J. Biosci., Vol. 4, Number 2, June 1982, pp. 209-218. © Printed in India.

Theoretical studies on β-lactam antibiotics VI*: Conformational analysis and structure-activity relationships of penicillin sulfoxides and cephalosporins

N. V. JOSHI** and V.S.R. RAO

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012

Abstract. Conformational energy calculations were carried out on penicillin α- and β-sulfoxides and Δ^2- and Δ^3-cephalosporins, in order to identify the structural features governing their biological activity.

Results on penicillin β-sulfoxide indicated that in its favoured conformation, the orientation of the aminoacyl group was different from the one required for biological activity. Penicillin α sulfoxide, like penicillin sulfide, favoured two conformations of nearly equal energies, but separated by a much higher energy barrier. The reduced activity of the sulfoxides despite the nonplanarity of their lactam peptide indicated that the orientations of the aminoacyl and carboxyl groups might also govern biological activity.

Δ^3- cephalosporins favoured two conformations of nearly equal energies, whereas Δ^2-cephalosporins favoured only one conformation. The lactam peptide was moderately nonplanar in the former, but nearly planar in the latter. The differences in the preferred orientations of the carboxyl group between penicillins and cephalosporins were correlated with the resistance of cephalosporins to penicillinases.

Keywords. Cephalosporins; penicillin sulfoxides; conformational analysis; structure activity relations.

Introduction

Penicillins, one of the most widely used anti-microbial agents, is known to act by inhibiting the enzyme(s) transpeptidase(s) and/or carboxypeptidase(s), which bring about the cross-linking reaction in peptidoglycan biosynthesis (Blumberg and Strominger, 1974). It has been suggested (Tipper and Strominger, 1965) and subsequently shown theoretically (Virudachalam and Rao, 1977) that penicillin is a structural analog of the natural substrate X-D-Ala-D-Ala.

The widespread use of penicillins has lead to the proliferation of penicillin resistant bacteria: these contain enzymes (penicillinases) which inactivate penicillins by hydrolysing the lactam peptide bond of the drug. Hence, a search has been on for developing drugs active against penicillin resistant bacteria. Cephalosporins, a class of compounds similar to penicillins, have been found to be

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* Contribution number 158 from Molecular Biophysics Unit

** Present Address: Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560 012.

resistant to the action of penicillinases, though their activity is low compared to penicillins. A detailed conformational analysis of cephalosporins, and their comparison with penicillin and its derivatives, is likely to throw light on the stereochemical basis of the resistance of cephalosporins towards penicillinases and their reduced antimicrobial activity.

It has also been observed that minor substitutions in the thiazolidine ring (as in penicillin α- and β- sulfoxides), can drastically reduce the biological activity: the reasons for this, however, are not clearly understood. In order to elucidate the structure-activity relationships in β-lactam antibiotics, conformational analysis of penicillin α- and β-sulfoxides has also been carried out, in addition to Δ^3- and Δ^2-cephalosporins.

Energy calculations

Choice of parameters

The molecule of penicillin sulfoxide is shown in figure 1, and the parameters used to describe it are shown in figure 2. Conformation of the thiazolidine ring is

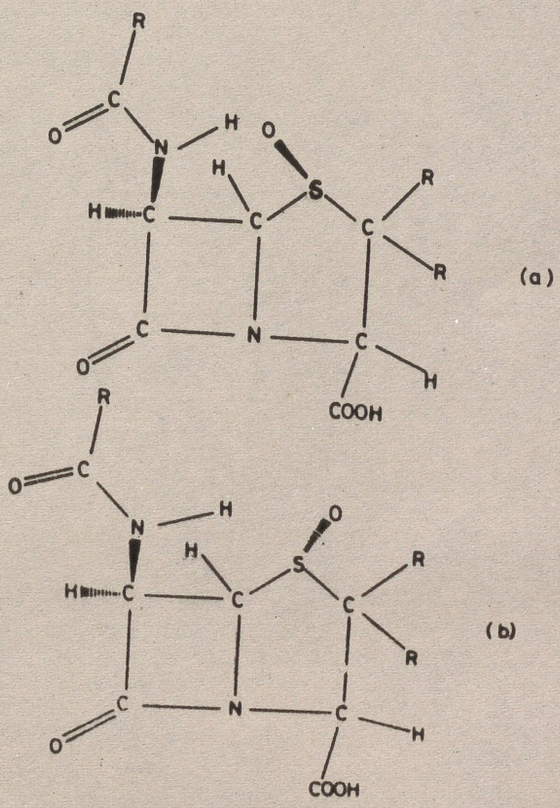

Figure 1. Structure of penicillin sulfoxide.

a . Penicillin β-sulfoxide.

b Penicillin α-sulfoxide. R denotes a methyl group.

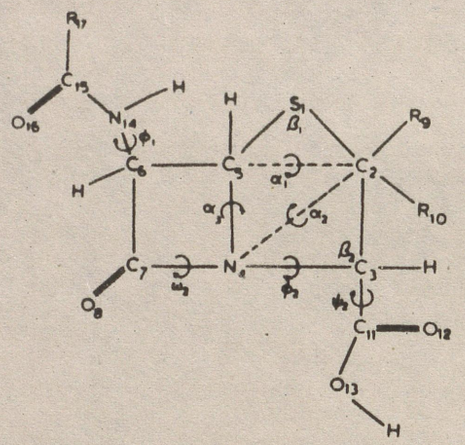

Figure 2. Numbering of atoms and conformational parameters for the thiazolidine ring. R denotes a methyl group.

described in terms of the two dihedral angles α_1 and α_2, denoting rotations about the virtual bonds C-5—C-2, C-2—N-4 respectively. The angle α_3 denotes the relative orientation of the lactam ring (assumed planar) with the C-5-C-2-N-4 plane. The aminoacyl group was fixed using the torsional angle θ_1(C-15-N-14-C-6-C-7). Conformation of the sulfide molecule was thus completely specified by the parameters shown in figure 2. The bond lengths were kept constant throughout the calculations, using crystal structure values.

Figures 3 and 4 depict Δ^3- and Δ^2-cephalosporin respectively. The parameters used to describe the conformation of the six membered dihydrothiazine ring are

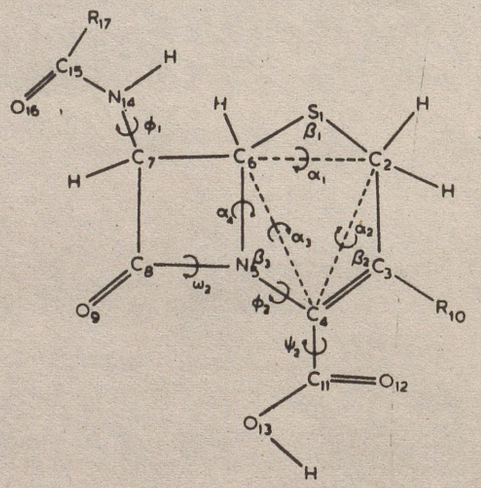

Figure 3. Conformational parameters for Δ^3-cephalosporin. R denotes a methyl group.

similar to those for the sulfoxide and are also shown in figures 3 and 4. Thus, α_1, α_2 and α_3 describe the six membered ring, while α_4 denotes the relative orientations between the lactam ring and the C-4-N-5-C-6 plane. The bond lengths were kept constant at the values obtained from crystal structure. The angle at the aminoacyl end, θ_1 was also kept constant at 160°, as it is energetically favoured, and also because it is unlikely to be affected by changes in the conformation of the dihydrothiazine ring. ψ_2 was kept constant at 30° as observed in simple peptides (Virudachalam and Rao, 1977).

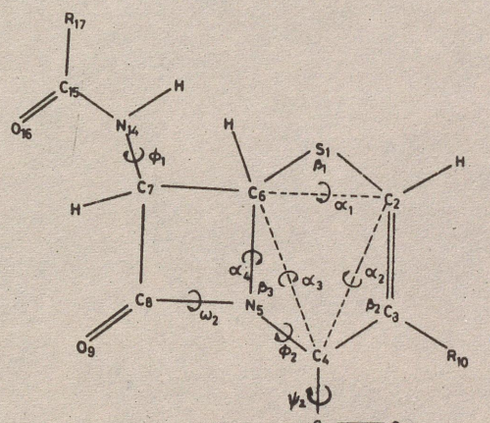

Figure 4. Conformational parameters for Δ^2-cephalosporin. R denotes a methyl group.

Conformational energy calculations

The total conformational energy was computed taking contributions from electrostatic and nonbonded interactions, as well as from bond angle and torsional angle distortions. The fractional charges on the atoms were taken to be the sum of the σ-*charges* (obtained by Del Re's (1958) method and π- charges (obtained using Huckel M O theory). Kitaigorodosky's (1961) functions were used to compute the nonbonded interaction energy. In the case of sulfoxides, to estimate the energy of hydrogen bond formation between the sulfoxide oxygen and aminoacyl N-H, the function proposed by Momany *et al.* (1975) was used. The other functions, as well as all the constants used in the present work, have been described earlier (Joshi and Rao, 1979; Joshi, 1980).

It is known from the earlier studies (Joshi *et al.*, 1978) that varying α_1 and α_2 over the range $-70°$ to 70° is adequate for sampling all the sterically allowed conformations of the thiazolidine ring. Hence, in the present work, α_1 and α_2 were varied over this range at 10° intervals. At every grid point (α_1, α_2), conformational energy was minimised with respect to β_1, β_2, α_3 and θ_1. Isoenergy contours were drawn in the α_1-α_2 plane.

Three variables (α_1, α_2 and α_3) are required to specify the conformation of the six membered dihydrothiazine ring in cephalosporins. However, due to the double bond, for a given (α_1, α_3), the range of α_2 is considerably restricted. Hence, in the present work, α_1 and α_3 were varied over a range $-70°$ to $70°$. At every grid point (α_1, α_3), α_2 was varied over a restricted range, and at each point, the energy was minimised with respect to the bond β_1, β_2, β_3 and with respect to the angle between the rings, α_4. Conformational energy surface of the dihydrothiazine ring was represented by isoenergy contours in the α_1-α_3 plane. A point on such a map corresponds to a conformation whose energy has been minimized with respect to β_1, β_2, β_3, α_4 and α_2. To indicate the small but significant variations in α_2 for a few low energy conformation the values of α_1, α_2, α_3 and the conformational energies were shown in tables 2 and 3 for Δ^3- and Δ^2-cephalosporins respectively.

Results and discussions

The conformational energy map of penicillin α-sulfoxide is shown in figure 5a. There are two minima, the global minimum occurs at $(\alpha_1, \alpha_2)=(40°, 15°)$ and a local minimum at $(-20°, -25°)$, which is about 0.5 Kcal. mol^{-1} higher in energy than the former. The global minimum corresponds to the C_2 puckered conformation of the thiazolidine ring, and the local minimum to the C_3 puckered conformation. For penicillin α-sulfoxide, no hydrogen bond is possible between the sulfoxide oxygen and aminoacyl N-H.

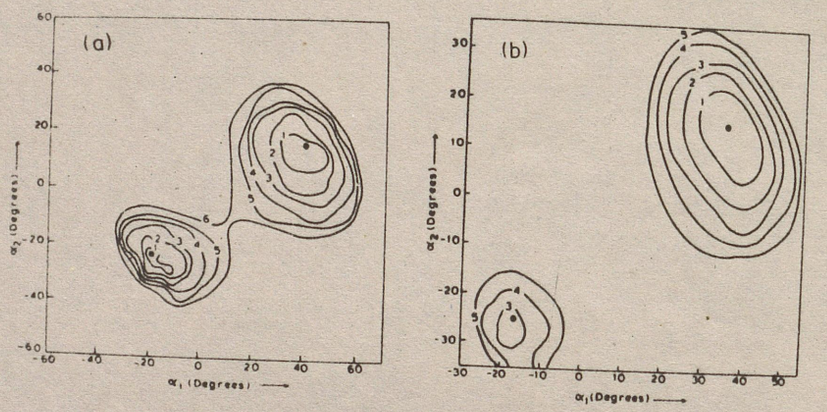

Figure 5. Conformational energy map.

a. Penicillin α sulfoxide.

b. Penicillin β sulfoxide.

Numbers on contours indicate energy in K cal mol^{-1}. Positions of the two minima are marked.

Table 1 shows the angles θ_1, θ_2 and ω_2 for penicillin sulfide, penicillin α-sulfoxide and penicillin β-sulfoxide. It is seen that for penicillin α-sulfoxide also, the lactam peptide is significantly nonplanar ($\omega_2 \sim 132°$) as in penicillin sulfide. Sweet and Dahl (1970) have proposed that the nonplanarity of the lactam peptide bond plays a

Table 1. Calculated dihedral angles of the amino acyl group, carboxyl group and the lactam peptide at the minimum energy conformations for penicillin sulfide and sulfoxides.

Conformation	Favoured orientation of aminoacyl group θ_1 (degrees)	Favoured orientation of carboxyl group [a] θ_2 (degrees)	Non-planarity of the lactam peptide ω_2 (degrees)
Penicillin sulfide C_2 puckered [b]	170	111	133
Penicillin sulfide C_3 puckered [b]	170	161	131
Penicillin α-sulfoxide C_2 puckered	170	108	131
Penicillin α-sulfoxide C_3 puckered	170	157	132
Penicillin β-sulfoxide C_2 puckered	−120	110	131
Penicillin β-sulfoxide C_3 puckered	−75	157	133

[a] $\psi_2 = -30°$ (see text.)
[b] (Joshi *et al.*, 1978).

major role in governing the biological activity of these antibiotics. By this criterion, penicillin α-sulfoxide should be as active as penicillin G or V; on the contrary, the sulfoxides are known to be much less active compared to the sulfides (Gorman and Ryan, 1972).

As can be seen from figure 5a the energy barrier separating the two minima is about six Kcal. mol^{-1}, which is higher by about 2 Kcal. mol^{-1}, than that obtained for penicillin sulfide. As a result, the rate of interconversion between the C_2 and C_3 puckered forms is about 20 times slower for the sulfoxides compared to the sulfides. This suggests that in solution, as the population of the biologically active C_3 puckered conformation decreases due to the interaction with the cross-linking enzymes, the rate at which it is restored due to the conversion from C_2 to C_3 puckered conformation will be much slower than that for the sulfide. This may account for the reduced activity of penicillin α-sulfoxide.

In penicillin β-sulfoxide the global minimum (figure 5b) occurs at (35°, 15°) as in penicillin α-sulfoxide and in penicillin sulfide, and corresponds to the C_2 puckered conformation. There is a possibility of hydrogen bond formation between the sulfoxide oxygen and the aminoacyl N-H groups. This is in agreement with X-ray crystal structure studies (Copper *et al.*, 1969).

The local minimum occurs at $(-15°, -25°)$, and corresponds to the C_3 puckered conformation. In this conformation also, a hydrogen bond between sulfoxide oxygen and the aminoacyl N-H group is possible. However, this conformation has about 2.5 Kcal. mole^{-1} higher energy than the global minimum. The energy barrier separating the two minima is about 7 Kcal. mol^{-1}.

Table 1 shows that the lactam peptide bond is nonplanar ($\omega_2 = 131°$) in both the conformations. Thus, if nonplanarity of the lactam peptide alone is important for biological activity, penicillin sulfide and both the sulfoxides should show more or less the same degree of biological activity. The fact that the activity of β-sulfoxide is considerably lower than that of α-sulfoxide suggests that other conformational features (such as orientations of aminoacyl and carboxyl groups) in the molecule may also have an important role to play in the biological activity.

Since the C_2 puckered conformation of β-sulfoxide is 2.5 Kcal. mole^{-1} lower in energy than the C_3 puckered conformation, in solution the population of the C_3 puckered conformation would be negligible. In fact, from NOE studies (Cooper *et al.*, 1969) it has been shown that penicillin β-sulfoxide exists in solution in the C_2 puckered conformation, which is different from the one required for biological activity. In addition to this, in the minimum energy conformation, the aminoacyl group favours a conformation ($\theta_1 \sim -90°$) different from the biologically active one ($\theta_1 \sim 180°$). This explains the greatly reduced activity of penicillin β-sulfoxide compared to the α-sulfoxide.

