Partial Pre-Determination of Caste in a Primitively Eusocial Wasp: Number of Empty Cells on Parent Nest Predict Probability of Egg Laying by Eclosing Females.

Raghavendra Gadagkar[1], C.Vinutha[1], Ashok Shanubhogue[2] and A.P.Gore[2]

[1] Centre for Ecological Sciences, Indian Institute of Science, Bangalore, 560012 INDIA and [2] Department of Statistics, University of Poona, Pune 411007, INDIA

December 1986

Technical Report No. 42

Centre for Ecological Sciences, Indian Institute of Science

Bangalore, 560012 INDIA

INTRODUCTION

Primitively eusocial insects (Michener, 1969) constitute attractive model systems for the study of reproductive competition, a major concern of Sociobiology (Fletcher and Ross, 1985; Hamilton, 1972; West-Eberhard, 1979; Wilson, 1971). The lack of morphological caste differentiation makes possible a considerable degree of plasticity in the social and reproductive roles that the adult insects can adopt. Newly emerged female wasps of the genus *Ropalidia* for instance, may leave their parental nests to found new ones either singly or in groups or may stay back and eventually challenge and replace the original queen (Gadagkar and Joshi, 1983; 1984; 1985; Gadagkar, 1985). Death or experimental removal of a queen often results in immediate take over of the queen's role by one of the subordinates and this can happen repeatedly leading to a succession of queens (West-Eberhard, 1969; Jeanne, 1972; Litte, 1971; Gadagkar, 1986; Strassman and Meyer, 1983).

A question of obvious interest is whether all females are potentially capable of assuming the role of the queen, an answer to which is clearly crucial before one begins to construct models for the complex mix of selfish and altruistic behaviours shown by these animals. Yet there has never been a direct test of this question even in the extensively studied temperate zone polistine wasps where, spring-time foundress associations show much of this flexibility. Here we report one attempt using the primitively eusocial wasp *Ropalidia marginata*. Our null hypothesis is that "all eclosing females are potentially capable of laying eggs but most are prevented from doing so by the dominant behaviour of their nestmates". Our experiments permit rejection of the null hypothesis and also suggest that the number of empty cells in the nest from which a female ecloses as well as its own rate of feeding during adult life are important predictors of whether a female can potentially become an egg layer.

MATERIALS AND METHODS

22 naturally occuring nests of *R.marginata* were collected, cleared of adults and maintained in the laboratory for eclosion of females. Upon eclosion the females were isolated into individual cages, never allowed to come into contact with any conspecific but provided the set of conditions under which this species readily initiates nests and produces large numbers of apparently healthy offspring in the laboratory (Gadagkar,R Unpublished results). In this manner the females were maintained until they began to lay eggs or died.

RESULTS

Of 197 female wasps so tested, 97 laid eggs (termed egg layers) while 100 females died without doing so (termed non egg layers) inspite of living, on the average (mean life span S.D = 87.4 66.5 days), longer than the time taken by an average egg layer to lay its first egg (61.7 37.8 days) (Student's t test, $p < 0.0005$). These results clearly indicate a heterogenity in fertilities of the wasps inspite of their never being subject to domination by conspecifics and thus call for rejection of the null hypothesis.

It is of obvious interest to understand the correlates, if any, of egg laying and non egg laying. The time of year during which an animal ecloses is clearly not such a correlate. The numbers of egg layers and non egg layers among females eclosing during different seasons are not significantly different from each other ($\chi^2 = 3.52$; df = 3, p> 0.05) (Table 1).

Each wasp in our experiment either lays an egg or dies without doing so. Our dependent variable is thus of binary nature and may be influenced by a variety of independent variables. To handle such a situation we use the method of logistic regression analysis (Cox, 1970). In the first model the following properties of the nest from which an animal ecloses are considered as independent variables potentially capable of influencing an animal's probability of egg laying : numbers of eggs, larvae, pupae, parasitised cells, empty cells, males and females. Only one regression coefficient, the one associated with the number of empty cells is significantly different from zero. (Table 2, p<0.02). In the second model the following variables associated with the individual animals themselves are considered as potentially capable of influencing an animal's probability of laying eggs : rate of food intake during adult life, inter-ocular distance, ocello-ocular distance, head width, head length, mesoscutellum width, mesoscutellum length and wing length, all but the first being indices of body size. Here the estimated regression coefficient associated with feeding rate is very significantly different from zero (Table 2, p<0.007). The coefficient associated with one of the indices of body size namely, ocelloocular distance is also significantly different from zero (p <0.05). This however is not as highly significant as the others and also appears, somewhat paradoxically, to negatively influence the probability of egg laying. Besides, none of the several other indices of body size

