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Juvenile hormone accelerates ovarian development and does not affect age polyethism in the primitively eusocial wasp, *Ropalidia marginata*

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Abstract

Juvenile hormone modulates post-imaginal reproductive division of labor in primitively eusocial species and promotes the production of queens (e.g., *Polistes*) while it modulates age polyethism and promotes the production of foragers in highly eusocial species (e.g., the honey bee). *Ropalidia marginata* is a primitively eusocial wasp that shows both post-imaginal regulation of reproductive division of labor as well as age polyethism. Hence, *R. marginata* is a particularly interesting model system to study the effect of juvenile hormone. We demonstrate here that a single, topical application of 100 µg of juvenile hormone-III per female wasp accelerates ovarian development of wasps held in isolation. Similar application to wasps released back on to their natal nests has no effect on their rate of behavioral development as witnessed from the age of first performance of feed larva, build, bring pulp and bring food. We conclude therefore that in *R. marginata*, juvenile hormone has retained its function of modulating reproductive division of labor and has not acquired the function of modulating age polyethism.

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1. Introduction

Eusocial insects (e.g., ants, bees, wasps and termites) exhibit overlap of generations, cooperative brood care and a reproductive caste differentiation into fertile queen castes and sterile worker castes. Primitively eusocial species (e.g., Polistine wasps and Halictine bees) are those in which the queen and worker castes are not morphologically differentiated, caste determination is largely post-imaginal and the workers are potentially capable of developing their ovaries, mating and replacing their queens, and in some cases, of initiating single foundress nests. On the other hand, highly eusocial species (e.g., most ants, termites and honey bees) are those in which the queen and worker castes are morphologically differentiated, caste determination is pre-imaginal and workers

seldom lay eggs, cannot mate and thus are incapable of replacing their queens (Wilson, 1971).

A phenomenon of particular interest in primitively eusocial species is the post-imaginal regulation of reproductive division of labor which permits queens to maintain reproductive monopoly in spite of the workers being potentially capable of reproduction (Röseler, 1991). Similarly, a phenomenon of special interest in highly eusocial species is the regulation of non-reproductive division of labor which, in honey bees for example, is strongly influenced by the age of workers; honey bee workers perform different tasks as they grow older, always beginning with intranidal tasks and ending with extranidal tasks. This phenomenon is referred to as age polyethism (Wilson, 1971; Seeley, 1995).

Juvenile hormone appears to modulate reproductive division of labor in primitively eusocial species and promote the production of queens (e.g., in *Polistes*, Barth et al., 1975; Röseler et al., 1980; 1984; 1985; in *Bombus*, Röseler, 1977; Bloch et al., 1996, 2000), while it modulates age polyethism and promotes the production of for-

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agers in highly eusocial species (e.g., in the honey bee, Robinson, 1987b; 1992; Sasagawa et al., 1989; Bloch et al., 2002; in Polybiine wasps, O'Donnell and Jeanne, 1993; O'Donnell, 1998). To the extent that queens and foragers represent opposite ends of the spectrum of division of labor in social insects, these two effects of juvenile hormone appear to be in "opposite" directions. For example, Robinson et al. (1992) suggested that the relatively novel function of regulating age polyethism seen in highly eusocial species may be incompatible with its more fundamental function of regulating reproductive division of labor in primitively eusocial species. What function juvenile hormone may perform in primitively eusocial species that show post-imaginal reproductive division of labor and also exhibit clear-cut age polyethism is therefore an interesting question (Robinson et al., 1992).

Ropalidia marginata is an old world, tropical, primitively eusocial polistine wasp, abundantly distributed in peninsular India. Consistent with its primitively eusocial status, queens and workers of *R. marginata* are not morphologically different and the workers are capable of developing their ovaries, mating and replacing their queens as well as of initiating single foundress nests (Gadagkar, 2001). However somewhat unexpected from its primitively eusocial status, *R. marginata* shows a remarkably well-developed, honey bee-like non-reproductive division of labor (age polyethism), with workers progressing as they grow older, from performing intranidal tasks (first feeding larvae and later building the nest) to extranidal tasks (first foraging for pulp and later for prey) (Naug and Gadagkar, 1998a, 1998b). Given that both post-imaginal regulation of reproductive division of labor and age polyethism are conspicuous phenomena in *R. marginata*, this species is therefore an ideal model system to investigate the evolution of the modulatory function/s of juvenile hormone.