Thus, on the basis of a detailed conformational analysis of the thiazolidine ring, the present study consistently explains both, the reduced biological activity of penicillin sulfoxides relative to the sulfides, and also the difference in the activities of the two sulfoxides.

The conformational energy map of Δ^3-cephalosporin is shown in figure 6a. The positions of minimum energy conformations, and the solid state conformation as

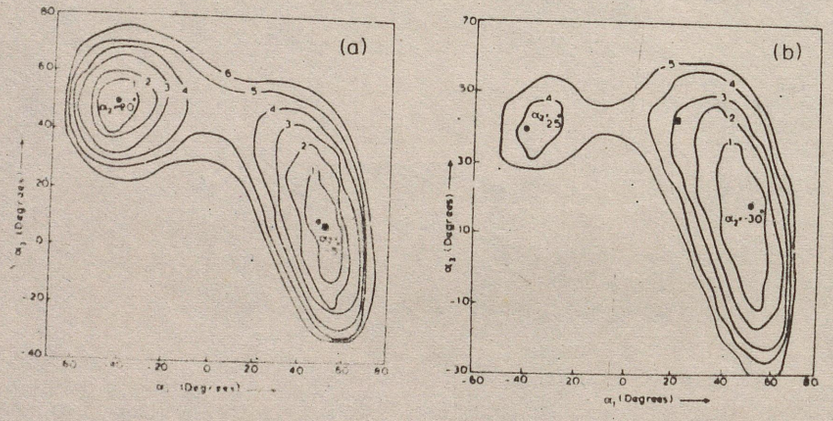

Figure 6. Conformational energy map.

a. Δ^1-cephalosporin and b. Δ^2-cephalosporin

Numbers on contours indicate energy in Kcal mol^{-1}. Positions of the two minima are marked. The value of α_2 (in degrees) at the minimum energy conformation is also shown in the figure. The solid state conformation is denoted by ■

observed in the crystal structure are also marked in the diagram. The global minimum occurs at (α_1, α_2, α_3) ($-40°$, $-20°$, $50°$). The local minimum occurs at ($50°$, $-5°$, $10°$) and is 0.4 Kcal. mole^{-1} higher in energy than the global minimum (table 2). Since this energy difference is small, the dihydrothiazine ring can

Table 2. Conformational angles and energies of some of the low energy conformations of Δ^3 cephalosporin.

No.	α_1 (degrees)	α_2 (degrees)	α_3 (degrees)	E Kcal. mol^{-1}
1	−40	−20	50	0.00 [a]
2	−50	−20	50	0.70
3	−40	−25	60	0.90
4	−30	−20	50	0.90
5	−40	−15	40	1.00
6	50	−5	10	0.40 [b]
7	50	−5	20	0.40
8	50	−5	0	0.70
9	60	−5	0	0.70
10	60	0	−10	0.90
11	60	0	10	1.10

[a] Global minimum. [b] Local minimum.

assume either of the puckered forms. The solid state conformation (Sweet and Dahl, 1970) corresponds to ($47°$, $-8°$, $8°$) and lies near the local minimum, indicating that the small energy difference may have been offset by lattice energy. The energy barrier separating the two minima is low (4-5 Kcal. mol^{-}), suggesting that the ring can easily flip over from one conformation to the other. Hence, in solution, both the conformations may exist in considerable proportions.

Recent molecular mechanics calculations of Boyd (1979), have also indicated that Δ^3-cephalosporin can exist in two minimum energy conformations, with the N_5-C_6-S_1-C_2 angle having values $51°$ and $-39°$. The present study shows that the values at the local and the global minima are $56°$ and $-21°$ respectively. However, according to Boyd's study, the latter conformation was about 2-4 Kcal. mol^{-1} higher in energy than the former, whereas the present study indicates that both have nearly equal energies. Since details of the molecular mechanics calculations were not reported, the reasons for the discrepancy between these two results cannot be discussed here.

The present study shows that the lactam peptide is more nonplanar at the global minimum ($\omega_2 \sim 161°$). However, both these are much less nonplanar than that in the penicillins ($\omega_2 \sim 130°$).

The orientations of the carboxyl group at the global and the local minimum are, respectively $\theta_2 \sim 90°$ and $\theta_2 \sim 35°$. Interestingly, these are quite different from those observed for penicillins in either of the conformations ($\theta_2 \sim 160°$ and $110°$ for C_3 and C_2 puckered conformations respectively).

Conformational energy map of Δ^2-cephalosporin is shown in figure 6b. The position of the energy minima and the solid state conformation are marked in the diagram. Table 3 shows α_1, α_2 and α_3 for some of the low energy conformations.

Table 3. Conformational angles and energies for some of the low energy conformations of Δ^2-cephalosporin.

No.	α_1 (degrees)	α_2 (degrees)	α_3 (degrees)	E (Kcal. mol^{-1})
1	50	−30	10	0.00 [a]
2	50	−30	20	0.00
3	60	−35	10	0.40
4	60	−35	0	0.40
5	50	−30	30	0.40
6	40	−25	30	0.50
7	40	−25	20	0.70
8	60	−35	20	0.90
9	−40	25	40	3.10 [b]
10	−30	20	40	3.40
11	−30	20	50	3.80

[a] Global minimum. [b] Local minimum.

The dihydrothiazine ring of Δ^2-cephalosporin shows two minimum energy conformations. As seen from table 3, the global minimum occurs at (50°, −30°, 10°). The local minimum occurs at (−40°, 25°, 40°) and has about 3 Kcal. mol^{-1} higher energy than the global minimum, indicating that the former would be favoured both in solid state and in solution. The conformation observed in the solid state (Sweet and Dahl, 1970) is similar to the one at the global minimum, but has about 2-3 Kcal. mol^{-1} higher energy. The value of θ_2 at the global minimum is ~90°, and at the local minimum is ~150°. At the global minimum, $\omega_2 \sim 170°$, suggesting that the lactam peptide is less nonplanar, compared to the penicillins. At the local minimum, $\omega_2 \sim 143°$, indicating considerable nonplanarity. However, due to its higher energy, this conformation is unlikely to be observed either in the solid state or in solution.

As mentioned earlier for the Δ^3-cephalosporins, in both the puckered conformations of the dihydrothiazine ring, the lactam, peptide bond will assume appreciable nonplanarity. On the other hand, in the preferred conformation of Δ^2-cephalosporin, the lactam peptide bond is less nonplanar. This can account for the observed difference in the biological activities of these compounds.

For Δ^3-cephalosporins, the aminoacyl group assumes approximately the same orientation as in penicillins, but the carboxyl group orientation is different. In fact, the values of θ_2 for Δ^3-cephalosporins (~35° and ~90°) are very much different from the one observed in the biologically active conformation of penicillin (~160°). Since Δ^3-cephalosporin is active (though less compared to

penicillin), the differences at the carboxyl group end suggest that the mode o binding of this drug with the cross linking enzymes may differ slightly from that o penicillins. Such differences in the mode of binding of molecules which differ ir the orientations of some of the groups are not unusual, e.g., binding of α and β anomers of N-acetyl glucosamine to lysozyme (Beddel *et al.*, 1970). However, it is not clear which of the conformations of the dihydrothiazine ring is associated with biological activity.

Our earlier studies (Joshi *et al.*, 1978) on specificity of penicillinases have indicated that for binding to penicillinases, the orientation of the carboxyl group (θ_2) should be 150°; as in penicilin (C_3 puckered) or clavulanic acid. However, in neither Δ^3-nor Δ^2 cephalosporins, the energetically favoured conformations have carboxyl group orientations near this value. These differences in the orientations of the $-COOH$ group perhaps account for the resistance of cephalosporins to penicillinases.

Acknowledgement

The authors thank the Department of Science and Technology, New Delhi, for financial support.

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Reprinted from:

The Biology of Social Insects

Proceedings of the Ninth Congress of the
International Union for the Study of
Social Insects, Boulder, Colorado, August 1982

**edited by Michael D. Breed,
Charles D. Michener,
and Howard E. Evans**

Westview Press / Boulder, Colorado

A Comparative Study of Social Structure in Colonies of *Ropalidia*

Raghavendra Gadagkar and N. V. Joshi,
Indian Institute of Science

Ropalidia is a large old world genus in the family Vespidae whose systematics has been studied by van der Vecht (1962) and Richards (1978). There is very little information on the biology of Ropalidia (summarised by Richards, 1978) and even less regarding social organization (summarised below). However, the genus Ropalidia is considered to be of special interest primarily because of its diversity in social organization and nest architecture. The genus contains both independent- and swarm-founding species with both open as well as enveloped nests (Jeanne, 1980; van der Vecht, 1962).

Some information on social organization is available for 2 African and 2 Indian species. In Africa, Roubaud (1916) reported that R.guttatipennis colonies consist of a mixture of morphologically indistinguishable females, some with functional ovaries and some without. The colonies of R.cincta studied by Darchen (1976) were monogynous and there was a dominance hierarchy with the queen at the top of the hierarchy and the males at the bottom.

In India, R.marginata has colonies which may be either monogynous or polygynous with the adults all morphologically identical. New colonies are founded by one or a group of females (Gadagkar et al. 1978). Gadgil and Mahabal (1974) showed that females with well developed ovaries tend to be among the heaviest individuals in a colony and hypothesized that workers spend more energy in food gathering but receive a disproportionately smaller share of the food and thus suffer from 'nutritional castration'. Gadagkar (1980) demonstrated that there is a dominance hierarchy in the colonies which influences division of labour in such a fashion that the dominant individuals including the queens spend most of their time sitting on the nest and at best show alarm reactions while the subordinate individuals spend a great deal of time making trips to places away from the nest to bring back food, building material, water etc. R.cyathiformis appears to be similar except that it is conjectured to be at a more primitive level of social organization because several individuals lay eggs as well as forage and therefore combine the roles of queen and worker (Gadagkar and Joshi, submitted).

Here we analyse time-activity budgets of individually identified wasps of R.marginata and R.cyathiformis by multivariate

statistical techniques such as principal components analysis and hierarchical cluster analysis and show that wasps of both species fall into three distinct clusters or behavioural castes which we call sitters, fighters and foragers.

MATERIALS AND METHODS

Observations were made on two colonies of R.marginata for 165 hours and six colonies of R.cyathiformis for 170 hours in Bangalore (13°00'N and 77°32'E). Individual wasps were identified by marking with a spot of paint.

Ad libitum sampling was used to describe the behavioural repertoire, instantaneous scanning to estimate the proportion of time spent in different behaviours and all occurrences of rare behaviours were recorded in separate sessions to calculate the frequencies with which these were performed (Altmann, 1974).

Time-activity budgets for 20 animals of R.marginata and 32 animals of R.cyathiformis were used in principal components analysis (Anderberg, 1973; Frey and Pimental, 1978) and hierarchical cluster analysis (using the single linkage algorithm, and Pearson product moment correlation as an index of similarity between animals, De Ghett, 1978).

RESULTS

The time-activity budgets (data not shown) reveal that every animal spends most of its time (85-100%) in the six activities, sitting plus grooming, sitting alert (antennae raised), mild alarm reaction (antennae and wings raised), walking, inspection of cells and temporary absence from nest. However, the manner in which an animal allocates its time between these activities is highly variable. For example, the time spent by an animal in sitting varied between 7-56% in R.marginata and between 0-67% in R.cyathiformis; the time spent in temporary absence from the nest varied between 0-69% in R.marginata and between 0-88% in R.cyathiformis. This suggests that most individuals are capable of, and do perform most activities and, the differences between individuals are likely to be quantitative rather than qualitative. For this reason we have subjected the time-activity budgets to multivariate analysis. The results of principal components analysis show that temporary absence from the nest and sitting have the highest weightage in the first two principal components respectively in R.marginata and temporary absence from the nest and sitting alert have the highest weightages in the first two principal components in R.cyathiformis respectively. 92.5% and 98.1% of the total variance are accounted for by the first two principal components in R.marginata and R.cyathiformis respectively. Wasps of both species fall into three distinct clusters when each animal is represented as a point in the coordinate space of the first two principal components (Figs.1 and 2). The distinctness of the three clusters was confirmed by the method of nearest centroid. The method of hierarchical cluster analysis gives identical clusters for both species (not shown). In both the species members of one cluster rank highest in sitting (see Figs.3 and 4)

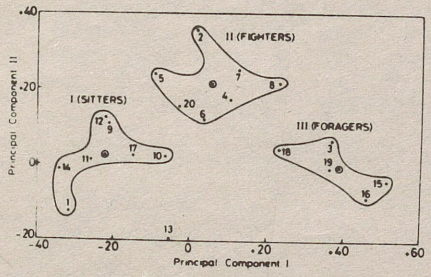

Figure 1. Behavioural castes in R. marginata obtained by principal component analysis. Each point represents an animal. ⊙ =centroid of the cluster

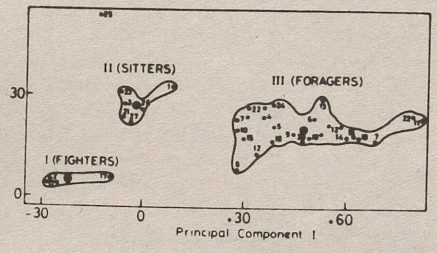

Figure 2. Behavioural castes in R. cyathiformis obtained by principal components analysis. Each point represents an animal. ⊙ =centroid of the cluster.

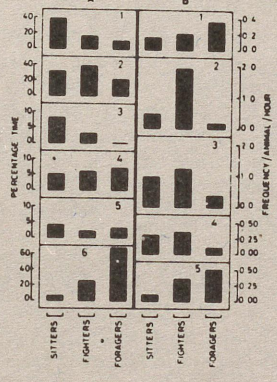

Figure 3. Mean behavioural profiles for the three clusters obtained in Fig. 1 for R. marginata. A, percentage time spent in the six activities used in the analysis: 1, sitting plus grooming; 2, sitting alert; 3, mild alarm reaction; 4, walking; 5, inspection of cells; 6, temporary absence from nest. B, mean frequency per hour of 5 other activities not used in the analysis: 1, bringing food loads; 2, attacking; 3, being attacked; 4, snatching food; 5, losing food.

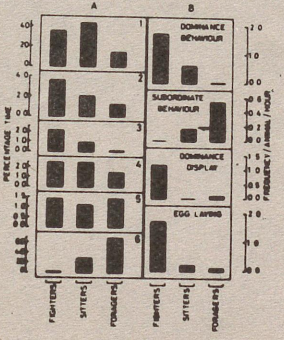

Figure 4. Mean behavioural profiles for the three clusters obtained in Fig. 2 for R. cyathiformis. A, percentage time spent in the six activities used in the analysis. Numbering as in Fig. 3. B, mean frequency per hour of 4 other activities not used in the analysis.

and we call these 'sitters'. Members of a second cluster rank highest in temporary absence from the nest. Wasps temporarily absent from the nest often returned with food, building material or water and hence we call these 'foragers'. The third cluster has the highest rank for sitting alert, an activity that is positively correlated with fighting (labelled as attacking in R.marginata and dominance behaviour in R.cyathiformis) and we have therefore labelled this cluster as 'fighters'.

