here every behaviour was treated as an event and no information on its duration was recorded.

All sampling sessions were begun and terminated by time-contingent rules using a stop watch accurate to 0.1 s. Observations were made for a total of 160 h. Ad libitum observations showed that the wasps were relatively inactive between 1800 and 0900 hours. Hence all sampling sessions were randomly chosen between 0900 and 1800 hours. All data were recorded on coding sheets in an 80-column format ready to be punched on computer cards. The data were analysed using a DEC 1090 computer at the Indian Institute of Science, Bangalore, India.

C. Analysis of Data

(1) **Behavioural repertoire.** The behavioural repertoire of *R. marginata* was classified into 37 distinct behavioural categories (Gadagkar, unpublished data; Gadagkar and West-Eberhard, in preparation). However, data on the following 11 categories alone were analysed in this study.

(i) **Sit and Groom.** By sitting is meant simply sitting quietly without doing anything in particular and without being alert to any external disturbance. In this position the wasps sit with their bodies held compactly in one plane and their legs and wings drawn close to the body with the antennae lowered. Grooming is always self-grooming and no allogrooming has been observed. The most frequent forms of grooming involve rubbing posterior legs against each other and against wings and abdomen, anterior legs against mouth parts, antennae and head, and antennae against mouth parts.

(ii) **Raise Antennae.** This involves sitting with wings drawn close to the body but the antennae raised above the body plane. There is a transition from Sitting to Raising Antennae when there is any disturbance.

(iii) **Raise Wings.** This involves sitting with both antennae and wings raised above the body plane. The legs are either folded or stretched so as to raise the body above the substratum. Raising Antennae is followed by Raising Wings if the disturbance continues.

(iv) **Walk.** The wasps walk from the face of the nest and back; when they walk they sometimes reach different cells in the nest and sometimes reach other adults sitting on different parts of the nest.

(v) **In Cells.** The wasps get inside the cells with only their head or the entire body either simply 'inspecting' the contents or receiving secretions from the larvae or transferring liquid to larvae.

(vi) **Absent from Nest.** Absence from the nest is considered here as one category although a wasp temporarily absent from the nest may return with food, building material, liquid or nothing.

(vii) **Bring Food.** As mentioned earlier the wasps sometimes bring back food, building material or liquid.

(viii) **Attack.** The wasps fight with each other. Attacking involves climbing over the opponent and sometimes chewing the dorsal part of its body, but more often bending itself over the head of the opponent and biting its mouth parts.

(ix) **Attacked.** The wasp that is being attacked is very subdued and keeps its body stiff with antennae, legs and wings all drawn close to the body.

(x) **Snatch Food.** This is the act of acquiring solid food from another individual. The other individual is said to **(xi) Lose Food.**

(2) **Time-activity Budgets.** With the data available it was possible to calculate the time–activity budgets of 20 wasps derived from the two

colonies for the six activities, Sit and Groom, Raise Antennae, Raise Wings, Walk, In Cells and Absent from Nest.

(3) Frequencies of rare behaviour. For behaviour that tended to occupy a very small proportion of time as observed from the scans, and for behaviours that are better treated as events rather than states, frequencies were computed from the all-occurrences recording sessions. These behaviours included Bring Food, Attack, Attacked, Snatch Food and Lose Food.

(4) Principal components analysis. From the time–activity budgets, every individual can be characterized by the proportion of time it spends in each of the six different behaviours. The method of principal components analysis (Frey and Pimental 1978) yields a new set of uncorrelated variables. Moreover, the new variables are maximally powerful in bringing out the differences between individuals in the sense that, of all the possible linear combinations, the proportion of total variance accounted for by each of the new variables is maximal (Anderberg 1973). It is often possible that only a small number of these new variables account for almost all the variance between the individuals.

(5) Hierarchical cluster analysis. Using the proportion of time spent in the six activities by different individuals as input data, Pearson product moment correlation was obtained as an index of similarity between pairs of individuals. Using these indices of similarity, a hierarchical cluster analysis was performed with the single linkage algorithm (DeGhett 1978).