Table 1. Numbers of Egg Layers and Non Egg Layers Among Animals Eclosing in Different Seasons

Season	No.of Egg Layers	No.of Non Egg Layers
Nov-Dec-Jan	41	55
Feb-Mar-Apr	30	22
May-June-July	9	9
Aug-Sep-Oct	17	14

Chi Square Value = 3.52 Degrees of Freedom = 3

$P > 0.05$

Legend to Table 2

Logistic Regression Analysis: Independent variables such as numbers of eggs, larvae etc. on the parent nest (model 1) or indices of body size and feeding rate (model 2) are modelled to influence the probability of egg laying by females such that:

$$\ln\left[p/(1-p)\right] = \beta_0 + \Sigma \beta_i X_i$$

The equation $p = e^{\beta_0 + \Sigma \beta_i X_i} / 1 + e^{\beta_0 + \Sigma \beta_i X_i}$ is solved using the maximum liklihood criterion: $L = \left(\prod_{i=1}^{m} p_i\right)\left(\prod_{i=m+1}^{n} \left[1-p_i\right]\right)$

where p is the probability of becoming an egg layer, 1-p the probability of becoming a non egg layer, β_0 = intercept, $\beta_i{}^s$ = the regression coefficients, X_i s the input variables, L the liklihood, animals 1 to m are the egg layers and animals m+1 to n the non egg layers.

Table 2. Logistic Regression Analysis: Determinants of probability of Egg laying by females of *R. marginata*.

Variable	Estimated Coefficient (Beta)	Standard Error	Z Value
Model 1: Nest Properties as Determinants of the Probability of Egg Laying By Eclosing Females			
Intercept	-0.1854	0.3335	-0.5558
No. of Eggs	-0.0072	0.0155	-0.4641
No. of Larvae	0.0060	0.0150	0.4020
No. of Pupae	0.0101	0.0225	0.4472
No. of Parasitised Cells	-0.1536	0.1489	-1.0315
No. of Empty Cells	0.0519	0.0218	2.3845**
No. of Males	0.1753	0.1771	0.9896
No. of Females	-0.0111	0.0194	-0.5700
Model 2: Feeding rate and Body size as Determinants of the Probability of Egg Laying			
Intercept	1.5631	4.1547	0.3762
Feeding rate	3.4993	1.2871	2.7188***
Inter ocular Distance	-6.4072	7.1170	-0.9003
Ocello Ocular Distance	-11.7276	5.9464	-1.9722*
Head Width	1.2445	1.4517	0.8573
Head Length	1.3271	1.4336	0.9257
Meso Scutellum Width	- 1.0160	1.5312	-0.6635
Meso Scutellum Length	1.1687	1.3327	0.8770
Wing Length	- 0.2291	0.3216	-0.7125

* = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.007$

tested by us appear to have any influence. We therefore prefer to defer interpretation of the significance of ocelloocular distance. Note that both models show good fits to the observed patterns of egg laying (Table 3).

Variables which are not properties of individual wasps but of the nests from which animals eclose can also be handled in another fashion. For each nest one can compute the proportion of egg layers among animals eclosing from that nest. Parametric and non parametric correlation coefficients are computed between (1) the proportion of egg layers among animals eclosing from a nest and (2) various nest properties such as those used in the logistic regression analysis or, simple transforms of these variables. The only correlation coefficients statistically significantly different from zero are once again those associated with the numbers of empty cells (Figs. 1 and 2 and Table 4). In summary, wasps that eclose from nests with relatively large numbers of empty cells and and those that feed well during their adult life have relatively high probabilities of becoming egg layers. Conversely wasps eclosing from nests with relatively few empty cells and those that feed poorly during their adult life have relatively low probabilities of becoming egg layers.

Admittedly we have tested virgin wasps under artificial conditions but, all conditions were identical for all the animals. Nevertheless, only about 50 % of the animals laid eggs making it very likely that caste is at least partially determined prior to eclosion. Postulating that the queen would in some way be involved in caste differentiation of her nest's brood, we propose the following

Legend to Table 3

The goodness of fit of the Logistic Repression models is tested as follows: p, the probability of becoming an egg layer is computed for each animal using the above equations. Dividing all probability values from 0 to 1 into 20 equal probability classes, the mean p value of all the animals falling in any probability class multiplied by the total number of animals in the experiment (197) gives the expected number of egg layers in that probability class.Similarly, 1- the mean probability multiplied by the total number of animals in the experiment gives the expected number of non egg layers in that probabilty class. The observed number of egg layers and non egg layers among animals falling in each probability class being known, a χ^2 test is performed. The observed and expected numbers of egg layers and non egg layers are not significantly different (model 1: $\chi^2 = 0.7142$, df = 4, $p>0.9$ and model 2: $\chi^2 = 5.9116$, df = 6, $p>0.1$) indicating the goodness of fit of the model.

