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DIVISION OF LABOUR AND ITS REGULATION IN A PRIMITIVELY EUSOCIAL WASP

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■ **Key Words** eusocial wasps, *Ropalidia marginata*, polyethism, juvenile hormone, reproduction

■ **Abstract** Social insect species that exhibit overlap of generations, cooperative brood care and reproductive caste differentiation have been termed eusocial. Among these, those that also exhibit morphological caste differentiation are termed highly eusocial while those that have morphologically identical queen and worker castes are termed primitively eusocial. Because queens and workers are not morphologically differentiated in primitively eusocial species, caste differentiation is often post-imaginal. The processes by which some individuals succeed in becoming reproductive queens while others end up as sterile workers in primitively eusocial species is of obvious interest. Such post-imaginal reproductive caste differentiation is absent in highly eusocial species because reproductive caste differentiation in these species is usually pre-imaginal. On the other hand, further differentiation of the workers into different kinds of task specialists is a phenomenon that has been extensively studied in highly eusocial species (Wilson, 1971). There has seldom been an opportunity to study both post-imaginal reproductive caste differentiation as well as post-imaginal division of non-reproductive labour in the same species because these two phenomena usually do not occur in the same species.

Ropalidia marginata is an old world tropical primitively eusocial wasp widely distributed in southern India. Although classified as primitively eusocial by the traditional criteria of overlap of generations, cooperative brood care, reproductive caste differentiation and absence of morphological differentiation between queens and workers (Gadagkar, 2001a,b), *R. marginata* appears to have acquired some features resembling highly eusocial species. One such feature concerns the mode of division of non-reproductive labour among the adult wasps. *R. marginata* exhibits a remarkably well-developed honeybee like age polyethism. Workers show a strong tendency to feed larvae, build the nest, forage for pulp and forage for food, in that order, as they age. The frequency and probability of performance of different tasks is strongly influenced by the age of the individual (Naug and Gadagkar, 1998a). Like in the honeybees, age polyethism in *R. marginata* is very flexible and this flexibility comes from the fact that workers are allocated tasks based more on their relative age rather than their absolute age. This can be demonstrated experimentally by creating artificial colonies containing only young individuals (young cohort colonies) or containing only old individuals (old cohort colonies). In young cohort colonies, precocious foragers, *i.e.*, some individuals who begin to forage at an early age when they would not do so in normal colonies, compensate for the absence of older individuals (Naug and Gadagkar, 1998b). In old cohort colonies, hard working nurses, *i.e.*, some individuals who feed larvae at rates higher than they would in normal colonies, compensate for the absence of younger individuals (Agrahari and Gadagkar, submitted).

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 honeybee). Since *R. marginata* shows both post-imaginal regulation of reproductive division of labor as well as age polyethism, it is a particularly interesting model system to study the effect of juvenile hormone. 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. It appears, 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 (Agrahari and Gadagkar, 2003).

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INTRODUCTION

Social insect species that exhibit overlap of generations, cooperative brood care and reproductive caste differentiation have been termed eusocial. Among these, those that also exhibit morphological caste differentiation are termed highly eusocial while those that have morphologically identical queen and worker castes are termed primitively eucocial. Because queens and workers are not morphologically differentiated in primitively eusocial species, caste differentiation is often post-imaginal. The processes by which some individuals succeed in becoming reproductive queens while others end up as sterile workers in primitively eusocial species is of obvious interest. Such post-imaginal reproductive caste differentiation is absent in highly eusocial species because reproductive caste differentiation in these species is usual pre-imaginal. On the other hand, further differentiation of the workers into different kinds of task specialists is a phenomenon that has been extensively studied in highly eusocial species (Wilson, 1971). There has seldom been an opportunity to study both post-imaginal

reproductive caste differentiation as well as post imaginal division of non-reproductive labour in the same species because these two phenomena usually do not occur in the same species.