Results

Time–activity budgets calculated for 20 individuals reveal that adults of *R. marginata* spend 85–100% (mean $\pm$ SD = 95.9 $\pm$ 4.4) of their time in the six activities Sit and Groom, Raise Antennae, Raise Wings, Walk, In Cells and Absent from Nest. However, the manner in which a given wasp allocates its time among these six activities is highly variable. For example, the queens (individuals 1 and 14) spend no time at all in Absence from Nest and among the other individuals; the time allotted to this activity varies from 4–69%. Similarly, the time spent in Sit and Groom varies from 7–56%, with the queen representing the highest value.

The results of principal components analysis performed using data on the time–activity budgets of 20 wasps are presented in Table I. The first principal component accounts for 72.3% of the total variance with Absence from Nest as its dominant term (weightage = 0.8289). The second principal component, whose dominant term is Raise Antennae (weightage = 0.8219), accounts for 20.2% of the total variance. Since the first two components together account for 92.5% of the total variance, we have represented each individual as a point in the coordinate space of the associated amplitudes of these two principal components. As seen from Fig. 1, the points fall into three obvious clusters. This has been confirmed by the method of nearest centroid; the distance between any individual and the centroid of the cluster to which it belongs is less than its distance from the other two centroids. Individual 13 alone does not fall into any of the three clusters. This we believe is because most of the data on this animal was collected when the nest was in the process of being abandoned because of extensive predation by *Vespa tropica*, a common predator of *R. marginata* colonies.

It must be emphasized here that the three clusters have emerged as a result of an objective analysis of the data in as much as no prior

Table I. Eigenvectors of Principal Components, Eigenvalues, Percentage of Variance, and Cumulative Percentage of Variance

	Principal components			
Behaviour	1	2	3	4
Sit and Groom	−0.5305	−0.5025	0.0118	0.5835
Raise Antennae	−0.1056	0.8219	−0.1701	−0.4060
Raise Wings	0.0152	0.1012	0.9052	0.1252
Walk	−0.1343	−0.0690	−0.2960	0.6004
In Cells	−0.0457	−0.0524	−0.2357	0.1169
Absent from Nest	0.8289	−0.2328	−0.0917	−0.3238
Eigenvalue	1.27×10^4	3.54×10^3	7.33×10^2	4.34×10^2
Variance (%)	72.30	20.20	4.18	2.48
Cumulative variance (%)	72.30	92.50	96.68	99.16

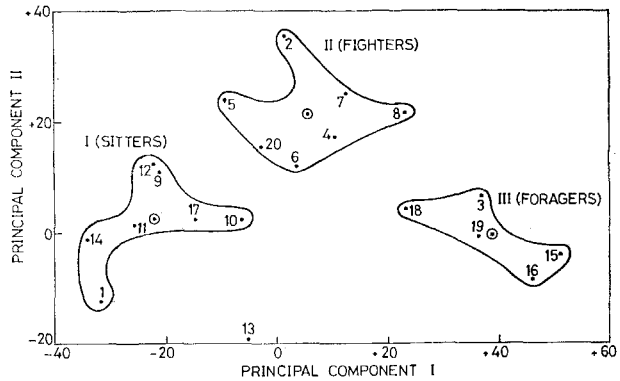

Fig. 1. Behavioural castes of *R. marginata*. Twenty wasps are shown as points in the coordinate space of the amplitudes associated with the first two principal components. The points fall into three clusters (or castes) by the criterion of nearest centroid. Circled dot = centroid.

assumptions were made regarding the criteria to be used for classification or the number of clusters required.

Absence from Nest is the dominant term in the first principal component, and clusters I and III thus represent two extremes for this activity, while cluster II is intermediate. Similarly, Raise Antennae is the dominant term in the second principal component and the members of cluster II are thus different from those of clusters I and III in the time spent with Raised Antennae. An independent method of classification, namely hierarchical cluster analysis using the Pearson product moment correlation as an index of similarity between individuals also gives identical clusters (Fig. 2). Individual 13 is again separated from all the others and one can recognize three clusters with the same composition as the clusters obtained from the principal components analysis. Although this method does not permit us to identify the distinguishng features of each cluster, we would like to interpret the complete concurrence of the two methods as an indication of the robustness of the clusters.