Table 3

Probability Class	Egg Layers		Non Egg Layers	
	Observed	Expected	Observed	Expected
Model 1: Nest Properties as Determinants of the Probability of Egg Laying				
0.00 - 0.05	0	0.0	0	0.0
0.05 - 0.10	0	0.0	0	0.0
0.10 - 0.15	0	0.0	0	0.0
0.15 - 0.20	0	0.0	0	0.0
0.20 - 0.25	1	1.0	4	4.0
0.25 - 0.30	0	0.0	0	0.0
0.30 - 0.35	7	6.1	12	12.9
0.35 - 0.40	3	2.3	3	3.7
0.40 - 0.45	10	11.6	17	15.4
0.45 - 0.50	31	32.2	37	35.8
0.50 - 0.55	15	14.6	12	12.4
0.55 - 0.60	8	7.0	4	5.0
0.60 - 0.65	15	14.9	9	9.0
0.65 - 0.70	0	0.0	0	0.0
0.70 - 0.75	0	0.0	0	0.0
0.75 - 0.80	0	0.0	0	0.0
0.80 - 0.85	7	7.2	2	1.8
0.85 - 0.90	0	0.0	0	0.0
0.90 - 0.95	0	0.0	0	0.0
0.95 - 1.00	0	0.0	0	0.0
Model 2: Feeding Rate and Body size as Determinants of Probability of Egg Laying				
0.00 - 0.05	0	0.0	0	0.0
0.05 - 0.10	0	0.0	0	0.0
0.10 - 0.15	0	0.0	0	0.0
0.15 - 0.20	0	0.2	1	0.8
0.20 - 0.25	3	1.6	4	5.4
0.25 - 0.30	1	4.2	14	10.8
0.30 - 0.35	3	3.5	8	7.5
0.35 - 0.40	9	10.2	18	16.8
0.40 - 0.45	12	8.9	9	12.1
0.45 - 0.50	13	13.4	15	14.6
0.50 - 0.55	12	10.0	7	9.0
0.55 - 0.60	12	11.0	7	8.0
0.60 - 0.65	8	10.6	9	6.4
0.65 - 0.70	10	9.5	4	4.5
0.70 - 0.75	9	7.4	1	2.6
0.75 - 0.80	3	2.3	0	0.7
0.80 - 0.85	1	3.3	3	0.7
0.85 - 0.90	0	0.0	0	0.0
0.90 - 0.95	0	0.0	0	0.0
0.95 - 1.00	1	1.0	0	0.0

Model 1: =0.71, df = 4, $p > 0.9$. Model 2: =5.92, df = 6, $p >0.1$f

Legend to Figures 1 and 2

Scatter diagram (Fig. 1) showing the proportion of egg layers among animals eclosing from different nests and the numbers of empty cells on each nest. Pearson product moment correlation = 0.37, a value not significantly different from zero (Student's t test, $p>0.5$) but much higher than the correlation obtained between the proportion of egg layers and other nest properties such as numbers of eggs, larvae, pupae, parasitised cells, males and females (correlation coefficient obtained with these other parameters range from -0.4 to 0.19). One data point (nest 14) marked with a circle in the figure shows a proportion of egg layers of 1.0 when the number of empty cells is 0, a situation radically different from the general trend of a weak positive correlation . Upon deletion of this data point the correlation coefficient between the number of empty cells and proportion of egg layers is significantly greater than zero (Student's t test, $p<0.05$) (see table 2). Besides correlations with no other nest properties approaches statistical significance when this or any other data point are deleted one at a time (Fig.2). This coupled with the results of the logistic regression analysis points to the role of the number of empty cells in predicting the probability of egg laying by these wasps. All correlation coefficients are henceforth calculated after deleting data for nest 14 (table 2).