Ropalidia marginata is an old world tropical primitively eusocial wasp widely distributed in southern India. Although classified as primitively eusocial by the traditional criteria of overlap of generations, cooperative brood care, reproductive caste differentiation and absence of morphological differentiation between queens and workers (Gadagkar, 2001 a,b), *R. marginata* appears to have acquired some features resembling highly eusocial species. One such feature concerns the mode of division of non-reproductive labor among the adult wasps. *R. marginata* exhibits a remarkably well-developed honey bee like age polyethism. Workers show a strong tendency to feed larvae, build the nest, forage for pulp and forage for food, in that order, as they age. The frequency and probability of performance of different tasks is strongly influenced by the age of the individual. However, the relative position of a wasp in the age distribution of the colony, rather than her absolute age, is a stronger predictor of her task performance. Soliciting behaviour (a form of trophallaxis) provides a plausible mechanism for the wasps to assess their relative ages (Naug and Gadagkar, 1998a).

Like in honeybees, age polyethism in *R. marginata* is very flexible and this flexibility comes from the fact that workers are allocated tasks based more on their relative age rather than their absolute age. This can be demonstrated experimentally by creating artificial colonies containing only young individuals (young cohort colonies) or containing only old individuals (old cohort colonies). In young cohort colonies, precocious foragers, *i.e.*, some individuals who begin to forage at an early age when they would not do so in normal colonies, compensate for the absence of older individuals (Naug and Gadagkar, 1998b). In old cohort colonies, hard working nurses, *i.e.*, some individuals who feed larvae at rates higher than they would in normal colonies, compensate for the absence of younger individuals (Agrahari and Gadagkar, Submitted). Juvenile hormone modulates post-imaginal reproductive division of labour 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 honeybee). Since *Ropalidia marginata* shows both post-imaginal regulation of reproductive division of labour as well as age polyethism, it is a particularly interesting model system to study the effect of juvenile hormone. A single, topical application of 100 g of juvenile hormone-III per female wasp accelerates ovarian development of wasps held in isolation. Similar application to wasps released back onto their natal nests has no effect on their rate behavioural development as witnessed from the age of first performance of feed larva, build, bring pulp and bring food. It appears therefore that in *R. marginata*, juvenile hormone has retained its function of modulating reproductive division of labour and has not acquired the function of modulating age polyethism (Agrahari and Gadagkar, 2003)

EUSOCIALITY

Social insects, such as termites, ants, bees and wasps have long intrigued biologists and laymen alike both because of the intricate organization and complexity of their colonies and also because of the extreme forms of cooperation

and altruism they display. To the particular delight of biologists aiming to unravel the evolution of social life, social hymenopterans display varying degrees of sociality ranging from the solitary all the way to large, complex societies, with thousands and even millions of individuals, functioning as a coordinated unit, aided by organized division of labour and communication, that invite comparison with human societies.

To focus attention on species with the highest accomplishments of social evolution, a category of "truly social" or "eusocial" species has been created. To qualify for the status of eusocial, any species must exhibit all three traits: (1) reproductive caste differentiation of colony members into fertile reproductives (queens, and also kings on the case of termites) and sterile workers, (2) cooperative brood care and (3) an overlap of at least two generations of adults in the same colony (Michener, 1969; Wilson, 1971).

It is customary to recognize two sub-divisions of the eusocial, the primitively eusocial and the highly eusocial. Primitively eusocial species generally have small colonies, queens and workers are not morphologically differentiated, caste determination is generally post-imaginal and queens suppress worker reproduction by physical aggression. On the other hand, highly eusocial species generally have large colonies, queens and workers and sometimes even different groups of workers are morphologically differentiated, caste differentiation is generally pre-imaginal and queens suppress worker reproduction (or, as is now more fashionable to say, queens honestly signal their reproductive superiority to workers) by means of pheromones (Wilson, 1971; Michener, 1974; Holldobler and Wilson, 1990; Bourker and Franks, 1995; Crozier and Pamilo, 1996; Gadagkar, 2001b).