DISCUSSION

Multivariate analysis of time-activity budgets reveals the presence of three clusters or behavioural castes namely sitters, fighters and foragers in R.marginata as well as R.cyathiformis. It is interesting to note that although data on egg-laying were not used in the analysis, both the queens of R.marginata (individuals 1 and 13) fall into the same cluster, 'sitters'. Thus R.marginata queens seem to do little other than egg-laying and spend most of their time sitting - perhaps the best strategy to develop their ovaries and maximise their egg-laying capacity. The rest of the sitters in R.marginata could either be hopeful queens or naive workers yet to be recruited into the worker force. This is being investigated by queen removal experiments. The situation in R.cyathiformis is somewhat different. One of the colonies was polygynous and some animals did both egg laying and foraging. However, individual 2 was the most dominant one which did most of the egg laying. Individual 17 was the next most dominant individual on the same colony which may be called a 'potential queen' because it later left this colony and went on to found a new colony in which she was the queen. The data on this animal after she became the queen on the new colony are treated separately in form of 17* in Fig.2. Note that individuals 2, 17 and 17* fall into the same cluster but, in this species they are the fighters. Although probably too early to generalise, it is very suggestive that in R.marginata, where the colonies studied were monogynous and the roles of queen and worker rather distinct, the queens are 'sitters'. In R.cyathiformis on the other hand, where one of the colonies was polygynous and the distinction between queen and worker was not always present, the 'queens' are 'fighters'. Reproductive competition must obviously be more intense in R.cyathiformis than in R.marginata. Details are being published elsewhere.

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Behaviour of the Indian social wasp *Ropalidia cyathiformis* on a nest of separate combs (Hymenoptera: Vespidae)

RAGHAVENDRA GADAGKAR AND N. V. JOSHI

Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560012, India

(*Accepted 28 January 1982*)

(With 5 figures in the text)

Observations were made on a nest of *Ropalidia cyathiformis* consisting of three combs. The number of eggs, larvae, pupae and adults were monitored at about 3-day intervals for a 2-month period. The behaviour of the adults was observed with special reference to the proportion of time spent on each of the three combs, the proportion of time spent away from the nest site and the frequencies of dominance interactions and egg laying. The adults moved freely between the three combs suggesting that all of them and all the three combs belonged to one nest. However, most of the adults preferred combs 2 and 3 over comb 1. Of the 10 animals chosen for a detailed analysis of behaviour, seven spent varying periods of time away from the nest site and often brought back food or building material. Five of the 10 animals laid at least one egg each but two adults monopolized most of the egg-laying. The animals showed a variety of dominance interactions on the basis of which they have been arranged in a dominance hierarchy. The dominant individuals laid most of the eggs and spent little or no time foraging, while the subordinate individuals spent more time foraging and laid few eggs or none. It is argued that *R. cyathiformis* is different from *R. marginata*, the only other Indian social wasp whose behaviour has been studied, in being at a more primitive stage of social organization.

Contents

Introduction

Social insects with their permanently sterile worker castes and many instances of altruistic behaviour have long been a source of fascination and intrigue for naturalists. Even Darwin

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recognized that the presence of sterile castes is difficult to explain on the basis of natural selection (Darwin, 1859). In recent years Hamilton (1964*a*, *b*) has argued that, although a sterile individual does not raise any of its own offspring, it can indirectly increase its genetic fitness by contributing to the survival of other individuals that share its genes. This has come to be known as the theory of *kin selection* (see West-Eberhard, 1975). Worker castes in Hymenoptera often help to raise their own sisters; and the peculiar mode of sex determination in Hymenoptera namely, *haplodiploidy*, makes her sisters in fact more closely related to a worker than her own potential offspring (see Wilson, 1971; Hamilton, 1972; Michener, 1974). Lin & Michener (1972) have proposed an alternative theory for the evolution of social behaviour in insects; they emphasize that, at least where complete sterility is not present, mutual advantage especially against predation may be an important factor favouring sociality. Alexander (1974), on the other hand, has argued that natural selection operates on the parents to produce a certain fraction of sterile offspring that would help the remaining fraction of fertile offspring to survive and reproduce better. Thus the emergence of a considerable body of theoretical ideas on the origin and evolution of sociality has intensified interest in the study of social insects (West-Eberhard, 1975; Wilson, 1975; Starr, 1979).

The social wasps belong to the family Vespidae which has about 40 genera (Richards, 1962, 1971, 1978; Jeanne, 1980). Of these, *Belonogaster, Parapolybia, Mischocyttarus, Polistes* and *Ropalidia* are of particular interest. They form a separate behavioural category characterized by simple, open nests that can be easily studied, and a rather primitive level of sociality because one or a small group of queens (inseminated females) found new colonies and rear the first brood without the aid of a swarm of workers. *Ropalidia* is considered a genus of special interest in this group (Jeanne, 1980). But, while *Belonogaster, Mischocyttarus* and *Polistes* have received considerable attention (West-Eberhard, 1969; Pardi & Piccioli, 1970; Piccioli & Pardi, 1970, 1978; Pickering, 1980; Jeanne, 1972; Litte, 1977, 1979; Strassmann, 1979), there is very little information on *Ropalidia* (Roubaud, 1916; Gadgil & Mahabal, 1974; Darchen, 1976; Gadagkar, 1980). This paper is the first report of observations on *Ropalidia cyathiformis* (Fab.).

Materials and methods

The animal

Ropalidia cyathiformis is a common paper wasp measuring about 8 mm from the tip of the head to the end of the abdomen. It builds small open nests of carton suspended by one or more pedicels on the walls of buildings or on the leaves of small plants such as croton. A nest is founded by one or a few females and large nests may contain 30 or more adults. The time taken for an egg to develop into an adult is about 9 weeks and the mean time of residence of adults on a nest is about 4 weeks.

The present study was carried out on a group of three small combs built on a metallic pole at the Indian Institute of Science, Bangalore (13°00′N and 77°32′E). The three combs, each with about 15–30 cells were within 2–3 cm of each other. As shown later, these combs formed a single nest and the adults moved freely between them.

Sampling methods

Three kinds of sampling methods were used in this study (Altmann, 1974).

***Ad libitum** sampling*

Initial *ad libitum* observations revealed that the adults not only moved between the combs but also

spent a considerable portion of time away from the nest site, at the end of which they often returned with food or building material. There were also many agonistic interactions on the basis of which the dominant and the subordinate individual could be recognized. It was therefore decided to employ unbiased sampling methods to quantify the extent to which each animal performed each of these activities. All the adults were individually identified by marking with one or more spots of quick drying paint without removing them from the nest. A census of all the wasps present was made on alternate days before 0530 hrs since none of the wasps ever left the colony before this time. During the census the comb on which each wasp was present was also recorded, and this information is used to calculate the proportion of time spent by the wasps on each of the combs at night; the wasps did not appear to move between the combs at night time.

Instantaneous scans

At randomly chosen times all the animals were instantaneously scanned to determine whether they were present on comb 1, 2, 3 or were temporarily away from the nest site.

All occurrences of some behaviour

Ad libitum observations showed that the wasps engaged in a number of kinds of dominance interactions (see results for description of the behaviour). To determine the frequency with which each animal dominated or was dominated by any other animal, every occurrence of a dominance interaction in randomly chosen 5-min intervals was recorded.

Since egg-laying was considered one of the most crucial activities in the present study, every instance of egg-laying seen during the entire period of observation was noted.

In all, 135 h of observations were spread evenly between 0630 and 1830 hrs during the 2-month period between 19 February and 19 April, 1980.

Results

The combs and their contents

The numbers of cells, eggs, larvae and pupae in each of the three combs are shown in Fig. 1. The total number of cells did not increase significantly on any of the combs during the period of study. On comb 1, the number of eggs varied around a mean of 3·4, the larvae around a mean of 6·3 and pupae between 0 and 1. In terms of the number of cells, comb 3 was bigger than comb 2 which in turn was bigger than comb 1. On both combs 2 and 3, the number of eggs varied around a mean of about 10, the larvae around a mean of about 12 and the pupae around a mean of about 3.

The adults

The number of adults present on these combs during the period of study (Panel (a) of Fig. 1) varied between 15 and 21. Ten of the longest lived animals, on which a considerable amount of information therefore exists, were selected for analysis of their behaviour. These animals are numbered from 1 to 10 and the time during which they were present is shown in Fig. 2.

Movement between combs

The adults moved freely between the combs. It was therefore of interest to determine whether different animals had special preferences for any of the combs. In other words did

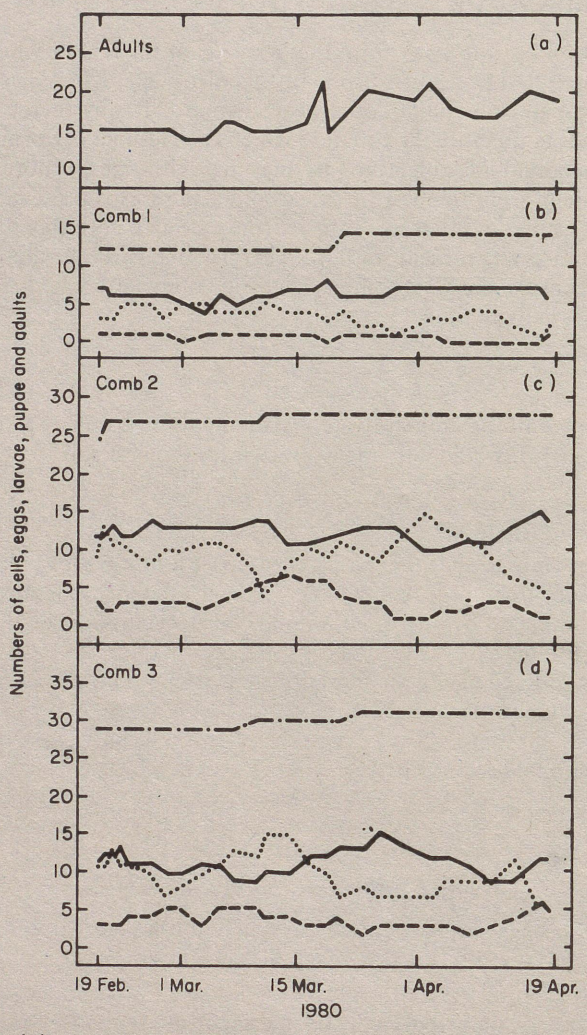

FIG. 1. (a) Numbers of adults (———); (b)–(d) cells (–·–·–), eggs (· · · ·), larvae (———) and pupae (– – – –) at different times on the nest and its constituent combs.

all the animals and all the combs belong to one nest? Figure 3 reveals that although the proportion of time spent on each of the combs varied between the animals, and between day and night, no animal or group of animals appeared to possess any of the combs at the exclusion of the others. Hence all the combs and all the animals may be said to belong to a single nest. Most of the animals however preferred combs 2 and 3 which were bigger and had a larger fraction of the brood than comb 1.

Time spent away from the nest site

The proportion of time spent away from the nest site by each of the ten animals (Fig. 4) was between 0 and 73%. Except animals 3 and 7, all spent at least some portion of their

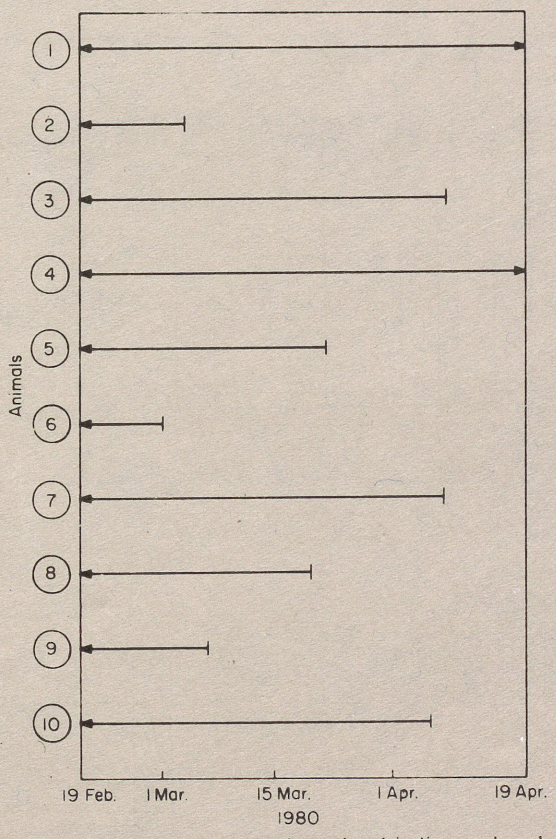

FIG. 2. The period of residence of adults on the nest. Arrowhead indicates that the fate of the animal beyond the time indicated is not known.

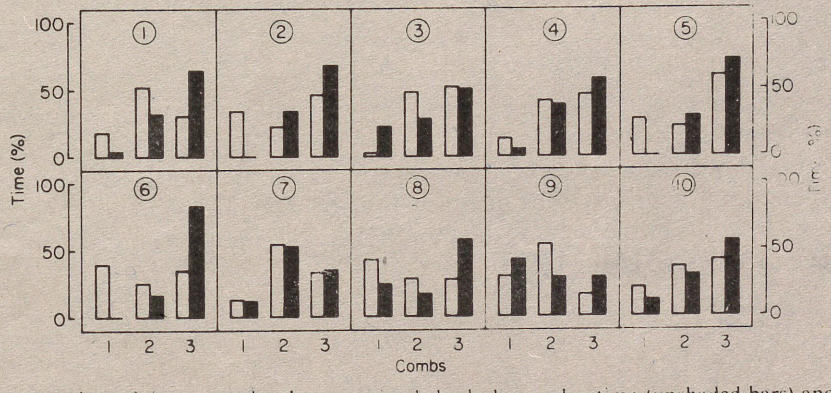

FIG. 3. The proportion of time spent by the ten animals both during day time (unshaded bars) and night time (shaded bars) on the three combs.

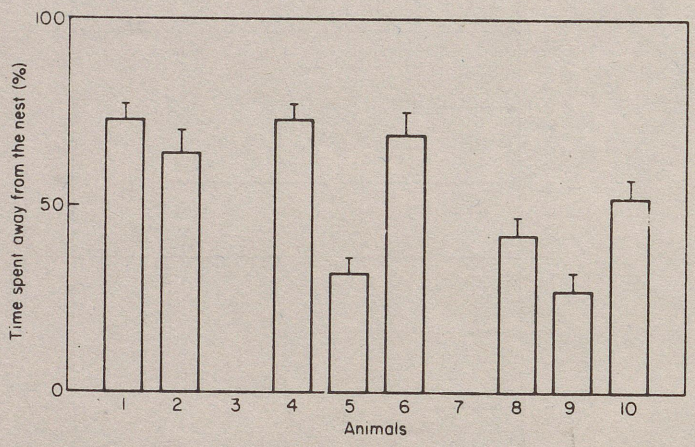

FIG. 4. The proportion of time (mean and standard deviation) spent away from the nest site by the ten animals.

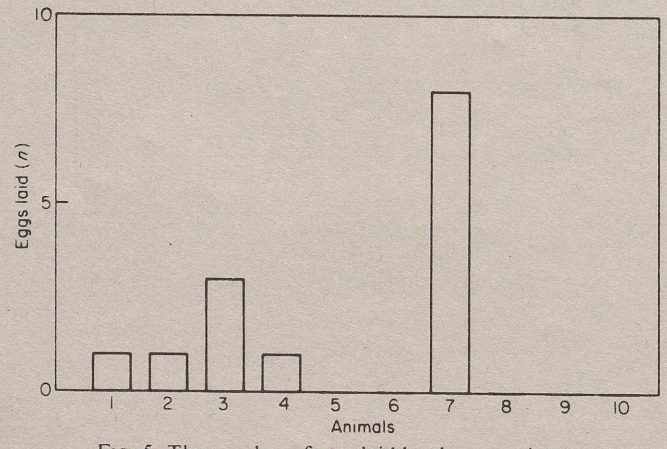

FIG. 5. The number of eggs laid by the ten animals.

time away from the nests. Wasps temporarily absent from the nests often returned with food or building material and even when they did not, they might have spent certain amount of time and energy and incurred certain risks in order to search for food. Moreover, foraging is very difficult to observe, let alone quantify directly. The proportion of time spent in being temporarily absent from the nests is therefore taken as an approximate index of time spent in foraging (see also Gadagkar, 1980).

Egg-laying

Five of the ten animals studied laid at least one egg (Fig. 5) showing that this was a polygynous nest. Animals 3 and 7, which did most of the egg laying, spent no time at all foraging (Fig. 4).