The mean profiles of the three clusters with reference to the six activities used in the classification are shown in Fig. 3A. Figure 3B shows the mean profiles of the same three clusters using the frequencies of the five other activities not used in the classification. Although it is not necessary for any one activity alone to show significant differences between the clusters (as the clusters have been obtained by the consideration of six activities simultaneously), it is obvious from Fig. 3A that the time spent in Sitting and Grooming, Raised Antennae, and Absence from the Nest are the most distinguishing attributes of clusters I, II and III respectively. The difference in the time spent in Raised Antennae between clusters I and II is rather small. On the other hand if we look at Fig. 3B, a high frequency of Attacking 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 and its frequency of attacking

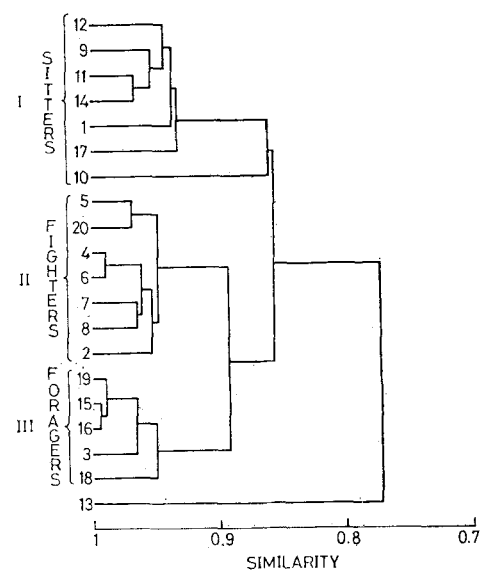

Fig. 2. Hierarchical cluster analysis of 20 adults of *R. marginata* numbered as in Fig. 1. The similarity between individuals shown in the Pearson product moment correlation calculated using the percentage of time spent in six activities as input data. The method of single linkage algorithm has been used in clustering.

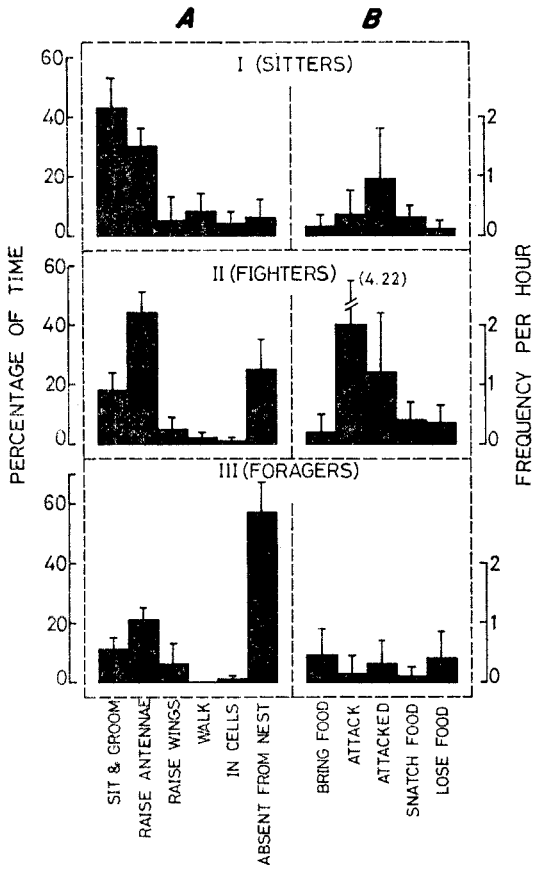

Fig. 3. Mean behavioural profiles of the clusters obtained in Figs 1 and 2. A, mean percentages of time spent in each of the six activities that were used in obtaining the clusters are shown for Sitters, Fighters and Foragers. B, mean frequencies per hour of the five activities that were not used to obtain the clusters are shown for Sitters, Fighters and Foragers.

other individuals ($P < 0.01$). Hence we wish to consider Attacking or Fighting as the distinguishing attribute of cluster II. Wasps in cluster III spend a great deal of time being Absent from the Nest. Because these wasps often bring back food loads or building material (confirmed by casual observations on wasps away from their nests), we consider Foraging as the distinguishing attribute of cluster III. Thus we see that the adults of R. *marginata* colonies can be classified into three behavioural castes which can be called Sitters, Fighters and Foragers.