2. Methods

2.1. Effect of juvenile hormone on ovarian development

Two large *R. marginata* nests (containing 75 and 135 pupae respectively) were collected from their natural nesting sites, brought to the laboratory, cleared of eggs, larvae and all adult wasps, and were checked daily for eclosion of wasps. Female wasps eclosing from these nests were randomly assigned to one of three treatments (see below) and isolated into well-ventilated transparent plastic jars (22 cm X 11 cm X 11 cm) and provided with *ad libitum* food, water and building material. The plastic jars were monitored daily until the wasps in them built a nest and laid an egg or died without doing so.

- Treatment 1 (JH treated): 100 µg of juvenile hor-

mone-III (Sigma Inc. USA) in 2 µl of acetone, applied on the abdomen on the day of eclosion (sample size = 34 wasps).

- Treatment 2 (Acetone treated): 2 µl of acetone applied on the abdomen on the day of eclosion (sample size = 35 wasps).
- Treatment 3 (Control): Isolated without application of either juvenile hormone or acetone (sample size = 28 wasps).

Different studies have used juvenile hormone-III or its analog, methoprene, in different doses (Barth et al., 1975; Robinson, 1987a; Robinson and Ratnieks, 1987; Sasagawa et al., 1989). Following (Giray et al., *in press*) we performed preliminary tests (data not shown) and found that the highest dose that did not cause increased mortality, in comparison to acetone or blank-treated wasps, was 100 µg/individual. We have used this maximum tolerated dose in the experiments reported here. The proportion of individuals that built nests and laid eggs as well as the time taken by these individuals to lay their first egg was computed separately for wasps in each treatment.

2.2. Effect of juvenile hormone on age polyethism

Three naturally occurring *Ropalidia marginata* nests were located and transplanted to the vespary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The vespary is a room measuring 9.3 m X 6 m X 4.8 m covered with wire mesh of dimension 0.75 cm X 0.75 cm, which prevents the major predator, *Vespa tropica*, from entering but allows *R. marginata* to fly in and out freely.

Behavioral observations consisting of, recording every occurrence of feed larva, build, bring pulp and bring food were conducted (see Gadagkar, 2001, for methods of observation). Experiments with the three nests lasted for 48, 104 and 50 days respectively, during which behavioral observations were made on 14, 30 and 15 days respectively. On each day of observation, 34 five-minute observation sessions were randomly distributed throughout the day. During the experimental period, adults that eclosed from the nests were removed in less than 24 hours after eclosion, uniquely marked with quick-drying paints and randomly assigned to one of two treatments, viz. juvenile hormone or acetone (see above). The treated individuals were isolated in ventilated plastic jars for about 2 hours for the acetone to evaporate. Subsequently these animals were returned to their nests of eclosion. Because there was no difference between acetone-treated wasps and control wasps in the isolation experiments described above, here we treated wasps only with acetone or juvenile hormone and did not have control wasps with neither treatment. Thirty eight, 35 and 16 wasps were treated with acetone in the three nests

respectively and 40, 34 and 16 wasps were treated with juvenile hormone in the three nests respectively. The observer was unaware of the treatment given to different animals until the end of all observations. The mean age of treated wasps at the time they performed their first acts of feed larvae, build, bring pulp and bring food was computed from these data.

