The role of larval nutrition in pre-imaginal biasing of caste in the primitively eusocial wasp *Ropalidia marginata* (Hymenoptera: Vespidae)

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Abstract. 1. When freshly eclosed females of the primitively eusocial wasp *Ropalidia marginata* (Lep.) are isolated, only about 50% of them build nests and lay eggs thereby suggesting a pre-imaginal biasing of caste.

2. Wasps that lay eggs take a very variable amount of time after eclosion to start doing so.

3. Females eclosing from nests where larvae are fed at a relatively higher rate are more likely to become egg-layers and are likely to take less time after eclosion to begin to lay eggs.

4. Thus, both forms of pre-imaginal biasing of caste, namely, differences in egg laying capacity and differences in the time taken to attain reproductive maturity, appear to be influenced by larval nutrition.

Key words. Larval nutrition, pre-imaginal caste bias, primitively eusocial wasp, *Ropalidia marginata*.

Introduction

A fundamental property of eusocial insects is the differentiation among colony members into a fertile reproductive caste and a sterile worker caste. In most highly eusocial insects there are marked and consistent morphological differences between the reproductive and sterile castes. Differential larval nutrition has long been proposed as the underlying process that gives rise to differential development of brood leading to such morphologically distinct castes

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(see reviews in Engelmann, 1970; Wilson, 1971; Spradbery, 1965, 1973; Brian, 1980; Wheeler, 1986). In most primitively eusocial species, however, there are few, if any, consistent morphological differences between the reproductive and worker castes. This suggests that the caste of an individual is not fixed at eclosion, thus leaving room for flexibility in the social roles it may adopt in adult life. Nevertheless, there is accumulating evidence that there is some pre-imaginal biasing of caste at least in some primitively eusocial species (see reviews in Gadagkar, 1990, 1991) and good reason to suspect that larval nutrition may be responsible for this (Hunt, 1991).

In one set of experiments, newly eclosed females of the primitively eusocial wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae) were isolated into individual cages and tested

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for any pre-imaginal effects on their ability to initiate nests and lay eggs. About 50% (150 out of 299) of the females so tested initiated nests and laid eggs while the remaining died without doing so (Gadagkar *et al.*, 1988, 1990). In addition, among the 150 egg-layers, the time taken after eclosion to start laying eggs varied considerably (mean $\pm$ SD = 65.9 $\pm$ 36.9 days). Here we test and confirm the prediction that differential larval nutrition is a factor that is responsible for both forms of pre-imaginal biasing of caste — that leading to differentiation into egg-layers and non-egg-layers as well as that leading to differentiation into early reproducers and late reproducers.

Materials and Methods

Six naturally occurring post-emergence colonies of *Ropalidia marginata* located in Bangalore (13:00:00 N, 77:32:00 E) and Mysore (12:25:00 N, 76:50:00 E), India, were studied between June 1986 and May 1987. Some characteristics of these nests are described in Table 1. Behavioural data presented in this paper are based on observations of individually identified wasps from the six colonies. These data were obtained during 144 5-min observation blocks per colony spread randomly between 08.00 and 18.00 hours over a period of 4–6 days. Colony N121 was attacked by the predatory hornet *Vespa tropica* before the observations could be completed and thus only seventy-two observation blocks were performed for this colony. This amounted to 6 h of observations on colony N121 and 12 h of observations on each of the remaining five colonies. Our methods used for making behavioural observations are described in more detail elsewhere (Gadagkar & Joshi, 1983). Data were pooled for all animals in a colony to compute the number of times larvae were fed per hour. Dividing this by the total number of larvae in the colony, we obtained the number of times an average larva is fed per hour in a colony (Table 1).

At the end of observations, the nests were collected and maintained in the laboratory after removing all eggs, larvae and adults. All females that eclosed from such nests were immediately isolated into individual 22 × 11 × 11 cm ventilated plastic jars. These isolated females were provided with a piece of soft wood as a source

Table 1. Characteristics of the six *R. marginata* nests used in this study.

Nest code	No. of eggs	No. of larvae	No. of pupae	No. of parasitized cells	No. of empty cells	Total no. of cells	No. of males	No. of females	No. of eclosing females tested	No. of egg-layers among females tested	No. of times an average larva was fed per hour
N120	28	20	13	0	7	68	0	8	12	8	0.48
N121	75	67	19	13	30	201	10	39	4	2	0.34
N123	39	52	41	2	1	135	0	41	32	21	0.24
N124	41	58	18	0	5	124	0	20	15	5	0.02
N129	62	18	19	0	3	102	0	8	16	10	0.17
N152	131	37	16	0	12	196	5	14	8	2	0.04

of building material and an *ad libitum* diet of final instar *Corcyra cephalonica* (Stainton) (Lepidoptera: Galleridae) larvae, honey and tap water from the same source. Records were maintained of the number of *C.cephalonica* larvae consumed by each wasp throughout the experiment. All wasps were observed for signs of nest building and egg laying every day (Table 1). These experimental procedures have been described in more detail elsewhere (Gadagkar *et al.*, 1988).