Discussion

Multivariate analysis of data on time–activity budgets of individually identified members of colonies of the social wasp *Ropalidia marginata* reveals the presence of three behavioural clusters despite the absence of any obvious morphological caste differentiation. These three behavioural clusters have been named Sitters, Fighters and Foragers on the basis of their most distinguishing features.

Sitters are those that spend much more time Sitting and Grooming than others. They do little or no foraging and seldom fight either with members of their own group or those of other groups (Fig. 4). It is important to note that the queens of both the colonies (individuals 1 and 14) belong to this group. However, there are other non-egg-laying members of this group. Whether these are 'hopeful queens' who may still have some chances or reproducing on their own (see West-Eberhard 1978), or naive workers yet to be recruited into the worker force, is not clear at present. These questions are being investigated by queen removal experiments.

Fighters are individuals that spend a large proportion of their time with Raised Antennae, and they also Attack other individuals frequently. They show the highest frequency of attacking other members of their own group and a lower frequency of attacking a Sitter or a Forager (Fig. 4). 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 investigator or by tachinid flies known to parasitize their brood (unpublished observations). It is conceivable that Attacking induces the other

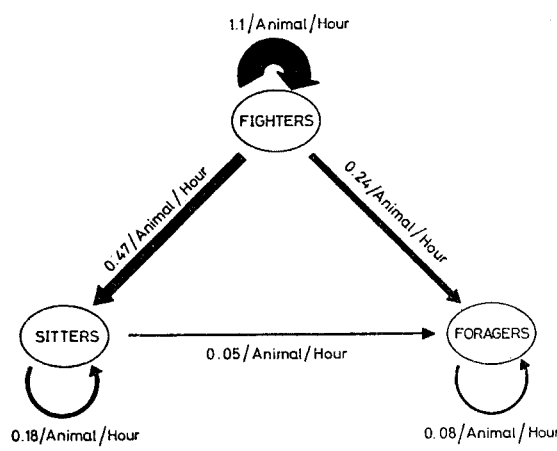

Fig. 4. Mean attacking frequencies within and between Sitters, Fighters and Foragers.

members of the colony to work. We have sometimes observed wasps leave the nest as a result of repeated attacks from nest mates and later return with food or building material. 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 Attacking and that of Snatching Food ($P < 0.01$) (see also Gadagkar 1980). Fighters may also have some chance of becoming egg-layers if the colony becomes large and polygynous or if some accident were to befall the queen. In *Mischocyttarus mexicanus*, for example, the next most dominant individual becomes the egg-layer if the queen is removed (Litte 1977). This is one possible reason why Fighters show the highest frequency of attacking another Fighter (Fig. 4). By means of attacking, a linear dominance hierarchy can be recognized among the members of a colony (Gadagkar 1980). In one instance we observed that a queen had disappeared from a nest and the most dominant individual of the remaining wasps immediately began to lay eggs (unpublished observations).

Foragers constitute the principal worker force of a colony. They show the lowest frequencies of Sitting and Grooming, Attacking and being Attacked (see also Fig. 4). Thus, they do not seem to be involved in reproductive competition with their nest mates and we suggest that they have the least chance of becoming egg-layers.

It should be pointed out that egg-laying itself was not used as one of the activities in the present analysis. This is because egg-laying occupies an extremely small proportion of the time available to a wasp and we were more interested in classifying individuals according to the manner in which they allocated their time between different activities. Moreover, differentiation into reproductive and non-reproductive castes is very well established in all eusocial insects. On the other hand, the fact that both the queens fall in the same cluster in spite of egg-laying not being included in the analysis can be considered as evidence of the biological significance of the kind of behavioural classification we have attempted.

The results of this study are remarkably similar to those of the only other similar study concerning social insects. Brothers and Michener (1974) collected behavioural data on the primitively social bee *Lasioglossum zephyrum* and subjected them to principal components analysis. Their results show that the bees can also be clustered into three groups which they call queens, guards and workers. It is interesting to speculate that the queens, guards and workers of *L. zephyrum* correspond to the Sitters (with queens as some of their members), Fighters (that also spend the maximum time with Raised Antennae) and Foragers respectively of *R. marginata*.

Further work is in progress to elucidate the effects of age of the animals and stage of colony development on such a behavioural caste differentiation.

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