3. Results

There was no significant difference between the juvenile hormone-treated, acetone-treated and control wasps in the proportion of individuals that built nests and laid eggs (Pair-wise G test, $P > 0.05$; Fig. 1, upper panel). However, juvenile hormone-treated wasps began laying eggs significantly sooner than acetone-treated or control wasps (Pair-wise t test, $P < 0.05$; Fig. 1, lower panel). There was no detectable influence of juvenile hormone on age polyethism. The ages of first performance of feed larva, build, bring pulp and bring food did not significantly differ between the juvenile hormone-treated and acetone-treated wasps. The same result was obtained for data from each of the three nests as well as for the data pooled from all the three nests (two-way ANOVA followed by Tukey–Kramer test, $P > 0.05$; Fig. 2). It should be noted that while there was no effect of juvenile hormone treatment on age polyethism, there was clear evidence of age polyethism itself in both the juvenile hormone-treated wasps as well as the control wasps; the age of first performance of feed larva was significantly lower than the age of first performance of build/bring pulp, which in turn was significantly lower than the age of first performance of bring food. And yet there was no difference in this regard between the juvenile hormone-treated and control wasps. It should also be noted that the age of first performance of different tasks observed in this study are nearly identical to those observed in the previous study that demonstrated age polyethism in *R. marginata* (Naug and Gadagkar, 1998b). The number of wasps which showed some of the tasks was rather small in some of the colonies. For this reason we have also analyzed the data pooled across the three nests and obtained identical results.

4. Discussion

R. marginata is a primitively eusocial wasp with evidence of some pre-imaginal caste bias. When freshly eclosed female wasps are isolated and provided the conditions necessary for initiation of single foundress nests, only 50% of the wasps successfully develop their ovaries, build nests and lay eggs (egg layers). The remaining 50% die without doing so even though they may live longer than those that do lay eggs (non-egg layers)

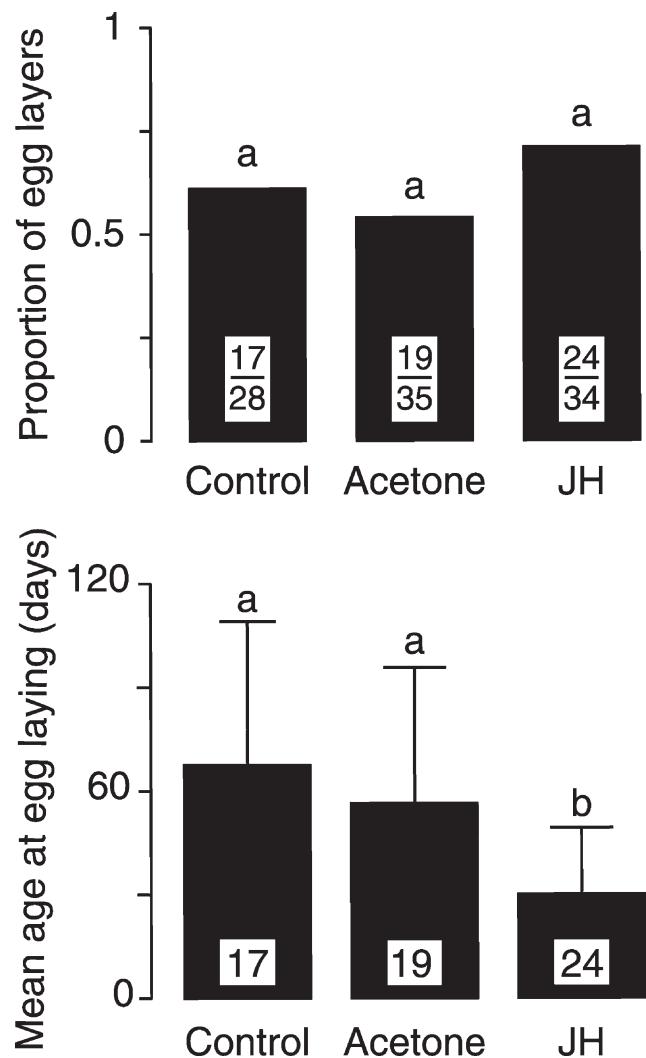


Fig. 1. Effect of juvenile hormone on ovarian development. Upper panel: Effect of juvenile hormone (JH) on egg laying in isolated wasps. The proportion of isolated wasps that lay eggs in the three treatments are not significantly different from each other as indicated by same letter on each bar (Pair-wise G test, $P > 0.05$). The number of egg layers and total number of wasps tested is indicated inside the bars. Lower panel: The mean age at egg laying of JH-treated wasps is significantly less than control and acetone-treated wasps, as indicated by letters on the bars (Pair-wise t test, $P < 0.05$). The number of wasps tested is indicated inside the bars.