Dominance behaviour

The wasps engaged in a variety of agonistic interactions. In the most common type of aggressive encounter one individual climbed on top of another and tried to bite its mouth parts. Such an animal is called dominant. The opponent (the subordinate animal) remained very still and kept its body as compact as possible. In a second kind of interaction, one individual (also called dominant) sat on top of another for several minutes. Sometimes one (again called dominant) sat close to another and held one of the latter's legs or antennae in its mouth. In other kinds of interactions one wasp (dominant) chased, nibbled or pecked the other (subordinate). These kinds of interactions were not distinguished in our records: an individual was said to be dominant if it behaved in any of the ways described above.

The hourly frequencies with which each of the ten animals were dominant or subordinate to any other individual are shown in Fig. 6. There appeared to be four kinds of animals. Animals such as 3 were often dominant and seldom subordinate. Animals such as 8 and 9 were seldom dominant and often subordinate. But animal 5, for example, showed a high frequency of both dominant and subordinate behaviour, while animals 1, 2, 4 and 6 had low frequencies of both.

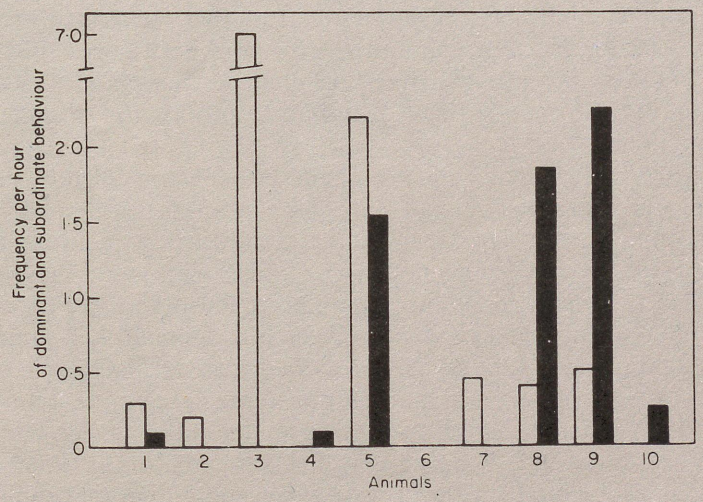

FIG. 6. The frequency per hour of dominance (unshaded bars) and subordinate behaviour (shaded bars) by the ten animals.

A dominance hierarchy for nine of the ten animals is given in Fig. 7. Animal 6, which was never involved in a dominance interaction, could not be represented. No instance of the reversal of a dominant/subordinate relationship was observed. Animals 3 and 7, which had the highest ranks, spent no time at all in foraging, and laid most of the eggs.

Discussion

The three combs of *R. cyathiformis* studied evidently belonged to one nest although most adults spent more time on combs 2 and 3. This species does not, however, always build multiple combs: we have also seen other nests with a single comb (unpublished observations).

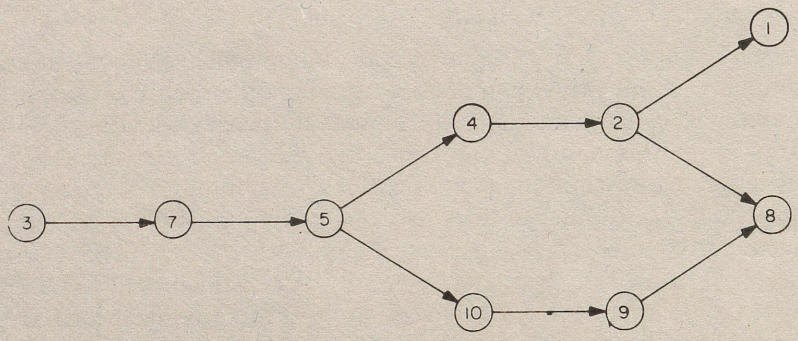

FIG. 7. Dominance hierarchy among the ten animals on the colony: 3 → 7 means that animal 3 is dominant over animal 7. Although the frequency of subordinate behaviour for 7 is shown to be zero in Fig. 6, data from *ad libitum* observations have also been used to construct the dominance hierarchy shown here and therefore 3 is shown to be dominant over 7.

The presence of multiple combs have been reported for other social wasps such as *Polistes canadensis* (Jeanne, 1979), *P. aterrimus* and *P. carnifex* (Rodrigues, 1968), *P. metricus* (Gamboa, 1981), *P. exclamans* (Strassmann, 1981) and *R. marginata* (Gadagkar, unpublished observations), but for none is there quantitative information on differences among adults in their comb preference.

The nest studied was polygynous; and five of the ten adults studied in detail each laid at least one egg. Polygyny is known in large nests of social wasps (see, for example, Richards & Richards, 1951). But in such nests there is a clear distinction between egg-layers and workers; other egg-layers in polygynous nests behave like queens in all respects and do no foraging (except when they cease to be queens and become workers; see for example West-Eberhard, 1978). In contrast, several individuals (such as animals 1, 2 and 4) in the nest of *R. cyathiformis* studied here, foraged as well as laid eggs. In addition to the time spent away from the nest (which is taken as an index of foraging), animal 1 for example was observed to bring back food and share it with other adults in the nest both before and after it was observed to lay an egg. It is believed that this is the first documented case of simultaneous egg-laying and foraging by a social wasp when more than one adult is present on a nest.

Although five of the ten animals studied laid eggs and seven of the ten animals foraged, there were considerable differences in the extent to which different animals did so. Animals 3 and 7 spent no time at all foraging and they laid most of the eggs; these animals were also at the top of the dominance hierarchy. Hence the dominant individuals spent the least amount of time foraging and laid most eggs, and the subordinate individuals spent more time foraging and laid few or no eggs. This is consistent with the behaviour of other social wasps such as *Polistes fuscatus* (West-Eberhard, 1969) and *R. marginata* (Gadagkar, 1980) for example.

Another important activity of this species is cannibalism. Several instances of adults eating eggs, larvae and even pupae (after breaking open the cap of the pupal cell) were observed (unpublished observations). It would be of interest to know which of the adults showed this behaviour, but we give no information on this for the following reason. Once one animal

initiates cannibalism by pulling out an egg, a larva or a pupa, several others present on the nest join it and feed on the already killed immature stage. Hence initiation of cannibalism is the significant act; but initiation of cannibalism itself was seldom seen.

Eighty-two species belonging to nine genera of social wasps have been recorded from India (Gupta & Das, 1977). The only other social wasp from India whose behaviour has been studied is, however, *R. marginata* (Gadagkar, 1980; Gadgil & Mahabal, 1974). It would, therefore, be of interest to compare *R. cyathiformis* with *R. marginata*. Small nests of *R. marginata* have a single egg-layer. Large nests have more than one individual with well developed ovaries. However, we have made no careful observations of such large nests. What follows therefore refers only to small monogynous nests in which there is always a clear distinction between egg-layers and workers with no overlap between these roles except when a single foundress is attending a newly founded nest. Furthermore, the egg-layer of a nest of *R. marginata* is dominant over all the other individuals: she does not normally come into contact with her nest mates, and they often withdraw from her presence. No instances of larval or pupal cannibalism have been observed in *R. marginata* except immediately after extensive predation by *Vespa tropica*.

In contrast, the nest of *R. cyathiformis*, which forms the subject of the present paper, had at least five egg-layers and there was no clear distinction between egg-layers and workers. The same individuals have been observed to lay eggs as well as forage within a short period. Moreover, even the most dominant individuals often engaged in actual physical interaction with other adults. The dominance interactions of this species appear to be much more varied than those of *R. marginata*. In addition, several instances of larval and even pupal cannibalism were observed in *R. cyathiformis*. It is therefore suggested that *R. cyathiformis* is at a more primitive stage of social organization than that of *R. marginata*. In fact, *R. cyathiformis* probably represents one of the most primitive levels of eusociality recorded among the Vespidae (except some stenogastrinae). More information on this species should therefore throw some light on the factors responsible for the origin of eusociality in wasps.

Summary

Three combs of the Indian social wasp, *Ropalidia cyathiformis*, built very close to each other were studied. The adults moved freely between the combs and it was concluded that the combs and adults belonged to a single nest. A variety of dominance interactions was observed among the adults on the basis of which they could be arranged in a dominance hierarchy. The most dominant individuals monopolized most of the egg-laying and spent little or no time foraging. The subordinate individuals spent more time foraging but some of them also laid some eggs. This species appears to be unusual among the social wasps studied in that some individuals did both egg-laying and foraging and therefore combined the roles of queen and worker. This is not usually known to occur except when a single foundress is attending a newly founded nest. On the basis of this and other evidence it is argued that *R. cyathiformis* is at a more primitive level of sociality compared to *R. marginata*, another Indian social wasp that has been studied.

We are very grateful to Professors Madhav Gadgil, S. A. Barnett and Charles Michener for critically reviewing the manuscript and to Dr J. Van der Vecht for identifying the species.

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J. Biosci., Vol. 4, Number 3, September 1982, pp. 377-390. © Printed in India.

Dosage compensation and sex determination in *Drosophila*: mechanism of measurement of the X/A ratio+

RAGHAVENDRA GADAGKAR*, VIDYANAND NANJUNDIAH***, N. V. JOSHI* and H. SHARAT CHANDRA* **

* Centre for Theoretical Studies, ** Microbiology and Cell Biology Laboratory and ICMR Centre for Genetics and Cell Biology, Indian Institute of Science, Bangalore 560 012, India and *** Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay 400 005

MS received 24 September 1982

Abstract. We propose a molecular mechanism for the intra-cellular measurement of the ratio of the number of X chromosomes to the number of sets of autosomes, a process central to both sex determination and dosage compensation in *Drosophila melanogaster*. In addition to the two loci, *da* and *Sxl*, which have been shown by Cline (*Genetics*, **90**, 683, 1978) and others to be involved in these processes, we postulate two other loci, one autosomal (ω) and the other, X-linked (π). The product of the autosomal locus *da* stimulates ω and initiates synthesis of a limited quantity of repressor. *Sxl* and π, both of which are X-linked, compete for this repressor as well as for RNA polymerase. It is assumed that *Sxl* has lower affinity than π for repressor as well as polymerase and that the binding of polymerase to one of these sites modulates the binding affinity of the other site for the enzyme. It can be shown that as a result of these postulated interactions transcription from the *Sxl* site is proportional to the X/A ratio such that the levels of Sxl^+ product are low in males, high in females and intermediate in the intersexes. If, as proposed by Cline, the Sxl^+ product is an inhibitor of X chromosome activity, this would result in dosage compensation. The model leads to the conclusion that high levels of Sxl^+ product promote a female phenotype and low levels, a male phenotype. One interesting consequence of the assumptions on which the model is based is that the level of Sxl^+ product in the cell, when examined as a function of increasing repressor concentration, first goes up and then decreases, yielding a bell-shaped curve. This feature of the model provides an explanation for some of the remarkable interactions among mutants at the *Sxl*, *da* and *mle* loci and leads to several predictions. The proposed mechanism may also have relevance to certain other problems, such as size regulation during development, which seem to involve measurement of ratios at the cellular level.

Keywords X-Chromosome transcription; sex-lethal mutations; maternal effect; RNA polymerase; size regulation.

Introduction

Bridges (1925) has shown that in *Drosophila melanogaster* the sexual phenotype is determined by the ratio of the number of X chromosomes (X) to the number of sets of autosomes (A). This X/A ratio, or Bridges' ratio, also regulates the rate at

+ Paper presented at a conference on 'Condensed chromatin and the human X chromosome', held at the Indian Institute of Science, Bangalore, December 14-16, 1981.

which most X-linked genes are transcribed (Lucchesi, 1973; Maroni and Plaut, 1973; Stewart and Merriam, 1978; Chandra, 1979). As a result, in flies with an integral number of chromosomes, the level of activity of enzymes coded by X-linked genes is proportional to the number of copies of the structural gene divided by the Bridges' ratio (Chandra, 1979). The end result of this regulatory process, known as *dosage compensation,* is that the phenotype resulting from two doses of a given X-linked gene in the female (AAXX) is equal to that resulting from one dose in the male (AAXY). This is the consequence of the single X chromosome in the male being transcribed at rougly twice the rate as each of the two chromosomes in the female. *D. melanogaster* is able to sustain wide variation in X/A ratios, and it has therefore been possible to show that dosage compensation operates over a variety of chromosome constitutions. Since both the sexual phenotype and dosage compensation appear to be cell-autonomous properties (Bridges, 1930; Lakhotia and Mukherjee, 1969), an intriguing feature of these two phenomena is the mechanism by which the X/A ratio is assessed within cells. Mutations which interfere with the capacity to measure the X/A ratio and, as a consequence, affect dosage compensation or sex determination, might provide insight into the molecular mechanisms involved in these processes.

Cline (1978, 1980) has made an elegant study of the following mutations which appear to fulfil such a purpose. (i) Daughterless (*da*) is a temperature-sensitive autosomal recessive (2-41.5) (Bell, 1954; Cline, 1976). Homozygous females leave behind only male offspring because the daughters die during embryonic development. Daughters can be rescued from *da/da* mothers following early injection of wild type (da^+) egg cytoplasm (Bownes *et al.*, 1977), suggesting that the daughterless phenotype is caused by the absence of some diffusible product coded for by the da^+ locus. (ii) Sex-lethal, male-specific (Sxl^{M1}), is an X-linked mutation (1-19.2) (Cline, 1978), lethal to males and, curiously, also a dominant suppressor of *da*. (iii) Sex-lethal, female-specific, (Sxl^{F1}), is also X-lined (Muller and Zimmering, 1960; Zimmering and Muller, 1961), 0.007 recombination units away from Sxl^{M1} (Cline, 1978). It was isolated as a dominant mutation but later studies have shown that it normally behaves as a recessive and that its occasional dominant character is dependent on some undefined elements of the genetic background and on certain environmental conditions (Cline, 1978).

Cline has shown that the effects of these mutations can be explained on the following bases. (a) A maternal factor is produced by da^+, the wild type allele of the *da* locus. In a fertilized egg whose X/A ratio corresponds to that of a female, this factor activates transcription at the *Sxl* locus. (b) The Sxl^{M1} locus is the control region of the *Sxl* gene and the Sxl^{F1} locus is the structural part. (c) The Sxl^+ product is essential for females and lethal for males. (d) The Sxl^{M1} mutation makes the synthesis of Sxl^+ product constitutive, that is, independent of stimulation by the da^+ factor. Based on these and other results, Cline has made the conjecture that the Sxl^+ product might itself be involved in dosage compensation and sex determination (Cline, 1978, 1979a). The mechanism by which X/A ratio is measured in the embryo is, however, an undefined aspect of Cline's interpretation.

In this paper we (i) present a model to show how measurement of the X/A ratio can be effected; (ii) show that the level of Sxl^+ product is proportional to the X/A ratio; and, (iii) postulate that there is a quantitative relationship between the sexual phenotype and the Sxl^+ product such that increasing cellular concentration of this product leads to increasing 'femaleness' while decreasing concentrations result in 'maleness'. The model also provides an explanation for the interactions among some of the related mutants affecting sex determination and dosage compensation.

The reasoning which led us to this model has been briefly outlined in a recent publication (Gadagkar *et al.*, 1981).