DIVISION OF LABOUR

Social insects have achieved spectacular ecological success, exceeding even vertebrates in biomass in some tropical habitats (Wilson, 1990). Division of labour is primary to colony organization in social insects. Division of labour and the consequent increase in ergonomic efficiency due to parallel processing of tasks is considered a major factor in the ecological success of social insects. The most fundamental division of labour within the colony is between the reproductive queen and sterile workers. The workers are either completely or partially sterile. In many species workers have lost the ability to mate and in some species workers have lost their ovaries altogether. Workers perform all tasks related to colony maintenance and growth such as building the nest, brood care, foraging, and nest defence (Wilson, 1971). Further division of non-reproductive labour among the workers may be based on physical or temporal castes. In physically differentiated castes, behaviour is primarily morphology-correlated and in temporally differentiated castes behaviour is age-correlated.

Division of non-reproductive labour increases the work efficiency of the colony by making it possible for workers to acquire special skills. A set of individuals specializing in performing a particular task is said to compose a particular caste. In some castes individuals are morphologically differentiated and consequently specialized for performing certain tasks. Therefore the allocation of workers to different castes is permanent. Colonies of most ants and termites include workers of two or more different sizes. Workers of a particular size class specialize

in the tasks for which they are more suited owing to their size. Small ant workers typically labour in the nest while bigger individuals defend and forage. Sometimes this form of division of labour involves elaborate morphological adaptations that go beyond simple differences in relative sizes, such as allometric growth of powerful jaws in castes specialized for defence. Such morphological polymorphism however, is not usually seen in bee and wasp societies (Oster and Wilson, 1978). Alternatively, individual allocation to a caste can be temporary and temporal, wherein morphologically identical individuals successively specialize to perform different tasks, each for limited period of their life. This is known as age polyethism and is one of the more common forms of division of labour observed in species with no morphological differences among the workers (reviewed by Jeanne, 1991; Robinson, 1992).

THE JUVENILE HORMONES

In spite of their relatively small brains, insects exhibit a rich diversity of behaviours. They have the capacity to respond to diverse environmental stimuli with great efficiency and flexibility. Much of insect behaviour is stimulated, modified or inhibited by hormones. The juvenile hormones are the most versatile hormones in the insect world. They play a role in almost every aspect of insect life - development and reproduction, metamorphosis, caste determination, regulation of behaviour, polyphenism, vitellogenin synthesis, ovarian development. Structurally most similar to retinoic acid, a morphogen in vertebrates, the juvenile hormones are unusual sesquiterpenoids with an epoxide group near one end and a methyl ester on the other. There are seven naturally occurring juvenile hormones, differing in the lengths of their side chains. Juvenile hormone-III, a 16-Carbon molecule, is the only form found in the Hymenoptera (reviewed by Nijhout, 1994).

As a developmental hormone, juvenile hormone controls switches between alternative pathways of development at several points in the insect life cycle. If juvenile hormone is absent during the critical period, gene expression changes and new processes begin and launch the insect on a new developmental pathway. The continuation of the current developmental state in the presence of juvenile hormone led Williams (1952) to refer to juvenile hormone as the "status quo" hormone. Developing larvae moult to another larval stage if they have high juvenile hormone titers during a critical period prior to each ecdysis. A drop in juvenile hormone titer is again required to achieve the transformation to pupa and then to adult. In adult insects, juvenile hormone serves as a gonadotropin. An increase in juvenile hormone titer either causes or hastens the onset of reproductive maturation. Juvenile hormone controls oocyte development by regulating both vitellogenesis in the fat body and vitellogenin uptake by developing oocytes. Juvenile hormone has been implicated in the control of many behaviours in different species, including receptivity and mating behaviour, oviposition behaviour, maternal behaviour, flight activity, and feeding (Nijhout, 1994 and the references therein).

POST-IMAGINAL REGULATION OF REPRODUCTIVE DIVISION OF LABOUR

Eusocial insects display many interesting phenomena that merit investigation. Here we focus on two such interesting phenomena namely post-imaginal regulation of reproductive division of labour and, (in the next section), age

polyethism (Table 1). As described above, reproductive caste differentiation means that only one or a small number of individuals in the colony reproduce. How does this come about? In most species studied, the difference between queens and workers is not a genetic one; any egg can potentially develop into a queen or a worker depending on the environment it encounters. In highly eusocial species such reproductive caste determination occurs in the pre-imaginal stage so that adults eclose with their fates (as queens or workers) fixed. It is such pre-imaginal caste determination that permits queens and workers to be morphologically distinct. Despite some pre-imaginal caste-bias (Gadagkar *et al.*, 1988), many or all females in primitively eusocial species are totipotent at eclosion with respect to queen and worker roles. How then do they differentiate into queens and workers? Post-imaginal caste determination or post-imaginal regulation of reproductive division of labour is brought about by aggressive interactions among the adult females, which may result from and/or influence their nutritional and hormonal status. It is the absence of pre-imaginal caste determination and the totipotency of the adults at eclosion that result in morphologically identical queens and workers.