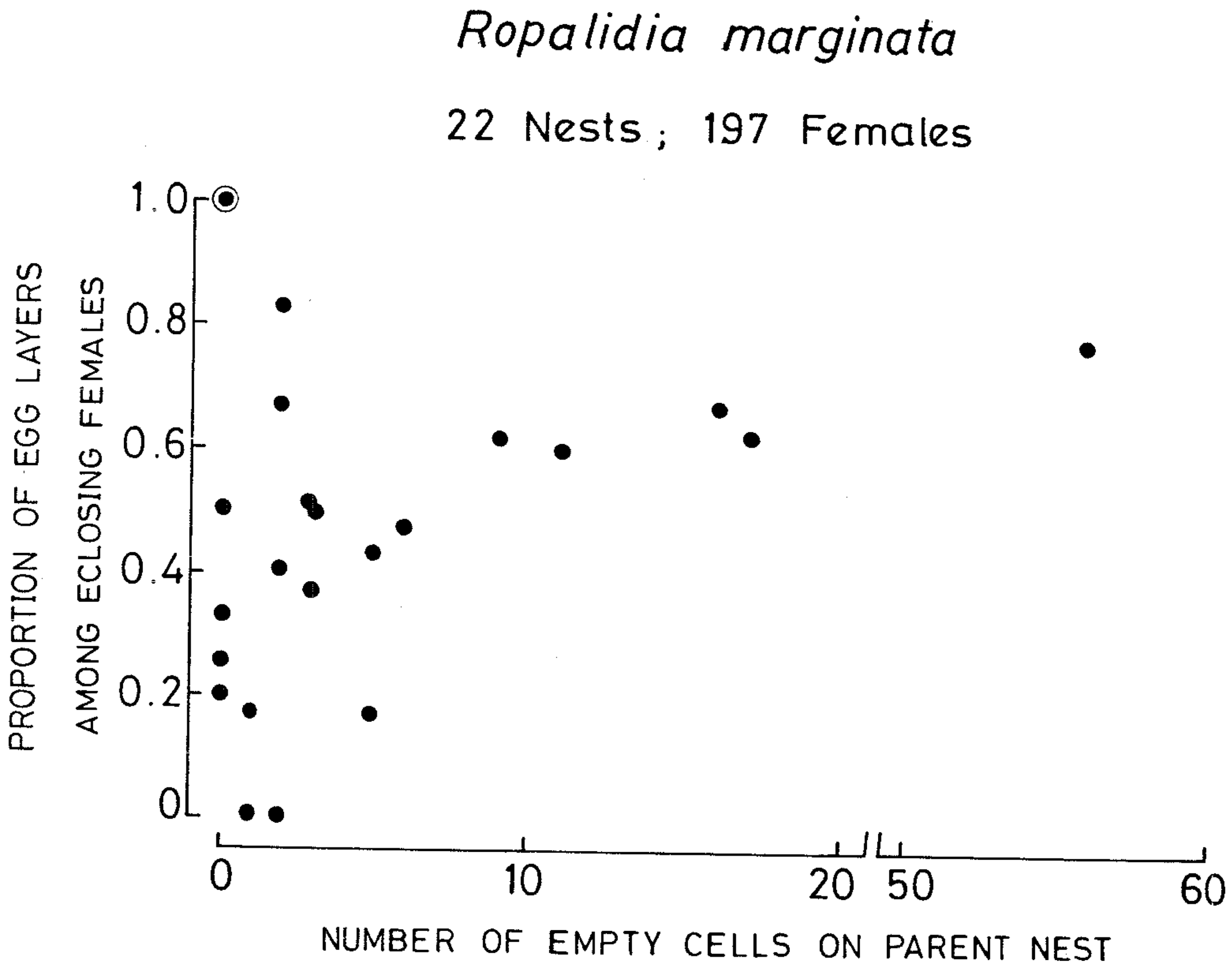
Ropalidia marginata
22 Nests ; 197 Females
PROPORTION OF EGG LAYERS
AMONG ECLOSING FEMALES
1.0
0.8
0.6
0.4
0.2
0
0
10
20
50
60
NUMBER OF EMPTY CELLS ON PARENT NEST