The model

Qualitative aspects

The model (figure 1) consists of five components: (i) the da^+ factor, produced in the mother and stored in the egg; (ii) a postulated autosomal site ω capable of

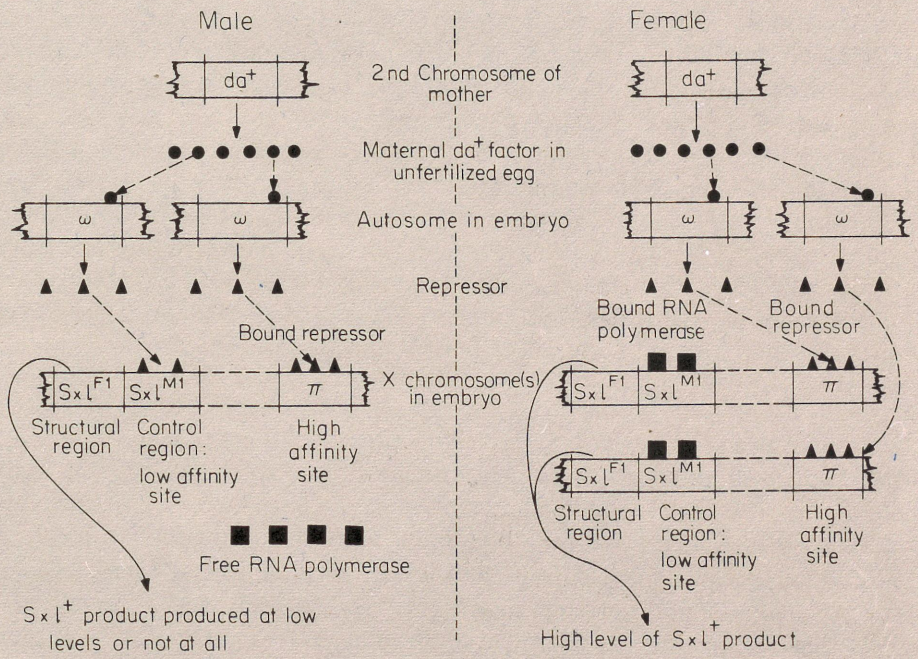

Figure 1. A model for the measurement of the ratio of the number of X chromosomes to the number of sets of autosomes.

The da^+ factor (●) is produced in excess by the maternal gene da^+ and stored in the egg. Following fertilization, this factor binds to a specific autosomal site ω in the embryo resulting in the production of a small quantity of repressor (▲). In the male embroyo (left half of the figure), there is only one X chromosome and therefore only one set of low affinity *Sxl* and high affinity π sites. The repressor is able to bind significantly to both these sites. As a result, on the average, little or no RNA polymerase (■) binds to the *Sxl* site and little or no Sxl^+ product is produced. In the female embryo (right half of the figure), there are two X chromosomes but the same quantity of repressor as in the male. This quantity of repressor is just sufficient to significantly block the π sites. RNA polymerase binds to *Sxl* and initiates synthesis of the Sxl^+ product. Females are viable at high levels of Sxl^+ product and males at low levels.

binding da^+ factor and releasing repressor; (iii) the *Sxl* locus, which in our model is the *low affinity site,* capable of binding both the repressor and RNA polymerase; (iv) a postulated ***high affinity site*** π, also on the X chromosome, capable of binding both repressor and RNA polymerase with a much higher affinity than the *Sxl* locus; and (v) ***RNA polymerase*** which binds to both the high and low affinity sites and whose binding to the low affinity site results in transcription at the *Sxl* locus. RNA polymerase binds to *Sxl* and π with an affinity which is less than that of the repressor for these two sites. Binding of RNA polymerase to either *Sxl* or π reduces its binding to the other site.

In the mother the da^+ locus produces an *excess* of da^+ factor which is stored in the egg. Following fertilization, the da^+ factor binds to ω; this results in the synthesis of a ***small*** quantity of repressor. Both male and female embryos have two copies fo ω and would therefore have the same quantity of repressor. In contrast, the number of *Sxl* and π sites is two each in the female and one each in the male. Repressor and RNA polymerase compete for binding to the low affinity *Sxl* and high affinity π sites. However, the affinity of the repressor for either of these sites is higher than that of the polymerase. Therefore the π site is preferentially bound by the repressor. Since the female has two π sites, these get bound to a significant extent by the repressor. However, repressor concentrations are limiting and therefore allow for polymerase binding to the low affinity *Sxl* sites; this leads to synthesis of significant amounts of the Sxl^+ product. In the male, on the other hand, there is only one copy each of the high and low affinity sites. The repressor thus practically saturates both these sites. Consequently, the low affinity *Sxl* site hardly binds RNA polymerase and little or no Sxl^+ product is produced. In a qualitative sense Sxl^+ product will therefore be made in the female but not in the male.

Our model also provides a ready explanation for the mutants discussed by Cline (1978). Eggs of *da/da* flies lack da^+ factor and therefore also lack repressor. In the absence of repressor, RNA polymerase binds preferentially to the π sites. As a result, affinity of the *Sxl* site for polymerase is reduced and thus a negligible amount of Sxl^+ product is produced. The Sxl^+ product being essential for females, this leads to the daughterless phenotype. The male lethal phenotype of Sxl^{M1} suggests that Sxl^+ product is produced in these males despite the low X/A ratio. Sxl^{M1} also acts as a suppressor of the daughterless phenotype. Both these effects of this remarkable mutation have a simple explanation in terms of our model: the mutation increases the affinity of the *Sxl* site for RNA polymerase, more RNA polymerase binds to it than in the wild type, and this leads to male lethality and survival of the daughters of *da/da* mothers.

A situation in which two sites compete for repressor and RNA polymerase resulting in the regulation of transcripion from these sites, is in fact known to exist in the bacteriophage lambda (Ptashne *et al.*, 1976, 1980; Walz *et al.*, 1976) where operator-promoter complexes for the genes *cro* and *cI* are close to each other and

are regulated by the same repressor. The *cro* operator-promoter complex, analogous to the π site in our model, has a higher affinity for repressor and RNA polymerase than the *cI* operator-promoter complex, analogous to the *Sxl* site. As a result, transcription from the *cI* promoter first increases and then decreases as a function of repressor concentration. In our model transcription from the *Sxl* site behaves in an identical fashion, also as a function of repressor concentration, and this leads to the model's many interesting features (see below).

Quantitative aspects

Relationship of level of Sxl^+ product to viability: Since Sxl^+ product is assumed to regulate the rate of transcription of the X chromosomes, the level of Sxl^+ product per X chromosome is used here as the standard of comparison among different genotypes. We assume that the viability of a genotype varies with the level of Sxl^+ product per X chromosome and that a male is maximally viable at levels of Sxl^+ product lower than that at which females are maximally viable. Males are assumed to be inviable at levels of Sxl^+ product above those in the intersex and females at levels below. Clearly, a number of factors other than the level of Sxl^+ product must be contributing to the reduced viabilities of metamales (AAAXY), metafemales (AAXXX) and intersexes (AAAXX). However, we assume that the only effect in terms of the contribution of Sxl^+ product is that increasing levels of Sxl^+ product reduce male viability and decreasing levels reduce female viability. This assumption is consistent with the observation that Sxl^{F1} males (presumably with no Sxl^+ product) are fully viable as are Sxl^{M1}/Sxl^{M1} females (Cline, 1978). Thus we define levels of Sxl^+ product above those occurring in the intersex as the region of female viability. Conversely levels of Sxl^+ product below those in the intersex are defined as the region of male viability. It should be noted that this represents a modification of Cline's (1978) 'all-or-none' assumption that Sxl^+ product is essential for females and lethal for males.

Computation of levels of Sxl^+ product: The calculations made in this paper refer to the binding equilibria between repressor and polymerase on the one hand and the low (*Sxl*) and high (π) affinity sites on the other. Binding is assumed to be Michaelian (non-cooperative) except that polymerase binding to either *Sxl* or π depresses its binding affinity to the other. Details are given in the legend to figure 2. The values of the parameters used as well as the range within which each can vary without affecting our conclusions are given in table 1. The result of these calculations is an expression for the equilibrium binding of RNA polymerase to the low affinity *Sxl* site. We assume that the level of this binding is directly reflected in the level of Sxl^+ product within the cell.

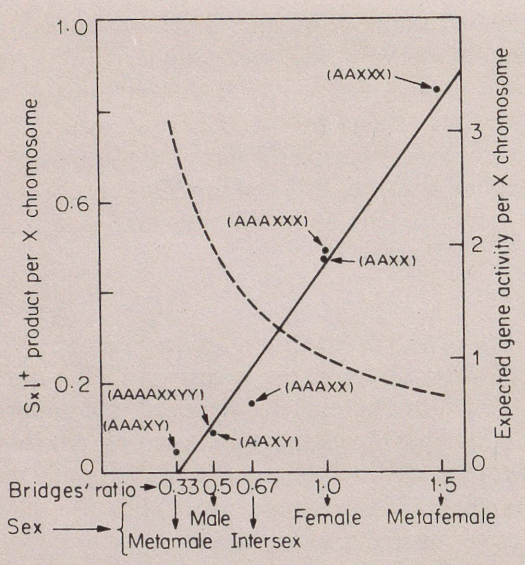

Figure 2. Levels of *Sxl*$^+$ product per X chromosome (left ordinate and solid line) and expected values of gene activity per X chromosome locus (right ordinate and broken line) (Chandra, 1979); both are expressed as a function of Bridges' ratio. Levels of *Sxl*$^+$ product were computed by solving for the following binding equilibria: $K_{LR} \times [L] \times [R] = [LR]$; $K_{LP} \times [L] \times [P] = [LP]$; $K_{HR} \times [H] \times [R] = [HR]$; $K_{HP} \times [H] \times [P] = [HP]$; where, [L], [H], [R] and [P] denote the free concentrations of low affinity sites (*Sxl*), high affinity sites (π), repressor, and RNA polymerase, respectively, and [LR], [LP], [HR] and [HP] are the concentrations of the bound complexes. The affinities of the reactions are denoted by K_{LR}, K_{LP}, K_{HR} and K_{HP} respectively.

The variable affinity in the binding of RNA polymerase to the two sites is simulated using the following equations:

$$K_{LP} = \frac{K^{\circ}_{LP}}{1 + \left(A \frac{[HP]}{[H_o]}\right)^n} \; ; \; K_{HP} = \frac{K^{\circ}_{HP}}{+\left(A \frac{[LP]}{[L_o]}\right)^n}$$

where, K°_{LP} is the affinity of RNA polymerase to the *Sxl* site when no polymerase is bound to the π site, K°_{HP} is the affinity of RNA polymerase to the π site when no polymerase is bound to the *Sxl* site; $[L_o]$, and $[H_o]$ are the total concentrations of *Sxl* and π, and A and n are constants. Thus, as binding of polymerase to the π site increases, K_{LP} decreases; when a fraction 1/A of the π sites is bound by polymerase, K_{LP} becomes half of K°_{LP} and, finally, when all the π sites are occupied by polymerase, the affinity falls by a factor of $1 + A^n$. Binding of polymerase to *Sxl* reduces its affinity for π in a like manner.

Conservation conditions yield the following set of equations: $[LP] + [LR] + [L] = [L_o]$; $[HP] + [HR] + [H] = [H_o]$; $[LR] + [HR] + [R] = [R_o]$; $[LP] + [HP] + [P] = [P_o]$; where, $[R_o]$ and $[P_o]$ are the total concentrations of repressor and polymerase respectively.

For various sets of constants, $[L_o]$, $[H_o]$, $[R_o]$, $[P_o]$, K_{HR}, K_{LR}, K°_{HP}, K°_{LP}, A and n, the above equations were solved iteratively. $[L_o]$ and $[H_o]$ were taken as unity and scaled with the number of X chromosomes while $[R_o]$ and $[P_o]$ were scaled with the number of sets of autosomes. After these computations were completed, the gene coding for RNA polymerase II was shown to be on the X chromosome (Greenleaf *et al.*1980). $[P_o]$ was therefore also scaled with the number of X chromosomes and the results do not alter any of our conclusions. The values of the various parameters used are given in table 1.

Table 1. Values of parameters used and their range of tolerance.

	Value used in the calculation	Range tolerated*
Repressor (R_0)	1.5	1.3 −1.7
RNA Polymerase (P_0)	5	2 −10
Affinity of polymerase for the low affinity site ($K°_{LP}$)	1	0.5 −2.0
Affinity of polymerase for the low affinity site in the Sxl^{M1} mutation	10	5 −40
Affinity of polymerase for the high affinity site ($K°_{HP}$)	100	10 −500
Affinity of repressor for the low affinity site (K_{LR})	100	50. −150
Affinity of repressor for the high affinity site (K_{HR})	10^5	2.5×10^4 −∞
A†	3.00	2.75 −3.50
n†	4.00	3.75 −4.50

* When the value of any parameter is outside this range, either the level of Sxl^+ product does not increase as a function of Bridges' ratio or the mutants do not behave as described in the text.

† These are constants in the equations used to simulate the variable affinity in the binding of RNA polymerase to the high and low affinity sites. See legend to figure 2 for details.

Numerical results

General Remarks

The level of Sxl^+ product per X chromosome increases in proportion to Bridges' ratio (figure 2). Triploids and tetraploids are extremely close to their diploid counterparts in their levels of Sxl^+ product per X chromosome, suggesting that it is indeed the X/A *ratio* rather than the level of X or A separately that is being measured. By applying the criteria for viability given earlier, one can see that (i) *da* is lethal in the female but not in the male; (ii) Sxl^{M1} is lethal in the male but not in the female; (iii) either one or two doses of Sxl^{M1} will rescue the daughters of *da/da* mothers and (iv) Sxl^{F1} is recessive because the level of Sxl^+ product per X chromosome in an Sxl^{F1}/Sxl^+ individual, though half of the normal level, is still within the region of female viability (table 2).

Mutations that can arise in the system

By varying the values of the parameters used beyond the assumed limits of viability of the wild type we are able to predict the kinds of mutations that can arise in this system (table 1). Many interesting consequences follow from the observation that the level of Sxl^+ product, when assessed as a function of increasing repressor concentration, first goes up and then comes down (figures 3A and B). Thus, whereas the region of female viability is confined to one continuous interval of repressor concentrations, males survive only at very low or high repressor levels.

Table 2. Sxl^+ product per X chromosome in wild type and mutant flies.

	Genotype	Sxl^+ product per X chromosome*
1.	*Wild type male*	*0.08*
2.	Sons of *da/da* mothers	0.10
3.	Sxl^{M1} male	0.40
4.	2, above, with Sxl^{M1}	0.52
5.	*Wild type female*	*0.47*
6.	Daughters of *da/da* mothers	0.09
7.	Sxl^{M1}/Sxl^{M1} female	0.68
8.	6, above, with Sxl^{M1}/Sxl^{M1}	0.47
9.	6, above, with Sxl^{M1}/Sxl^+	0.28
10.	Sxl^{F1}/Sxl^+ female	0.24
11.	*Intersex* (AAAXX)	*0.15*
12.	Intersex offspring (AAAXX) of *da/da* mothers	0.13
13.	11, above, with Sxl^{M1}/Sxl^{M1}	0.48
14.	12, above, with Sxl^{M1}/Sxl^{M1}	0.60
15.	*Metamale* (AAAXY)	*0.05*
16.	15, above, with Sxl^{M1}	0.34
17.	*Metafemale* (AAXXX)	*0.84*
18.	Metafemale offspring (AAXXX) of *da/da* mothers	0.08

* These values were calculated as described in legend to figure 2. The parameters used are those listed in table 1.

Thus we have the apparently paradoxical situation in which a partial reduction in repressor levels leads to male-specific lethality, whereas totally eliminating the repressor restores viability to the male but results in female lethality. These predictions are mirrored respectively in the male-specific autosomal lethal, ***mle*** (Belote and Lucchesi, 1980a, b; Fukunaga *et al.*, 1975; Tanaka *et al.*, 1976) and in the *da* mutation (Cline, 1978). The other autosomal, male-specific lethals ***msl-1***, ***msl-1***^{b}, ***msl-2*** (Belote and Lucchesi, 1980a, b) are expected to be similar to ***mle*** in this respect.

The basis for the curious interaction between the *da* and Sxl^{M1} mutations is also brought out by Figs. 3A and B. Sxl^{M1} increases the level of Sxl^+ product above that of the wild type over the entire range of repressor concentrations. This has the effect of making the male nonviable over the entire range and of restoring viability to those females in which the repressor concentration is ***simultaneously*** lowered to near zero levels. Sxl^{M1}, therefore, is a male-specific lethal mutation which also has the property of rescuing the daughters of *da/da* mothers.