There is growing evidence that juvenile hormone modulates post-imaginal regulation of reproductive division of labour in primitively eusocial species (Table 1). For *e.g.*, juvenile hormone acts as a gonadotropin in the bumble bee, *Bombus terrestris*. In the absence of a queen, the onset of ovarian development in worker bumble bees is preceded by a sharp elevation in rates of juvenile hormone biosynthesis and circulating titers (Röseler and Röseler, 1978; Bloch *et al.*, 1996, 2000). Egg-laying individuals have high titers, while reproductively inactive individuals have low titers of juvenile hormone (Bloch *et al.*, 2000). Treatment with juvenile hormone also promotes ovarian development in the primitively eusocial sweat bee, *Lasioglossum zephyrum* (Bell, 1973). Several investigations in the primitively eusocial wasp genus *Polistes* also demonstrate the role of juvenile hormone as a gonadotropin. During nest foundation, female wasps establish a linear dominance hierarchy and the most dominant female becomes the functional queen of the colony (Röseler *et al.*, 1985). The probability that a female will be dominant in the colony is strongly correlated with the size of her corpora allata, the source of juvenile hormone (Röseler *et al.*, 1984, 1985). Treatment with juvenile hormone significantly increases the aggressiveness of females in *Polistes annularis* (Barth *et al.*, 1975).

Table 1. Two interesting phenomena in eusocial insects

Phenomenon	Primitively Eusocial	Highly Eusocial	<i>Ropalidia marginata</i>
Post-imaginal regulation of reproductive division of labor	Present	Absent	Present
Age polyethism	Absent, poorly developed or poorly studied	Present	Present

AGE POLYETHISM

Age polyethism or the systematic change in task specialization of workers as they grow older, is the best known mechanism of division of labour, especially in those species where task specialization is not based on morphological sub-castes among the workers. The honey bee is a prime example of age polyethism where worker bees perform different tasks as they grow older, always beginning with intranidal tasks and ending with extranidal tasks (Wilson, 1971). There is good evidence that age polyethism is regulated by juvenile hormone (Table 1). Titters of juvenile hormone gradually increase during adult life so that high titters are correlated with foraging behaviour. Application of juvenile hormone to young adult worker bees causes them to shift their behaviour from nursing and to become precocious foragers (reviewed in Robinson, 1992). Juvenile hormone titters are low in bees that work in the hive performing brood care ("nursing") and other activities, and high in foragers (Robinson, 1987b, Robinson *et al.*, 1989; Huang *et al.*, 1991, 1994; Huang and Robinson, 1995). Experiments designed to understand the role of juvenile hormone in age-related division of labour suggest that treatment with juvenile hormone-III (or its mimic methoprene) on the first day of adult life could induce a worker bee to forage precociously (Jaycox, 1976; Robinson, 1985, 1987a; Robinson and Ratnieks, 1987; Sasagawa *et al.*, 1989). Results of the juvenile hormone treatment experiments as well as the juvenile hormone measurements suggest that an elevated juvenile hormone titer is required for bees to mature into foragers.

Juvenile hormone has also been shown to be involved in the regulation of age-related division of labour in other highly eusocial social insects. O'Donnell and Jeanne (1993) report that treatment with juvenile hormone analogues affect division of labour in colonies of the highly eusocial tropical wasp, *Polybia occidentalis*. Wasps treated on day 1 of adulthood began foraging at younger ages, just as in honey bees.