The model used to perform logistic regression analysis, to establish larval nutrition as a determinant of the probability of egg laying by eclosing wasps, is also described elsewhere (Gadagkar *et al.*, 1988; Shanubhogue & Gore, 1987). The independent variable, namely the number of times an average larva is fed per hour in a nest, was modelled to influence the probability of egg laying by eclosing females in that nest such that

$$\ln [p/(1 - p)] = \beta_0 + \beta_1 X$$

The equation

$$p = e^{\beta_0 + \beta_1 X}/1 + e^{\beta_0 + \beta_1 X}$$

was solved using the maximum likelihood criterion:

$$L = (\prod_{i=1}^{m} p_i)(\prod_{i=m+1}^{n} [1 - p_i])$$

where p is the probability of becoming an egg-layer, $1 - p$ is the probability of becoming a non-egg-layer, β_0 is the intercept, β_1 the regression coefficient, X the independent variable, L the likelihood, 1 to m the egg-layers and $m + 1$ to n the non-egg-layers. The goodness of fit of the model was tested as follows: p the probability of becoming an egg-layer was computed from the above equations for each of the eighty-seven animals. Dividing the probability values from 0 to 1 into twenty equal probability classes, the mean p values of all the animals falling in any probability class multiplied by the total number of animals in the experiment (87) gives the expected number of egg-layers in that probability class. Similarly, [1 − (mean probability)] × (total number of animals in the experiment) = expected number of non-egg-layers in that probability class. The observed number of egg-layers and non-egg-layers among animals falling in each probability class being known, a χ^2 test was performed.

Results

Eighty-seven freshly eclosed females of the primitively eusocial wasp *Ropalidia marginata* were tested for their ability to initiate nests and lay eggs when isolated into laboratory cages. As in previous experiments (Gadagkar *et al.*, 1988, 1990), about half of them (47 out of 87) built nests and laid eggs while the remaining forty died without doing so, in spite of living, on the average (128.6 ± 94.0 days), longer than the time taken by the egg-layers to start laying eggs (73.4 ± 64.1 days) (*t* test, $P < 0.01$). However, the probability that an individual develops into an egg-layer or a non-egg-layer was significantly influenced by the rate at which an average larva was fed per hour in their nest of origin (Table 2, $P < 0.03$). The goodness of fit of the model used in the logistic regression analysis was confirmed by the fact that the expected numbers of wasps in each probability class calculated from the model were indistinguishable from the corresponding observed numbers (Table 3, $P > 0.05$).

As in earlier experiments (Gadagkar *et al.*, 1988, 1990), and, as mentioned above, there was a large variation in the time taken by the egg-layers to start laying eggs, ranging, in this experiment, from 5 to 277 days after eclosion (mean $\pm$ SD = 73.4 ± 64.1 days). There is a weak but statistically significant negative correlation between the time taken by an animal to start laying eggs (age at reproductive maturity) and the rate at which larvae are fed in the nest from which they eclose (Fig. 1, $P < 0.01$).

Discussion

The results presented in this paper show that pre-imaginal caste bias takes two forms. First, some animals lay eggs while others die without doing so. It is reasonable to expect that those animals that build nests and lay eggs under the conditions of these experiments will have a relatively high probability of becoming egg-layers under natural conditions compared to those animals that die without laying eggs. Second, among the egg-layers there is considerable variation in the time taken to lay eggs. It is similarly reasonable to expect that such of the egg-layers in these experiments that started laying eggs soon after eclosion will have a higher chance of capitalizing on egg-laying oppor-

Table 2. Logistic regression analysis. Larval nutrition as a determinant of the probability of egg laying by eclosing females of *R.marginata*.

Variables	Estimated coefficient (β)	Standard error	Test statistic	P
Intercept	−0.5612	0.3963	−1.4160	0.1556
No. of times an average larva is fed per hour	3.5462	1.6525	2.1459	0.0316

Table 3. Goodness of fit of the model tested in Table 2. Probability classes with zero in all four columns are not shown. There is no significant difference between the observed number of egg-layers and the number expected under the model ($\chi^2 = 0.25$, df = 1, $P > 0.05$). To compute χ^2, adjacent rows were pooled when the expected value was less than 5.