The observation that the level of Sxl^+ product first increases and then decreases with repressor concentration is central to explaining yet another curious result. This is the recent finding of Skripsky and Lucchesi (1980) that females of the genotype ***mle/mle;*** Sxl^{F1}/Sxl^+ develop, with a low penetrance, male secondary sexual characteristics (sex-combs). Referring to figure 3, the bell-shape of the curve implies that if the effect of the ***mle*** mutation is to partially reduce repressor concentration, it would lead to unacceptably high levels of Sxl^+ product in the male. In the female, on the other hand, the levels are slightly reduced but still within the region of viability. In combination with one dose of Sxl^{F1}, which by itself reduces the level of Sxl^+ product by one half, ***mle/mle*** would further lower the

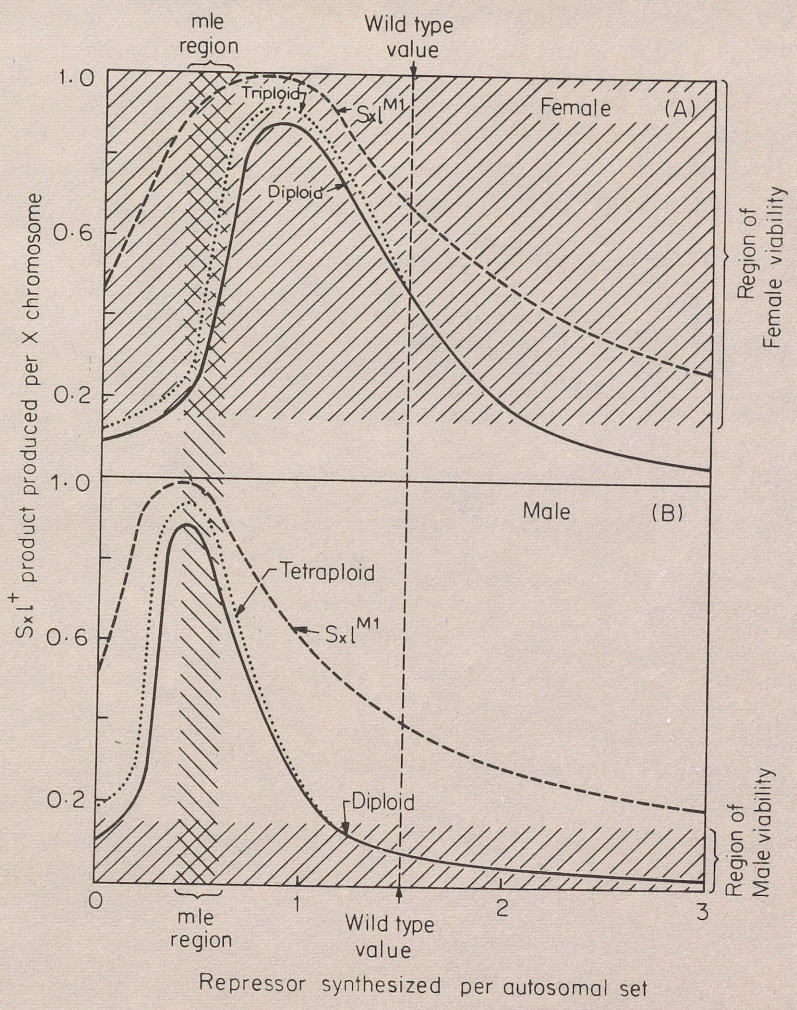

Figure 3. Levels of *Sxl*$^+$ product per X chromsome as a function of repressor synthesized per autosomal set in the female (A) and male (B).

Solid lines refer to the wild type, dotted lines to tetraploid male and triploid female and broken lines to the *Sxl*^{M1} male and female. The levels of *Sxl*$^+$ product corresponding to the regions of male and female viability and also the levels of repressor corresponding to the ***mle*** mutation are shown as hatched areas. Note that the range in levels of *Sxl*$^+$ product tolerated by a male is much narrower than that tolerated by a female. Metafemales have an extreme female phenotype and a rate of transcription per X chromosome lower than that in the normal female (Lucchesi *et al.* 1974; Stewart and Meriam, 1975). In terms of our model metafemales should have a level of *Sxl*$^+$ product higher than that of a normal female. Thus the level in normal females is expected to be somewhat below 1.0, the theoretical maximum value for *Sxl*$^+$ product per X chromosome. *Sxl*^{F1} is a recessive mutation (Cline, 1978). Since *Sxl*^{F1}/*Sxl*$^+$ females (presumably with half the wild type levels of *Sxl*$^+$ product) are fully viable, the level of *Sxl*$^+$ product in an intersex should be less than 0.5. The male, as a result, can only have a relatively narrow region of viability, ranging from zero to some value below 0.5 of *Sxl*$^+$ product per X chromosome. The *da* mutation corresponds to a zero level of repressor. The value of $[R_o] = 1.5$, considered as the wild type value, is indicated by the broken vertical line running through both panels.

level of Sxl^+ product and bring it to the neighbourhood of the male value. Consequently, such flies, if they survive, ought to show male-like characters.

The consequences of varying the affinities of the *Sxl* and π sites for repressor and RNA polymerase have been examined by us. A summary of these results and the properties of the various mutations, known as well as predicted, and the interactions among them are given in summary form in table 3 and figure 4.

Table 3. Properties of mutations, known and predicted.*

Mutation	Reference	Phenotype	Expected change at the molecular level	Can be rescued by	Will rescue the effects of
Sxl^{F1}	Cline (1978)	Female lethal	Inactive *Sxl* product	None	Sxl^{M1}, *mle*
da	Cline (1978)	Daughterless	No repressor, leading to low levels of Sxl^+ product in females	Sxl^{M1}	K_{LR} *low*
mle	Belote and Lucchesi (1980a, b); Fukunaga *et al.* (1975); Tanaka *et al.* (1976)	Male lethal	Lower than normal levels of repressor, leading to high levels of Sxl^+ product in males	Sxl^{F1}, K_{HP} *high* or K_{HR} *low*	None
Sxl^{M1} or K_{LP} *high*	Cline (1978); also see figure 4A	Male lethal	Higher than normal levels of Sxl^+ product in males	Sxl^{F1}	K_{HP} *high*, K_{HR} *low*, *da*
K_{LR} *low*	Predicted, see Fig. 4B	Male lethal	Higher than normal levels of Sxl^+ product in males	*da*	K_{HP} *high*, K_{HR} *low*
K_{HP} *high*	Predicted, see figure 4C	Female lethal	Lower than normal levels of Sxl^+ product in females	Sxl^{M1}, K_{LR} *low*	*mle*
K_{HR} *low*	Predicted, see figure 4D	Female lethal	Lower than normal levels of Sxl^+ product in females	Sxl^{M1}, K_{LR} *low*	*mle*
ω-	Predicted	Female lethal	No repressor, leading to lower than normal levels of Sxl^+ product in females	Sxl^{M1}	K_{LR} *low*

* K_{LP} and K_{LR} are, as defined in legend to Figure 2, the affinities of the *Sxl* site to RNA polymerase and repressor respectively; similarly, K_{HP} and K_{HR} are the affinities of the π site to the polymerase and repressor respectively. The mutation K_{LP} ***high*** is one which results in an increase in K_{LP}; K_{LR} *low* is a mutation which results in a decrease in K_{LR}; K_{HP} ***high*** results in an increase in K_{HP} whereas K_{HR} *low* leads to a decrease in K_{HR}.

Changes in the affinity of the repressor or polymerase to these sites can be brought about by muations in the sites themselves or by mutations affecting the properties of the repressor or polymerase. The former would behave as X-linked mutations and cannot be rescued by injection of cytoplasm from wild type eggs into defective eggs while the latter would behave as autosomal mutations and can be rescued by injection of cytoplasm from wild type eggs. The mutation Sxl^{M1}, which results in an increase in K_{LP}, is expected to be of the former kind because it is X-linked.

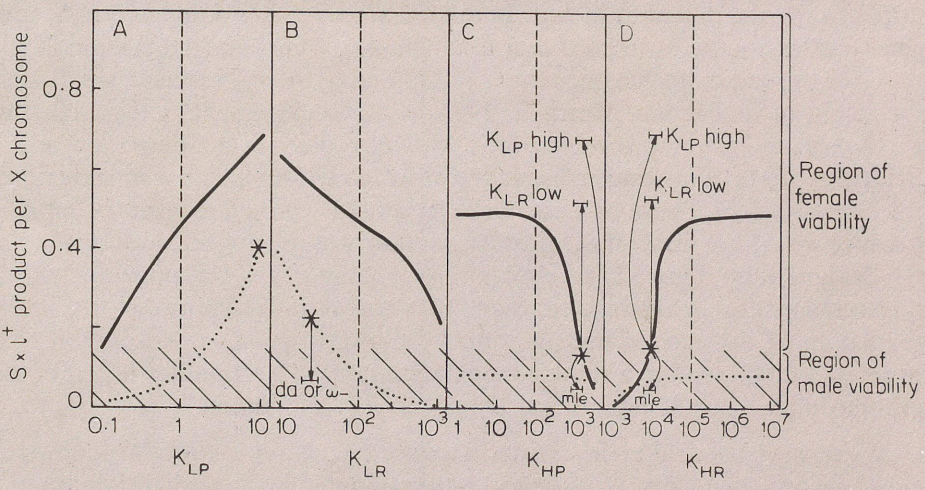

Figure 4. Levels of *Sxl*$^+$ product per X chromosome in the female (solid line) and male (dotted line) as a function of variations in the values of K_{LP} (Panel A), K_{LR} (Panel B), K_{HP} (Panel C) and K_{HR} (Panel D).

The panels A, B, C and D illustrate respectively the consequences of the four mutations *K_{LP} high, K_{LR} low, K_{HP} high* and *K_{HR} low*. The values considered as wild type for each of these affinities are indicated by broken vertical lines running through the middle of each panel. The values considered as mutant for each of these affinities are indicated by an asterisk in each panel. The region of male viability is indicated by the hatched areas whereas the region of female viability is unhatched. The two male-specific lethal mutations *K_{LP} high* and *K_{LR} low* can rescue females carrying either of the two female-specific lethal mutations *K_{HP} high* and *K_{HR} low* by restoring high levels of *Sxl*$^+$ product in them. This is indicated by means of long vertical arrows in panels C and D. On the other hand neither of the two female-specific lethals can rescue the male-specific lethals *K_{LP} high* and *K_{LR} low* because they do not bring about any significant reduction in the high levels of *Sxl*$^+$ product occurring in such genotypes. As shown by the arrow in Panel B, the mutations *da* and ω rescue males carrying the male-specific lethal mutation *K_{LR} low* by bringing down the amount of *Sxl*$^+$ product to a level at which males are viable. The *mle* mutation fails to rescue females carrying either of the two female-specific lethal mutations (*K_{HP} high* and *K_{HR} low*) because it further brings down the already low level of *Sxl*$^+$ product. This is shown by the short arrows in panels C and D. In all panels, the arrows indicate the levels of *Sxl*$^+$ product reached as a result of combining the mutation denoted against the arrow with the mutation illustrated in the panel. The values of affinities used here to represent the different mutations are arbitrary. See also table 3.

Discussion

The model discussed here provides a molecular mechanism for understanding how the X/A ratio can be measured in the cells of a developing embryo. The measurement is effected by means of a series of interactions initiated by the *da*$^+$ factor which result in a characteristic levels of *Sxl*$^+$ product in the cell. This *Sxl*$^+$ product is assumed to function as an inhibitor in regulating the rate of transcription of X-linked genes (Cline, 1978).

We wish to leave open the question whether the *Sxl*$^+$ product regulates transcription of the X chromosome *en bloc* or whether there are several *Sxl*$^+$-like products regulating transcription in different sets of X-linked genes (see Chandra, 1979 for a review). We have also not discussed the consequences of duplications and deletions of the *Sxl* locus (Cline, 1978) because we do not know whether the

duplications and deletions include both the *Sxl* and π sites or not. Since the model requires that the two sites, *Sxl* and π, function in a coordinated fashion, it is not possible to predict the consequences of separating them. Nor have we discussed the results of Stewart and Merriam (1975) which seem to suggest that in flies with 2½ X chromosomes the relationship between dosage compensation and the Bridges' ratio breaks down irrespective of which chromosome arm is retained as the extra segment. These data cannot be simply interpreted in terms of our model because we do not know if the postulated relationship between Sxl^+ product and the Bridges' ratio (figure 2) also breaks down in these flies. It is possible that while this relationship is retained in such flies, breakdown occurs at the level of regulation of the rate of transcription by the Sxl^+ product. Resolution of this problem will depend to a significant extent on our understanding whether the X chromosome is regulated in a piecemeal or *en bloc* manner.

We now wish to make a few remarks regarding the implications of our model for the broader problems of dosage compensation and sex determination. We postulate that increasing levels of Sxl^+ product promote a female phenotype and, correspondingly, decreasing levels, a male phenotype. Independently of its effect on sexual phenotype, increasing levels of Sxl^+ product per X chromosome would lead to decreasing levels of X-linked gene products. Thus we consider the Sxl^+ product as having two primary roles, one in determining the sexual phenotype and the other, in dosage compensation. There are several other genes affecting sex determination (Baker and Ridge, 1980). The picture we have is that the Sxl^+ gene product initiates the pathway determining the sexual phenotype and that the other genes act subsequently.

Three predictions can be made about the role of the Sxl^+ product in dosage compensation. (i) Flies carrying the mutation Sxl^{F1} should have little or no Sxl^+ product. The rate of transcription of the X chromosome in such flies should therefore be higher than that in individuals carrying the wild type allele. This is consistent with the recent observations of Lucchesi and Skripsky (1981). (ii) Flies carrying the mutation Sxl^{M1} should have higher levels of Sxl^+ product than wild type individuals (table 2). The rate of transcription of the X chromosome in such flies should therefore be lower than in their wild type counterparts. Lucchesi and Skripsky (1981) have studied males of this genotype but their data did not permit them to distinguish between a lower rate of transcription and under-replication of the X chromosome. (iii) We expect the mutation *mle* to interfere with dosage compensation in the male by lowering the rate of transcription of the X chromosome; in the female, on the other hand, this mutation should have little or no effect (figures 3A and B). This is consistent with recent experimental data (Belote and Lucchesi, 1980a).

Three classes of data have a bearing on the relationship between Sxl^+ product and sex determination. One has to do with the sexual phenotype of flies carrying one or more of the mutations which form components of our model. For example, if Sxl^+ product is also involved in sex determination, we would predict that the sexual phenotype of (i) Sxl^{M1}/Sxl^{M1} and Sxl^{M1}/Sxl^+ females would shift in the direction of metafemales; (ii) Sxl^{F1}/Sxl^+ females would shift in the direction of

intersexes; and (iii) daughters of *da/da* mothers rescued by a single copy of Sxl^{M1} would more closely resemble intersexes than those rescued by two copies of Sxl^{M1}. A second class has to do with the effects of mutations which modulate the level of Sxl^{+} product in flies which already have an abnormal sexual phenotype. For example, an individual of the constitution AAAXX, which would normally develop as an intersex, might be expected to develop as a male under the influence of *da* and as a female under the influence Sxl^{M1} (table 2). This is in fact the observation of Cline (1981). Reasoning along the same lines, we predict that *da* would decrease the 'femaleness' of a metafemale while Sxl^{M1} would decrease the 'maleness' of a metamale along a male-female continuum (table 2). The third class of results pertains to the sexual phenotype in islands of mutant cells of one sex in a genetic background consisting of wild type cells of the other sex. In gynandromorphs, or in mosaics with viable Sxl^{M1} male tissue in a background of Sxl^{M1}/Sxl^{+} female tissue, our model suggests that the male tissue should exhibit phenotypic features of a female. Similarly, when viable, Sxl^{F1}/Sxl^{F1} female tissue within a Sxl^{F1}/Sxl^{+} background would show male characteristics. These are indeed the observations reported by Cline (1979a, b).