FIG. 1

Ropalidia marginata

CORRELATION BETWEEN

PROPORTION OF EGG LAYERS AND NEST PROPERTIES

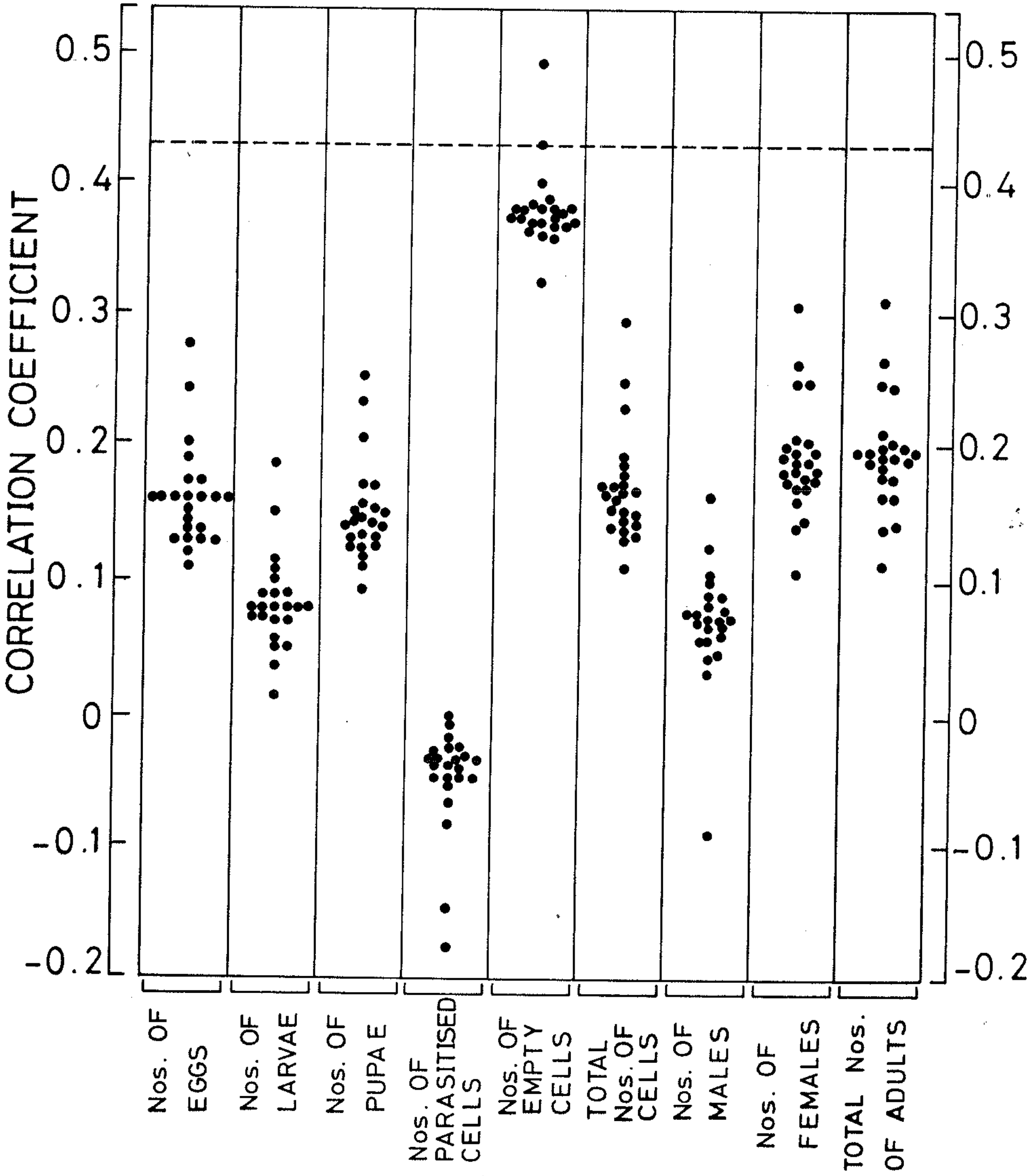

FIG 2

Legend to Table 4

Pearson product moment correlations are computed between (1) the proportion of egg layers among animals eclosing from each nests (p) and (2)various properties of the nests (n) (cases 1-4). The dependence of p on n could however be non linear. We have therefore investigated three simple non linear relationships and calculated correlation coefficients between the following suitably transformed variables : case 2 = $\ln(1-p)$ vs n: case 3 = $\ln(1-p)$ vs $\ln(1+n)$; case 4 = $1/(1-p)$ vs n. To take into account the possibility that some of our variables may not be normally distributed Kendall's coefficient of rank correlations are also computed (case 5).

Table 4. Correlation Coefficients between the proportion of egg layers eclosing from different nests and various properties of the nests.

Nest Property.	Case: I	II	III	IV	V
No. of eggs	0.27	-0.18	-0.12	0.05	0.13
No. of Larvae	0.19	-0.10	-0.12	-0.01	0.15
No. of Pupae	0.23	-0.14	-0.25	0.03	0.28
No. of Parasitised Cells	0.01	-0.01	-0.08	0.00	-0.06
No. of Empty Cells	0.49*	-0.54**	-0.56**	0.52**	0.40***
Total No. of Cells	0.29	-0.21	-0.21	0.09	0.23
No. of males	0.13	-0.09	-0.05	0.03	-0.17
No. of Females	0.31	-0.25	-0.26	0.16	0.29
Total No. of Adults	0.31	-0.25	-0.26	0.16	0.27