(Gadagkar et al., 1988). Among the egg layers there is considerable variation in the time taken after eclosion to lay the first egg. There is evidence that the quantity of food received by young larvae influences the time taken to lay eggs in their adult life such that better-nourished larvae develop into early egg layers while relatively poorly nourished larvae develop into late egg layers (Gadagkar et al., 1991). The time taken after eclosion to begin laying eggs can have a significant effect on the fitness of wasps choosing the solitary nest-founding strategy while it has little or no effect on the inclusive

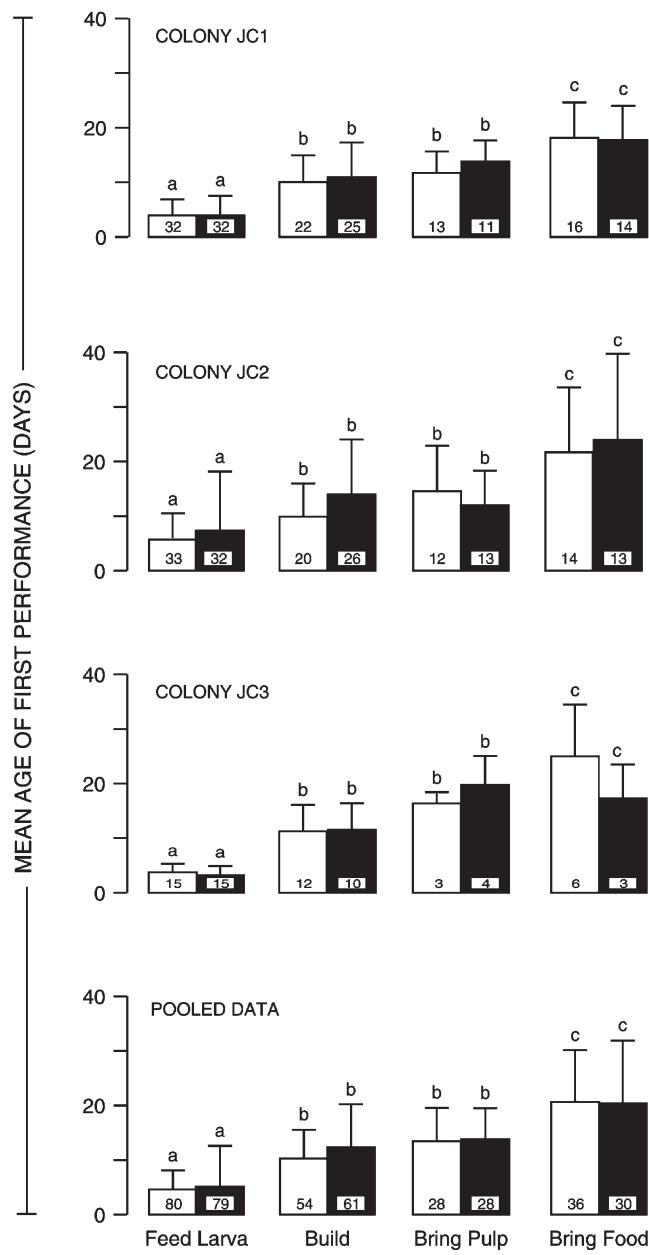


Fig. 2. Effect of JH on behavioral development. Mean and standard deviation of age at first performance of different behaviors in three colonies and in the pooled data. In each panel, bars carrying the same letter are not significantly different from each other. There is no significant difference between JH-treated wasps (open bars) and acetone-treated wasps (filled bars) in all cases (two-way ANOVA followed by Tukey–Kramer test $P>0.05$). Number of wasps tested are indicated inside the bars.

fitness of wasps choosing the worker strategy (Gadagkar, 1991).

In the present experiment, topical application of juvenile hormone did not alter the proportion of egg layers and non-egg layers among freshly eclosed wasps isolated and tested, as described above. This does not, however, mean that juvenile hormone did not affect ovarian development in these wasps. There was a significant reduction

in the age at egg laying of juvenile hormone-treated wasps as compared to acetone-treated and control wasps. We conclude therefore that in *R. marginata*, juvenile hormone modulates post-imaginal reproductive division of labor and promotes the production of queens as has been observed in *Polistes* and *Bombus*.