Finally, the following predictions can be made about the interaction of Sxl^{F1} with Sxl^{M1} and *mle*. Males carrying the mutations Sxl^{M1} or *mle* are inviable because, according to the model, there would be an overproduction of the Sxl^{+} product. Sxl^{F1} is assumed to be a mutation in the structural part of the *Sxl* locus leading to the production of inactive *Sxl* product. Since Sxl^{F1} males—which presumably have no Sxl^{+} product at all—are viable, it follows that Sxl^{F1} should rescue male embryos carrying Sxl^{M1} or *mle*. This prediction appears to be confirmed by the behaviour of two new alleles at the Sxl^{F1} region (Cline, 1981). Both these mutant alleles rescue Sxl^{M1} males from lethality. Data are not yet available for the interaction of *mle* with Sxl^{M1}.

We wish to point out that while some of the predictions made here are a direct consequence of the molecular mechanism we have proposed for the measurement of the X/A ratio, others follow from our quantitative approach to Cline's qualitative model for the behaviour of the *Sxl* and *da* mutations (Cline, 1978).

To account for certain experimental observations on levels of alcohol dehydrogenase activity in maize, Schwartz (1971) has proposed a 'gene competition' model which has certain features similar to our model for the measurement of the X/A ratio. According to Schwartz, the level of gene activity is related to the availability of a factor for which a group of genes competes. This factor, he assumes, is present in limiting concentrations. Schwartz's experimental results, which are consistent with this interpretation, suggest that such models are plausible in eukaryotic systems. Schwartz has in fact suggested that such a gene competition model may explain certain features of dosage compensation in *D. melanogaster* (Schwartz, 1973).

A feature of our model for dosage compensation is that it permits the measurement of *ratios* of the concentrations of two molecular species. This is brought out by the observation that the levels of Sxl^{+} product in the triploid female and the tetraploid male remain very close to those in their diploid counterparts throughout the range of repressor concentrations (figures 3A and B). In many

developing systems, the fate of a cell depends on its relative position with in the cell mass (Wolpert, 1971). A means for a cell to determine its relative position is to measure, for instance, the ratio of two substances ('morphogens') whose concentration gradients across the cell mass are in opposite directions. The consequences implied in looking at the problem of regulative development in such a manner are being examined by us.

Acknowledgements

We thank Dr. Arthur Chovnick and Dr. Rasika M. Harshey for drawing our attention to certain recent papers on this subject. We also thank Dr. Martin Johnson for critically commenting on an earlier version of this paper.

This work was supported by grants to H.S.C. from the Indian Council of Medical Research, to V.N. from the Indian National Science Academy and to both H.S.C. and V.N. from the Department of Science and Technology, Government of India.

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Proc. Indian Acad, Sci. (Anim. Sci.), Vol. 91, Number 6, November 1982, pp. 539–552.

Observations on the natural history and population ecology of the social wasp *Ropalidia marginata* (Lep.) from Peninsular India (Hymenoptera: Vespidae)

RAGHAVENDRA GADAGKAR, MADHAV GADGIL,
N V JOSHI and A S MAHABAL*
Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560 012, India
* Zoological Survey of India, Pune 411 005, India

MS received 23 June 1982

Abstract. *Ropalidia marginata*, the most common Indian social wasp, belongs to a crucial stage of social evolution showing no obvious morphological caste differentiation but a behavioural caste differentiation and a dominance hierarchy that appears to influence division of labour. The nests consist of a single open comb that can sometimes have up to 500 cells and 10 pedicels. Nests are initiated and abandoned all round the year. Initiation is by 1–20 foundresses, 1–4 being the most common number. There is a great deal of variation in brood developmental times both within and between nests. Male progeny disappear from the nest soon after emergence while daughters stay on at the parent nest for a mean period of about a month. Small nests have a single egg layer while large nests have two or more females with well developed ovaries that presumably lay eggs. Most nests are short-lived, small nests being highly susceptible to failure. Large nests are less susceptible to failure but the emergence of multiple egg layers reduces the average relatedness of workers to the brood which presumably is the cause for large scale emigrations from these nests. An interaction of ecological and soical factors therefore appears to determine the growth of a nest.

Keywords. Social wasp ; *Ropalidia marginata* ; natural history ; population ecology; hymenoptera ; caste differentiation.

1. Introduction

Recent years have witnessed a great surge of interest in social hymenoptera because the emergence of a considerable body of theoretical ideas (Hamilton 1964 a,b ; Lin and Michener 1972 ; Alexander 1974) have raised hopes that herein lies the key to understanding the evolution of social behaviour (West-Eberhard 1969, 1975 ; Wilson 1971, 1975 ; Jeanne 1972, 1980 ; Michener 1974 ; Trivers and Hare 1976 ; Litte 1977, 1979, 1981 ; Starr 1979). Bees and wasps are of special interest in this connection because they exemplify a series of stages in the evolution of

sociality from the completely solitary to the highly advanced eusocial species (see Evans and West-Eberhard 1970 ; Michener 1974 ; Wilson 1971).

Ropalidia marginata is the commonest social wasp of Peninsular India (Van der Vecht 1962). This species shows cooperative brood care, reproductive caste differentiation and overlap of generations (Gadgil and Mahabal 1974 ; Gadagkar 1980 ; Gadagkar and Joshi 1982b, 1983a ; Gadagkar unpublished observations) and hence can be called *eusocial* according to the classification of Michener (1969). There is no obvious morphological differentiation between egg layers and non egg layers (Gadgil and Mahabal 1974) and division of labour is brought about by a dominance hierarchy among the females belonging to a nest (Gadagkar 1980). Analysis of the time-activity budgets of adults on *R. marginata* nests has in fact revealed the presence of a behavioural caste differentiation in this primitively eusocial wasp (Gadagkar and Joshi 1982b, 1983a).

Apart from these few recent studies there is very little information in the literature about this interesting genus (Roubaud 1916 ; Carl 1934 ; Darchen 1976 ; Belvadi and Govindan 1981 ; Gadagkar and Joshi 1982a,c, 1983b). Moreover, in addition to understanding reproductive differentiation and social organization, information on the dynamics of initiation, growth and extinction of colonies is essential before we even begin to speculate about the factors that might be responsible for the origin and maintenance of sociality. We present in this paper the results of our observations on the natural history as well as population ecology of *Ropalidia marginata* in Peninsular India.

2. Materials and methods

2.1. *Study sites*

In all we have observed 125 nests of *Ropalidia marginata* from the cities of Pune (18° 30′ N and 73° 53′ E) (45 nests) and Bangalore (13° 00′ N and 77° 32′ E) (80 nests) at various times over a period of nine years from October 1971 to October 1980.

2.2. *Population fluctuations*

Our population observations include records of the numbers of pupae and adults in a nest maintained at roughly 8–10 day intervals. Such observations were maintained on three nests in Pune from October 1971 to May 1973 and for 35 nests in Bangalore from October 1974 to October 1976. The 35 nests in Bangalore were all located on the windows of one building about 20,000 sq.ft. in area and a height of about 40 ft. Our records of the population in this site also provide information on (i) seasonal variations in numbers of adult wasps, pupae and nests, (ii) seasonality of initiation and abandoning of nests and (iii) life spans of nests.

2.3. *Brood developmental times*

For one nest in Pune and two nests in Bangalore the contents of each cell in the nest were noted to provide estimates of developmental times of the eggs, larvae and pupae.

2.4. *Period of residence of adults on nests*

Every adult on two nests in Bangalore was marked with a unique spot of quick drying paint immediately upon emergence without removing it from the nest. A census of all the adults present on the nests was taken on alternate days from November 1979 to June 1980 to provide records of the total period of residence of 60 females and 3 males.

2.5. *Collection of nests*

28 nests in Pune and 31 nests in Bangalore were collected taking precaution not to bias the sampling in favour of any particular size class of nests and to collect entire combs along with all the adults and immature stages. The numbers of pedicels, cells, eggs, larvae, pupae and adults were determined. The adults were sexed and the females were dissected to determine the state of development of their ovaries. The females were classified arbitrarily into 3 catagories : those with undeveloped ovaries, those with moderately developed ovaries and those with well developed ovaries on the basis of maximum ovariole width. Those classified as 'with well-developed ovaries' appeared to have mature eggs and were probably laying eggs. These females are designated as egg layers. We do not however know if all females with well-developed ovaries actually laid eggs.

3. Results

3.1. *The nest and its structure*

R. marginata builds nests with simple, open (Gymnodomous according to the classification of de Saussure (1853–59) and Richards and Richards (1951)) combs the construction of which begins with the laying down of the pedicel which is usually 5–10 mm long and about 1 mm thick. The first cell is constructed at the tip of this pedicel and the subsequent cells are added either all round the first cell or only on one side so that in larger combs the initial pedicel may either end up being approximately in the centre of a layer of cells or at one extreme end. As the comb grows in size the initial pedicel is enlarged in width and may grow up to about 5–6 mm in diameter in large combs. In addition to enlarging the original pedicel, new thin pedicels (about 1 mm in diameter) that reinforce the attachment of the comb to the substratum are added at several points. Most small combs ($<$ 100 cells) have a single pedicel while large combs ($>$ 100 cells) often have more than one pedicel (table 1). The largest comb we have recorded had about 500 cells and 10 pedicels, the latter also being the largest number of pedicels recorded on a comb.

All but one of the nests recorded, has a single comb per nest. In one case however, there were two combs within about 20 mm of each other and the adults clearly moved between these two combs.

3.2. *Initiation of nests*

Nests of *R. marginata* are initiated and abandoned all round the year (table 2). New nests are initiated by 1–20 females, 1–9 being the commonest number (figure 1).

Table 1. Nests with different number of pedicels

Number of cells in nest	Frequency of nests with different number of pedicels					
	1 Pedicel	2 Pedicels	3 Pedicels	4 Pedicels	7 Pedicels	10 Pedicels
1–100	17	3	...	...	...	...
101–200	1	...	2	...	...	...
201–300	...	...	...	...	...	...
301–400	1	1	...	1	...	...
401–500	...	1	2	...	1	1

Table 2. Year-round initiation and abandoning of nests*

Month	Number of nests initiated	Number of nests abandoned
January	4	3
February	0	3
March	0	1
April	3	2
May	5	2
June	1	8
July	1	2
August	8	7
September	3	1
October	2	1
November	4	2
December	0	0

Data pooled from observations throughout the study period both in Bangalore and Pune.

In many cases the initial single foundress appears to be joined by other females within a few days of initiation of the nest. When newly emerging females were marked with spots of paint, it was noticed that some of the newly emerged individuals were not spending every night on the parent nest but were occasionally missing for 2–3 days before returning to it. It is possible that these individuals had been visiting other newly founded nests on the nights they were absent. There were emigrations of large number of adults from nests which had grown to more than 40–50 adults in size. Groups of individuals from these exoduses probably constitute the initial set of foundresses for many nests.

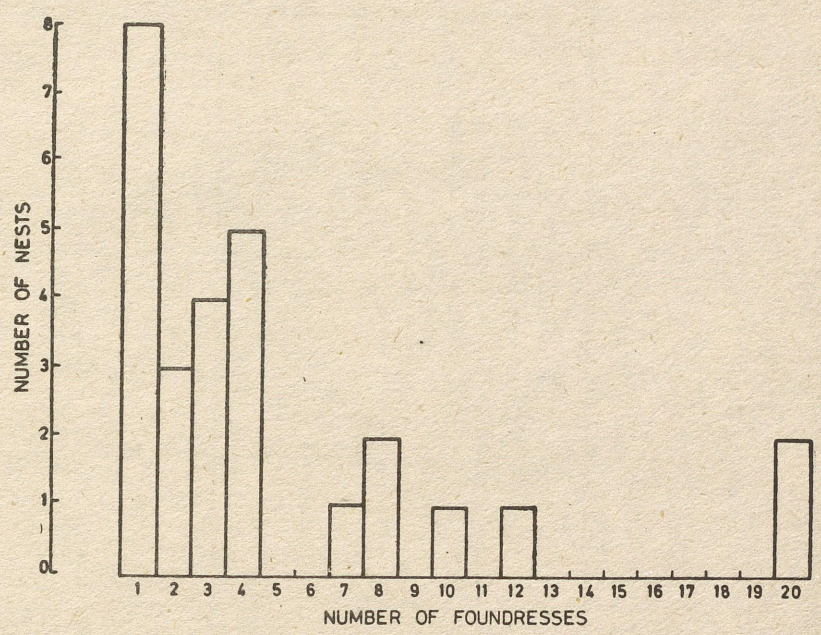

Figure 1. Frequency distribution of the number of nests with different numbers of foundresses.

3.3. *Brood developmental times*

The accurate determination of brood developmental times is beset with a number of problems and the estimates given here are only to be treated as first approximations. The duration of the egg, larval and pupal stages both in Pune and Bangalore are given in table 3. In each stage the duration in Pune is much less than in Bangalore. This difference could either be a genuine difference due to different environmental conditions in Pune and Bangalore. However, it cannot be ruled out that the differences are simply a result of small sample sizes in terms of the number of nests studied. The data in Pune in fact represent a single nest and that in Bangalore two nests. The difference could therefore be simply a manifestation of different stages in the nest cycle or of different local conditions. The nest in Pune, may have been located close to a good food source and therefore the difference may not even reflect differences between Pune and Bangalore as such.

The data both from Pune and Bangalore show a very great degree of variation. The standard deviations are close to half or sometimes more than half of the mean. The wide variation in egg developmental times is primarily because there is a significant degree of egg cannibalism which remains undetected. Eggs are eaten and replaced by new ones and several consecutive replacements may occur before an egg successfully hatches into a larva. The variation in larval developmental times almost certainly reflects differences in food supply. A larva can complete development and pupate in as little as 7 days in Bangalore under laboratory conditions when the adults feeding the larva are provided with an *ad libitum* food supply (Gadagkar, unpublished observations). The variations in pupal develop-

Table 3. Brood developmental times*

	From weekly observations in Pune			From daily observations in Pune			From weekly observation in Bangalore		
	Mean	Standard deviation	Sample size	Mean	Standard deviation	Sample size	Mean	Standard deviation	Sample size
Egg	18	13	1221	12	8	64	27	15	43
Larva	15	10	1052	10	5	37	22	7	28
Pupa	16	8	1071	14	6	45	29	11	16

* All means and standard deviations are in days.

mental times are the hardest to understand. The hypothesis that a strong correlation between larval and pupal developmental times is the cause of this variation is not borne out because we find that the correlation coefficients between larval and pupal developmental times are not significantly different from zero at 5% level. This is true even in the large sample size from weekly observations in Pune.

3.4. *Duration of residence of adults on the nest*

In all nests in which the newly emerging adults were marked it was observed that the males always disappeared within two to four days of emergence. While some females disappeared very soon after emergence, others stayed on at the parent nest for long periods of time. On two nests all the emerging adults (a total of 75 females and 3 males) were marked. The 3 males disappeared from the nest within 2, 3 and 4 days respectively of emergence. Of the 75 females we have information on the duration of residence on the nest for 60 females that disappeared before the end of our study. The frequency distribution of residence times for these 60 females is shown in figure 2. This corresponds to a (mean ± SD) residence time of 27 ± 23 days and a range from 1–160 days. When a wasp disappears from one nest it may either have died (mortality) or initiated or joined another nest (emigration). In our records these two components cannot be distinguished directly. The (mean ± SD) age-specific day to day probability of remaining at the same nest (inset, figure 3) has a value of 0·95 ± 0·04 which is nearly constant with age. This seems to suggest that mortality as opposed to emigration forms a very large component of our estimates. The reasoning behind this is that mortality seems to occur during the foraging trips because the wasps simply do not return to the nest at the end of the day. Perhaps they are lost or preyed upon. It is reasonable to assume that the probability of these events would be

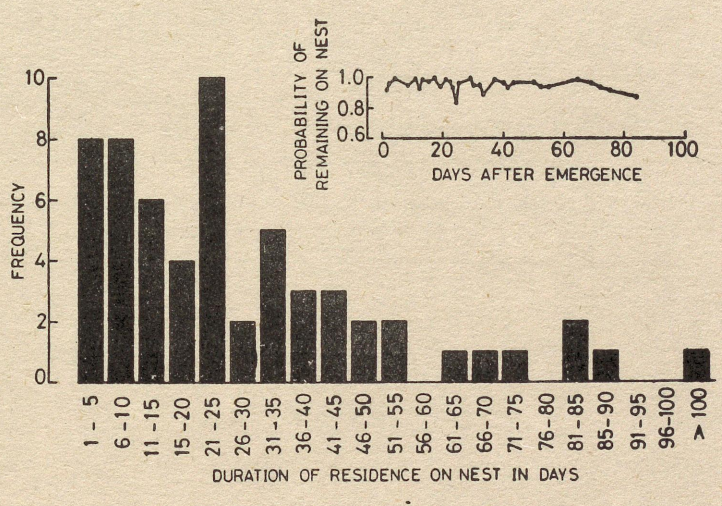

Figure 2. Frequency distribution of residence times on a given nest of 60 female wasps of *R. marginata*. The age-specific day-to-day probability of remaining at the same nest (inset) of 0·95 ± 0·04 is nearly constant with age. Note that most of the points lie between 0·90 and 1·0.

independent of the age of the animal but that the probability of emigration to found or join another nest would show some age dependence.