Student's t test * = $p<0.05$; ** = $p<0.02$; *** = $p<0.01$

conceptual model for what might be called partial pre-determination of caste (Fig. 3) (For a recent overview of caste determination in social Hymenoptera (see Wheeler, 1986). In a normal healthy nest cells almost never remain empty. Queens usually lay eggs in cells vacated by pupae within hours of their becoming vacant. When new cells are being constructed, an egg is usually laid even while the new cell is barely large enough to hold an egg; proper shaping and elongation of the cell take place only after the cell has an egg in it. We believe therefore that the accumulation of empty cells in a nest, especially when the number of empty cells is more than just one or two that could be left by chance alone, is a very strong indicator of the queen's declining influence on the nest. This could happen due to old age or poor health of the queen. We suggest that it could also happen temporarily and in a programmed fashion during a certain phase of the nesting cycle that may be devoted to the production of reproductives. Tropical species such as *R.marginata* have perennial nests and the little that we do know about their nesting cycle is consistent with the idea that workers and reproductives are produced alternately in a series of cycles (Gadagkar et al, 1982).

Whatever the cause, our model envisages that the queen's declining influence activates a set of processes (designated X in Fig 3) which, on one hand cause the brood to develop into adults programmed to feed more and differentiate into high probability reproductives and on the other hand cause empty cells to accumulate in the nest. To complete the scenerio we must imagine that when the queen is young and healthy or when the nest is in a phase devoted largely to the production of workers, the queen's influence would be high. As a

Legend to Figure 3

A conceptual model for partial pre determination of caste in the primitively eusocial wasp R.marginata. The left half of the figure depicts the set of processes that are expected to operate in nests which are in a phase of worker production while the right half depicts the set of processes that might operate in nests in the queen production phase. The main point made in this model is that the numbers of empty cells are not hypothesised to be causal factors in caste determination but indirect consequences of the same set of processes that lead to caste determination. The rate of feeding by the adults, on the other hand, is postulated to be a link 'in the programming for caste determination. The underlying processes that are involved in such programming for caste determination are of course unknown and thus simply labelled X and Y.