Probability (of becoming an egg-layer) class	Egg-layers		Non-egg-layers	
	Observed	Expected	Observed	Expected
0.35−0.40	5	5.7	10	9.3
0.40−0.45	2	3.2	6	4.8
0.50−0.55	9	8.1	7	7.9
0.55−0.60	21	18.3	11	13.7
0.65−0.70	2	2.6	2	1.4
0.75−0.80	8	9.1	4	2.9
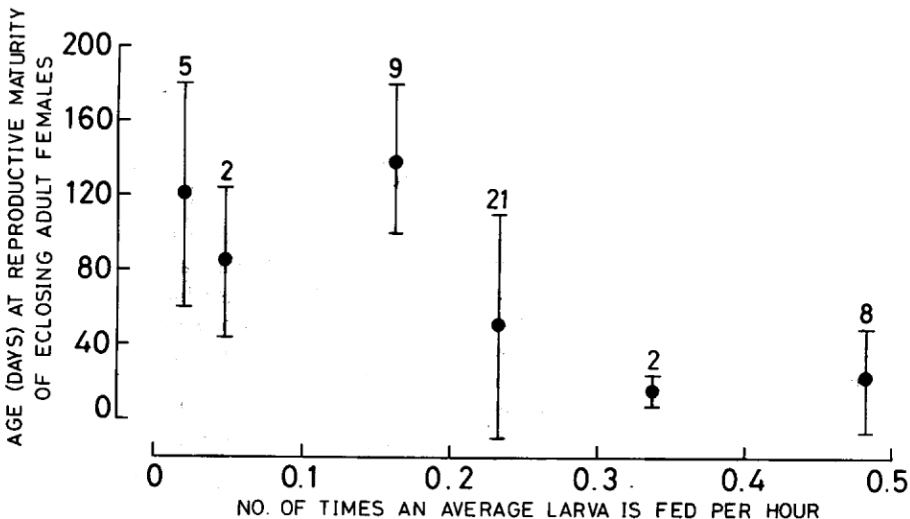
Fig. 1. There is a weak but statistically significant negative correlation between time taken by an animal to start laying eggs (= age at reproductive maturity) (shown as mean ± SD) and the rate at which larvae are fed in the nest from which they eclose. (Pearson product moment correlation $r = -0.42$; df = 45; $P < 0.01$.) The numbers on top of each bar represent sample sizes.

tunities under natural conditions compared to those egg-layers in the experiment that took a very long time to start laying eggs. Both these forms of pre-imaginal caste bias appear to be mediated at least partly by larval nutrition. The higher the rate at which larvae are fed in a nest, the greater is the probability that eclosing females from that nest will become egg-layers and the smaller is the time required for them to start laying eggs. To the extent that these laboratory results can be extrapolated to natural conditions, the results presented here not only provide some support to the roles of parental manipulation (Alexander, 1974) and sub-fertility (West-Eberhard, 1975) in promoting the origin of eusociality (Gadagkar, 1990, 1991) but also suggest a mechanism by which such factors may operate (Hunt, 1991).

In previous studies it was shown that nests that have more empty cells are likely to produce a greater proportion of egg-layers, and nests that had fewer empty cells were likely to produce a greater proportion of non-egg-layers. A link was hypothesized between the state of the queen and the number of empty cells that she may leave behind such that a young and healthy queen was not expected to leave behind too many empty cells while an old and weak one was expected to do so. But the nature of the link, if any, between the number of empty cells left behind by the queen and her ability or otherwise to prevent her daughters from developing into potential reproductives was not clear. Indeed, it was suggested that the number of empty cells was merely '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 determination (or biasing)' (Gadagkar *et al.*, 1990, page 184).

In the light of the results presented here, however, an attractive hypothesis causally linking the number of empty cells with caste biasing, a hypothesis also independently proposed by Hunt (1991), suggests itself. Nests begin with relatively young and healthy queens who may leave behind few or no empty cells. Such nests may also have a modest worker force which furthermore will be busy expanding the nest to match the queen's egg-laying rate. The food that is brought to the nest at this time and shared between the adults and the larvae may therefore be limited, leading to a bias in the nature of larval development thus programming

them to become workers. In course of time the colony will have a large worker force and also require less effort in expanding the nest. Besides, at such a time the queen, either on account of old age and weakness or for the purpose of producing reproductives, may slow down her rate of egg laying leading to accumulation of empty cells and soon to a reduction in the number of larvae to be fed relative to the amount of food available. Larvae at this time may thus be better nourished and hence be programmed to develop into egg-layers. Such a hypothesis is consistent with what is known about colony cycles (Jeanne, 1972), variation in larval and adult nutrition through colony development (West-Eberhard, 1969; Wilson, 1971; Wheeler, 1986) and with the postulated role of nutrition in social evolution (Pardi & Marino Piccioli, 1970, 1981; Hunt, 1984, 1989, 1990, 1991; Hunt *et al.*, 1987; Rossi & Hunt, 1988). The time is clearly ripe for more direct laboratory experimentation to examine the link between larval nutrition and adult caste in primitively eusocial wasps.

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