3.5. *Reproductive differentiation*

Our dissections from the harvested colonies indicate that although there is no morphological differentiation amongst the females, there is a marked differentiation amongst them in terms of development of ovaries. In all the nests, a majority of the females possessed rudimentary, completely undeveloped ovaries, while only 1 to 6 females possessed moderately or well developed ovaries. In most cases, the females with well-developed ovaries tended to be heavier in weight than the other females (Gadgil and Mahabal 1974). In addition there is a dominance hierarchy amongst the females at a nest with the dominant females doing less foraging (Gadagkar 1980). There is extensive food sharing at the nests of *R. marginata*, and since frequency of dominance behaviour and snatching food are significantly correlated (Gadagkar and Joshi 1983a) it is quite plausible that the dominant individuals get a disproportionately greater share of the food, while expending less energy on foraging. They may thus be able to grow heavier and develop their ovaries, while the less dominant individuals, the workers, suffer from 'nutritional castration'.

The number of females with developed ovaries does not bear any clear relation to the total number of females on the colony ; while it shows evidence of an increase with the number of cells in the comb (table 4). Thirty out of 32 nests with less than 100 cells had a single egg-layer, while 14 out of 17 larger nests had 2 or more. The number of cells in a comb is a good indicator of the age of the colony, while the number of females in a colony keeps constantly fluctuating because of periodic large scale emergence and emigrations. We may therefore conclude that the number of egg-layers in a colony increases with the age of the colony. Initially, at the founding, a single female dominates and monopolises

Table 4. Nests with different numbers of egg layers

Number of cells in nest	Frequency of nests with different numbers of females with well developed ovaries (egg-layers)					
	1 egg-layer	2 egg-layers	3 egg-layers	4 egg-layers	5 egg-layers	6 egg-layers
1–100	30	2	1	0	0	0
101–200	2	1	1	1	1	2
201–300	1	0	0	1	0	0
301–400	2	1	0	1	0	0
401–500	0	1	1	0	0	1

all egg-laying ; as the colony develops, this monopoly is broken and other females again the heavier, more dominant ones, also begin to lay eggs.

3.6. *Population fluctuations*

Nests of *R. marginata* that have long life spans are characterised by continuous fluctuations in the number of adults. Figures 3 and 4 represent the population changes at two nests which grew to a considerable size and lasted for two years or more. In both cases the number of adults on the nests increased initially and following one or more mass emigrations, remained fluctuating for several months at less than 20 adults. In the case of the first nest (figure 3) there were four clear cut instances of mass emigrations. These involved 30 or more adults leaving the nest perhaps to initiate other nests nearby. In the case of the second nest (figure 4), there was a single major exodus, apparently in direct response to predation on the nest by *Vespa tropica*. This large wasp feeds on eggs, larvae and pupae of *R. marginata*. The particular nest depicted in figure 4 was under continual observation, and it is known that the mass exodus followed the first ever visit of the predator to the nest. The predator continued to regularly visit this nest thereafter, and apparently kept the population in check for a year or so. Beyond this period, the nest failed to grow further, although the visits of the predator apparently ceased.

We have rather complete information on population fluctuations at one site in Bangalore where we observed all the nests present at that site for a period of 104 weeks. In all, 35 nests were observed at this study site. The number of nests and the total population of adults and pupae present in all the nests at different times at this site are shown in figure 5. The total population of adults varied between 70 and 400, the population of pupae between 0 and 340 and the total number of nests present at any given time varied between 8 and 16. The largest number of adults were present during January to April in both years. However, the number of pupae and that of the nests seemed to fluctuate rather widely.

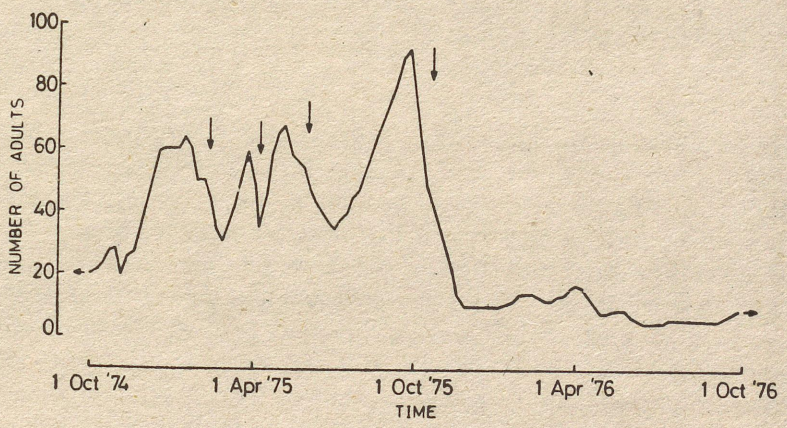

Figure 3. Number of adults at a *Ropalidia marginata* nest in Bangalore. The arrows indicate mass exoduses.

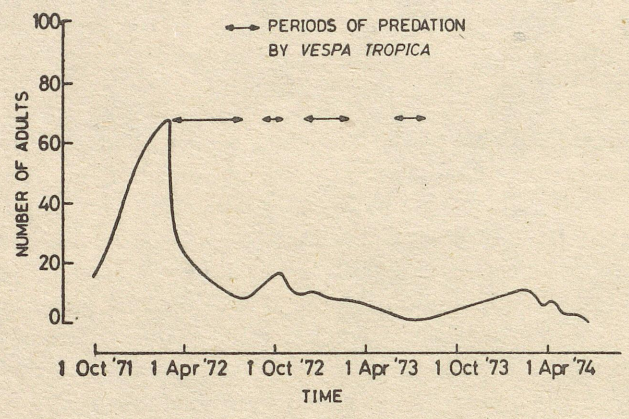

Figure 4. Number of adults at an *R. marginata* nest in Pune. There was a single exodus following the first instance of predation on the nest by *Vespa tropica*. Arrows indicate periods of regular predation by this wasp.

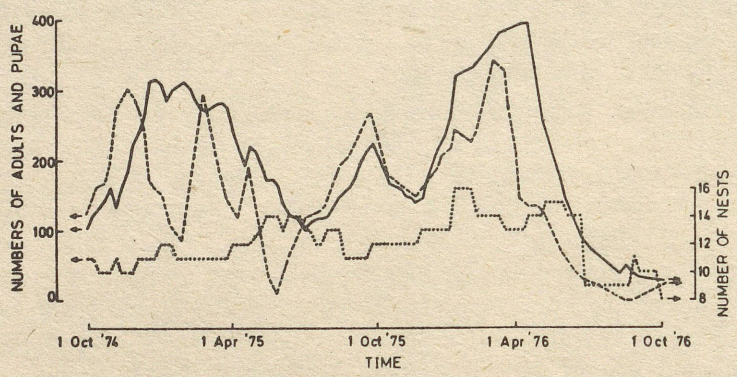

Figure 5. The total number of adults (left ordinate and solid line) and pupae (left ordinate, broken line) and the total number of nests (right ordinate and dotted line) present at different times at a single study site in Bangalore over the period of 104 weeks.

The long lived nests represented in figures 3 and 4 are only a small proportion of the total nests. Most of the nests in fact have a shorter life span. The total life span of 18 nests is known because both the initiation and abandoning of these nests occurred during the period of study. The frequency distribution of total life span of these nests (figure 6A) shows that most nests (70%) have a live span of 10 weeks or less. There was only one nest among these that survived for longer than 30 weeks. However, estimates of the total life spans obtained from any finite period of study is likely to be biased in favour of short lived nests. The distribution of minimum life spans, *i.e.*, where either initiation or abandoning alone were observed (figure 6B) reveals that 10 out of 16 additional nests survived for longer than 30 weeks. Moreover, for one nest neither initiation nor abandoning was observed ; in other words, it survived for longer than 104 weeks (the dura-

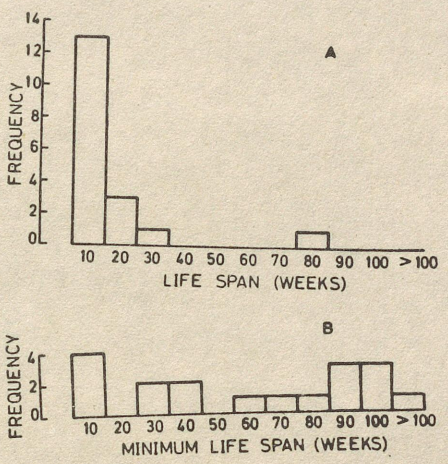

Figure 6. Frequency distribution of total life spans (A) and minimum life spans (B) of *R. marginata* nests. Total life span is defined as the time interval between initiation and abandoning of a nest and is therefore known only for those nests for which both initiation and abandoning occurred during the period of study. Minimum life span is given only for those nests for which either the initiation or abandoning alone is known.

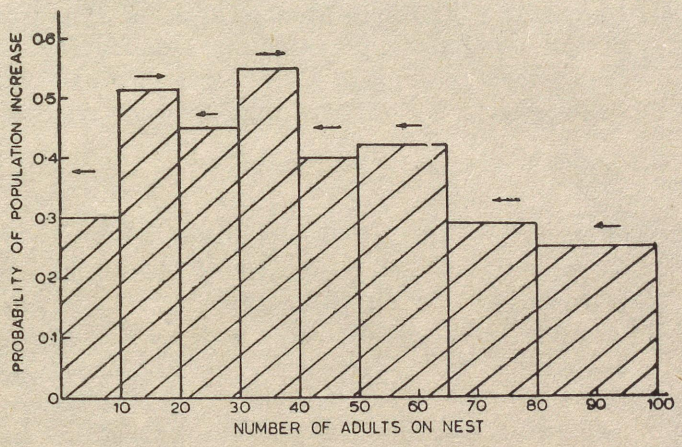

Figure 7. Probability of increase in adult numbers as a function of number of adults already present. The arrows indicate the expected change on the mean in the number of adults in colonies of various sizes.

tion of observation). Thus, although most nests are short lived, some do survive for very long periods of time.

Figure 7 presents further analysis of the population fluctuations. Here we present the probability of increase in the number of adults at a nest as a function of the number of adults already present. These probabilities have been computed by pooling together our data for the 8 nests monitored for over 2 years. As can

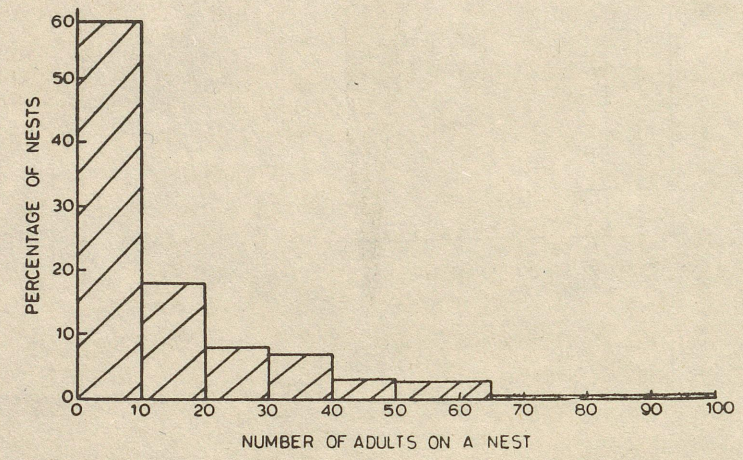

Figure 8. Frequency distribution of the total population of *R. marginata* nests in terms of the number of adults present on the nest.

be seen, the smallest nests have the lowest probability (only 0·3) of further increase in number. They are thus nests most susceptible to extinction. The only sizes at which the nests have near even or better than even chance of increase are between 10 to 40. Thus, a nest which has increased to this level may further increase rapidly till it crosses 40 adults. Beyond this, the nests tend to have a high probability of decrease (due to mass exoduses). The resultant size frequency distribution of nests is presented in figure 8. The vast majority of the nests have less than 10 adults, most go extinct without getting beyond this stage.

4. Discussion

According to the theory of kin selection (Hamilton 1964a, b ; 1972 ; West-Eberhard 1975) the rationale for the development of sociality in ants, bees and wasps lies in their haplodiploid system of sex determination. Because of this, a female wasp is genetically more closely related to her sister than she is to her daughter, and it is therefore more 'advantageous' for a female wasp to help her mother raise daughters which would be her sisters, than to attempt to raise daughters by herself. It is believed that this is why females are selectively favoured to stay on with their mother and help her with the colony labour. At the same time, sons are more closely related to females than brothers are ; hence the workers would have a tendency to lay male eggs, and the males themselves would not share in the colony labour (Hamilton 1964a, b ; Wilson 1971 ; West-Eberhard 1975 ; Trivers and Hare 1976).

Wasp nests with multiple foundresses and multiple egg layers do not fall neatly in this scheme, particularly if the egg-laying females themselves were not close relatives. We however know that in the case of *Polistes* the foundresses do in fact tend to be sisters (West-Eberhard 1969 ; Ross and Gamboa 1981). This system of multiple foundresses can evolve if the nests are highly susceptible to failure in the early stages of growth. Then, if the coming together of several

females increases the probability of success of a nest by a factor of 1·5 or more, sisters may band together, and relinquish reproduction to the most dominant female as the female brood they are raising will be related to them as nieces with coefficient of relatedness = 0·375. If a single female remains reproductive, the workers of the later brood will be raising their sisters with coefficient of relatedness = 0·75.

If, however, more than one of the founding sisters starts to lay, the workers will now be raising a brood related at least in part to them as first cousins coefficient of relatedness = 0·19. At this point the workers may find it more advantageous to leave the nest and attempt to initiate one on their own. This tendency will increase with an increase in the number of egg-layers in the nest.

As discussed earlier, the small nests of *R. marginata* are in fact highly susceptible to failure, hence the banding together of several foundresses is expected. We have no evidence that these are sisters, though this is plausible as new nests are very often founded close to old ones and the foundresses are likely to be sisters who leave together in an exodus from a nest.

We have also shown that there is a tendency for mass exoduses from nests with over 40 adults. This may be related to these being older nests with multiple egg-layers in which the average degree of relationship between the workers and the brood would tend to be low, making it less advantageous for the workers to stay on at nest. Difficulties of sustaining a larger number of adults on the food resources of the home range could be ruled out as a major factor since the new nests are often founded next to the parental nest and must therefore utilize much the same food resources.

In conclusion it appears that ecological pressures render small nests highly susceptible to failure and therefore necessitate the banding together of several females. As the nest grows in size, a single female can no longer dominate it to the level of exclusively monopolizing all egg-laying. With the emergence of multiple egg-layers the workers are at less of an advantage in remaining on the nest and hence begin to leave in significant numbers producing large population fluctuations. An interaction of ecological and social pressures thus determine the course of growth of a nest.

Acknowledgements

We are grateful to O W Richards for his kind help in identifying the *R. marginata* and *V. tropica* material.

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