A MODEL FOR CASTE PRE-DETERMINATION IN THE PRIMITIVELY EUSOCIAL WASP *ROPALIDIA MARGINATA*

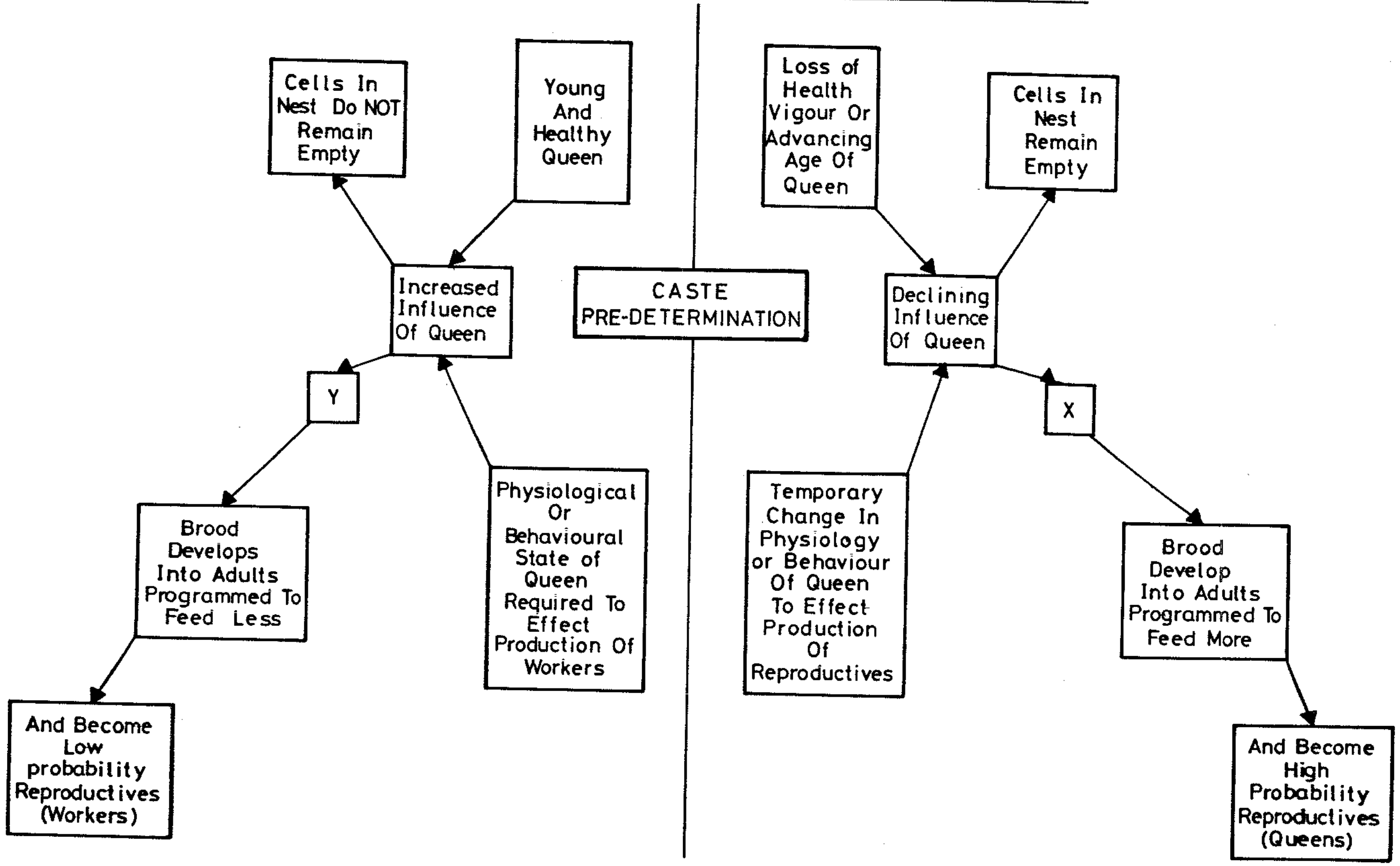

FIG.3

consequence an alternate set of processes (designated as Y in fig 3) would become activated so that (1) the brood would develop into adults programmed to feed less and thus become low probability reproductives (workers) and (2) empty cells would not accumulate in the nest. It should be emphasized that we expect the numbers of empty cells to be an indirect consequence of the same set of processes that lead to the production of reproductives or workers and, not to be causally related to caste differentiation. On the contrary, the differential rates of feeding exhibited by egg layers and non egg layers might be thought of as being a link in the chain of events leading to caste differentiation. It should also be emphasized that we do not believe that caste pre-determination is by any means an all or none process. While we suspect that at eclosion wasps have different probabilities of becoming egg layers there is still undoubtedly a great deal of flexibility in the roles that they would ultimately adopt.

But why do empty cells accumulate when the queen's influence is expected to be low and when reproductives are being produced ? One possibility is that empty cells accumulate because the queen's influence is low. In other words the queen's physiology does not permit her to lay enough eggs to keep all the cells occupied. The other possibility is that empty cells accumulate because reproductives are being produced ; the time when reproductives are being produced is expected to be a period of intense reproductive competition and therefore a period of differential egg eating potentially leading to the accumulation of empty cells. There is little one can say at this stage regarding the possible mechanism of caste pre-determination. The fact that there is no detectable influence of body size on probabilities of egg laying makes it somewhat unlikely (though not necessarily impossible) that trophic factors are involved. Chemical communication between the queen (and perhaps other dominant females as well) and the brood, including the developing pupae, is entirely within the realms of possibility. Exocrine glands on the gastral sternites of social wasps are well known (Vecht, 1968; Jeanne et al, 1983; Landolt and Akre, 1979) , as are a variety of behaviours involving rubbing of the abdomen on the nest surface (Gamboa and Dew, 1981). What is more, the functional significance of some of the glands as well as the behaviours await elucidation (Downing and Jeanne, 1985).

Belonogaster griseus is the only other primitively eusocial insect where caste pre-determination has been suggested. Pardi and Marino Piccioli (Pardi and Marino Piccioli, 1981) found that 'queen-like' females are on the average larger, have a slightly wider thorax, are fertilised more often, reach greater fertility, are more dominant, more oophagous and are mainly nest founding while 'worker-like' females have the opposite traits and forage more often. The results reported here for *R.marginata* and those reported by Pardi and Marino Piccioli for *B.gresieus* provide evidence for the existence of females quite heterogeneous in their fertilities, which by itself should facilitate the evolution of sociality as suggested by the sub-fertility hypothesis of West-Eberhard (1975), a hypothesis that has been supported by quantitative modelling (Craig, 1983) but whose acceptance has hitherto been limited due to lack of empirical evidence for sub-fertility itself (Sullivan and Strassman, 1984).

Acknowledgements

We thank N.V.Joshi, W.D.Hamilton, Mary Jane West-Eberhard and Madhav Gadgil for many helpful discussions. Supported in part by a grant from the Department of Science and Technology, (Principal Investigator : R.G.).

References

Cox, D.R. 1970. The Analysis of Binary Data. Chapman and Hall.