Of all the primitively eusocial species studied so far, *R. marginata* has the best-developed, honey bee-like age polyethism (Naug and Gadagkar, 1998a, 1998b). And yet we find no effect of similar topical application of juvenile hormone on age polyethism. Unlike what has been observed in the honey bees, juvenile hormone did not significantly affect the age of first performance of any task examined and certainly did not produce precocious foragers. This result is admittedly based on a single application of a single dose of juvenile hormone. However, the fact remains that the same single application of a single dose did produce a significant acceleration of ovarian development. We conclude therefore that in *R. marginata*, which exhibits both post-imaginal regulation of reproductive division of labor as well as age polyethism, juvenile hormone modulates the former but not the latter.

Because juvenile hormone was hitherto known only to modulate ovarian development in primitively eusocial species and age polyethism in highly eusocial species, it was proposed that juvenile hormone might have acquired a novel function, and one that is probably incompatible with its original function, in the course of evolution from the primitively eusocial to the highly eusocial state (Robinson et al., 1992). This has been referred to as the novel function hypothesis by West-Eberhard (1996), (see also, Bloch et al., 2002). Because the essence of hypothesis proposed by Robinson et al. (1992) is that juvenile hormone can have only one of the two functions in any species it should perhaps be referred to as the “single function” hypothesis rather than the novel function hypothesis. Alternatively, it has also been suggested that juvenile hormone can modulate age polyethism without losing the ability to modulate ovarian development. This may be possible because the function of modulating ovarian development may be performed in one set of individuals (queen-destined individuals) while the function of modulating age polyethism may be performed in a different set of individuals (worker-destined individuals). This has been termed as the “split function” hypothesis (West-Eberhard, 1996). Neither primitively eusocial species such as *P. dominulus*, where there is a clear evidence for post-imaginal regulation of reproductive division of labor but no evidence of age polyethism, nor species such as honey bees where there is no post-imaginal regulation of reproductive division of labor but there is clear age polyethism, can be used to distinguish between the novel or single function hypothesis and the split function hypothesis. Species such as *R. marginata*, which appear to be

intermediate between primitively and highly eusocial stages, and exhibit both post-imaginal regulation of reproductive division of labor as well as age polyethism, are suitable for discriminating between the two hypotheses. In such a species, if juvenile hormone regulates any one, but only one of the two phenomena, the novel or single function hypothesis may be said to be supported. On the other hand, if juvenile hormone regulates both the phenomena, then the split function hypothesis may be said to be supported.

There appears to be some recent evidence that juvenile hormone may influence both age polyethism as well as ovarian development in the primitively eusocial wasp *Polistes canadensis* (Giray et al., *in press*). To the extent that juvenile hormone modulates ovarian development and does not affect age polyethism in *R. marginata*, the split function hypothesis is not supported in our study. Since only one of the two phenomena, namely, reproductive division of labor is modulated by juvenile hormone in *R. marginata*, we conclude that the novel or single function hypothesis is supported in the present study. Why is it that in *R. marginata*, juvenile hormone does not modulate age polyethism (with or without losing the ability to modulate ovarian development)? This is somewhat surprising in light of the fact that *R. marginata* is relatively advanced by primitively eusocial standards. Not only is there a well-developed age polyethism but colony sizes sometimes approach one hundred individuals and queens (though not morphologically differentiated) appear to regulate worker reproduction through pheromones rather than through physical aggression (Suman, Bhadra and Gadagkar, unpublished observations). On the other hand, because post-imaginal regulation of reproduction remains important in *R. marginata*, juvenile hormone cannot also lose its original function. Why then has it not acquired a “split function” of modulating both ovarian development and age polyethism? We think that this may be because, in spite of evidence for pre-imaginal caste bias (Gadagkar et al., 1988), many adult wasps can become either queens or workers depending on the social environment they find themselves in, i.e., there are no clear-cut, queen-destined and worker-destined individuals in natural colonies of *R. marginata* (Gadagkar, 2001).

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