Craig, R. 1983. Subfertility and the evolution of Eusociality by Kin Selection. J.Theor.Biol. 100, 379-397.

Downing, H.A. & Jeanne, R.L. 1985. Communication of status in the social wasp, *Polistes fuscatus* (Hymenoptera:Vespidae). Z.Tierpsychol. 67, 78-96.

Fletcher, D.J.C. & Ross, K.G. 1985. Regulation of reproduction in Eusocial Hymenoptera. Ann.Rev.Entomol. 30, 319-343.

Gadagkar, R. 1985. Evolution of Insect Sociality - a review of some attempts to test modern theories. Proc.Ind.Acad.Sci.(Anim.Sci.)94, 309-324.

Gadagkar, R. 1986. Social structure and the determinants of queen status in the primitively eusocial wasp *Ropalidia cyathiformis*. In Proc.X Internatl.Congr.IUSSI (Munich,in press).

Gadagkar, R. & Joshi, N.V. 1983. Quantitative ethology of Social Wasps: Time-Activity budgets and caste differentiation in *Ropalidia marginata* (Lep.)(Hymenoptera: Vespidae). Anim.Behav. 31, 26-31.

Gadagkar, R. & Joshi, N.V. 1984. Social Organisation in the Indian Wasp *Ropalidia cyathiformis*.(Fab.)(Hymenopter:Vespidae). Z.Tierpsychol.64, 15-32.

Gadagkar, R. & Joshi, N.V. 1985. Colony fission in a social wasp. Curr.Sci. 54, 57-62.

Gadagkar, R.,Gadgil,M.,Joshi,N.V.and Mahabal,A.S. 1972. Observations on the natural history and population ecology of the social wasp *Ropalidia marginata* (Lep.) from peninsular India. (Hymenoptera: Vespidae). Proc.Indian Acad.Sci. 91, 539-552.

Gamboa, G.J. & Dew, H.E. 1981. Intra colonial communications by body oscillations in the paper wasp, *Polistes metricus*. Insectes Sociaux. 28, 13-26.

Hamilton, W.D. 1972. Altruism and related phenomena mainly in the social insects. Ann.Rev.Ecol.Syst. 3, 192-232.

Jeanne, R.L. 1972. Social Biology of the Neotropical wasp, Mischocyttarus drewseni. Bull.Mus.Comp.Zool.Harv.Univ. 144, 63-150.

Jeanne, R.L.,Downing, H.A. & Post,D.C. 1983. Morphology and function of sternal glands in polistine wasps (Hymenoptera: Vespidae). Zoomorphol. 103, 149-164.

Landolt, P.J. & Akre, R.D. 1979. Occurrence and location of exocrine glands in some social vespidae. (Hymenoptera:). Entomol.Soc.Am. 72, 141-148.

Litte, M. 1981. Social Biology of the Polistine wasp Mischocyttarus labiatus: Survival in a Colombian rain forest. Smiths.Contr.Zool. No.327.

Michener, C.D. 1969. Comparative social behaviour of bees. Ann.Rev.Entomol. 14, 299-342.

Pardi, L. & Marino Piccioli, M.T. 1981. The biology of Belanogaster (Hymenoptera, Vespidae) : 4. Caste differences in Belanogaster griseus and the position of this genus among social wasps. Monit. Zool. Ital. Suppl. 14, 131-146.

Strassmann, J.E. & Meyer, D.C. 1983. Gerontocracy in the social wasp, polistes exclamans. Anim.Behav. 31, 431-438.

Sullivan, J.D. & Strassmann, J.E. 1984. Physical variability among nest foundresses in the polygynous social wasp, Polistes annularis. Behav.Ecol.Sociobiol. 15, 249-256.

Vecht, J.Van der. 1968. the terminal gastral sternites of of female and worker social wasps (Hymenoptera: vespidae). Koninkl.Nederl.Akademie Van Wetenchappen-- Amsterdam. Proc.Ser.C. 71, 411-422.

West-Eberhard, M.J. 1969. Social Biology of Polistine Wasps. Misc.Publ.Mus.Zool.Univ.Mich. 140, 1-101.

West-Eberhard, M.J. 1975. The Evolution of Social Behavior by Kin Selection. Quar.Rev.Biol. 50, 1-33.

West-Eberhard, M.J. 1979. Sexual Selection, social competition, and evolution. Proc.Am.Phil.Soc.123, 222-234.

Wheeler, D.E. 1986. Developmental and physiological determinants od caste in social Hymenoptera: Evolutionary implications. Am.Nat. 128, 13-34.

Wilson, E.O. 1971. The Insect Societies. Harvard University Press.