THE CONUNDRUM OF JUVENILE HORMONE ACTION

As described above, a phenomenon of particular interest in primitively eusocial species is the post-imaginal regulation of reproductive division of labour, which permits queens to maintain reproductive monopoly, in spite of the workers being potentially capable of reproduction. Similarly a phenomenon of special interest in highly eusocial species is age polyethism, which permits colonies to adaptively allocate workers to different tasks even though all workers are morphologically identical. The evidence available so far suggests that juvenile hormone modulates reproductive division of labour in primitively eusocial species and promotes the production of queens while it modulates age polyethism and promotes the production of foragers in highly eusocial species. To the extent that queens and foragers represent opposite ends of the spectrum of division of labour in social insects, these two effects of juvenile hormone appear to be in "opposite" directions and that is the conundrum of juvenile hormone action (Table 1).

POSSIBLE SOLUTIONS TO THE CONUNDRUM OF JUVENILE HORMONE ACTION

Since juvenile hormone was hitherto known only to modulate ovarian development in primitively eusocial species and age polyethism in highly eusocial

species, there was not such a serious conceptual problem in reconciling its two functions. It was argued that juvenile hormone might have acquired a novel function (regulation of age polyethism), and one that is probably incompatible with its original function (regulation of ovarian development), 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). 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 (Agrahari and Gadagkar, 2003). Alternatively, it has also been suggested that juvenile hormone can modulate age polyethism without losing the ability to modulate ovarian development. This, it has been argued, may be possible because the function of modulating ovarian development is performed in one set of individuals (queen-destined individuals) while the function of modulating age polyethism is performed in a different set of individuals (worker-destined individuals). This has been termed as the "split function" hypothesis (West-Eberhard, 1996). In order to distinguish between the novel function or the single function hypothesis, we need a species that simultaneously exhibits both phenomena, post-imaginal regulation of reproductive division of labour and age polyethism, and therein lies the importance of *R. marginata*.

ROPALIDIA MARGINATA

Ropalidia marginata is an old world, tropical, primitively eusocial polistine wasp abundantly distributed in South India (Fig. 1). New colonies are founded throughout the year by a single foundress or a group of foundresses. In a single foundress nest, the lone female builds the nest, cares for the larvae, forages and feeds the developing brood, guards the nest, and, like any solitary wasp mother, she brings her offspring to adulthood, all by herself. In multiple foundress nests there is division of labour. One and only one individual functions as the sole egg layer or queen, who begins her career as a very aggressive individual attacking and biting her nestmates. Once she begins to lay eggs however, she becomes a strikingly docile individual who nevertheless maintains her reproductive monopoly, very likely through the use of pheromones (Gadagkar, 2001b). At any given time only one individual monopolizes all egg laying; the rest of the individuals, in all colonies studied, do not lay eggs while the queen is present.

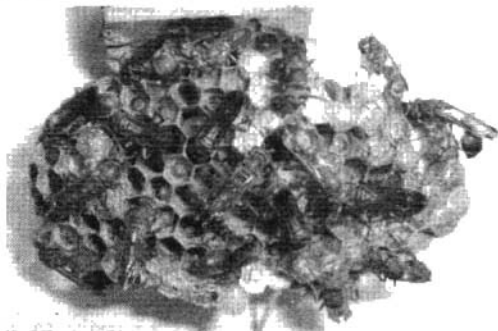


Fig. 1. A typical nest of *Ropalidia marginata* (Photo: R. Gadagkar)

POST-IMAGINAL REGULATION OF REPRODUCTIVE DIVISION OF LABOUR IN *R. MARGINATA*

Dissection of all the female wasps in a colony of *R. marginata* and examination of their ovaries confirms the behavioural observation of monogyny. Most colonies have three kinds of individuals: a queen with well-developed ovaries and at least one mature oocyte in each of the six ovarioles; a subset of individuals with partially developed ovaries containing several developing oocytes; but usually no fully mature oocytes and another subset of individuals with completely undeveloped ovaries containing thread like ovarioles without any discernible oocytes. In Fig. 2 data on several measures of body size as well as several measures of ovarian development have been subjected to principal components analysis and the relative positions of the members of a colony have been plotted in the space of the first two principal components. We see clearly that the queen, though among the largest individuals in her colony, is not distinguished in any qualitative way from her nestmates (Fig. 2A).

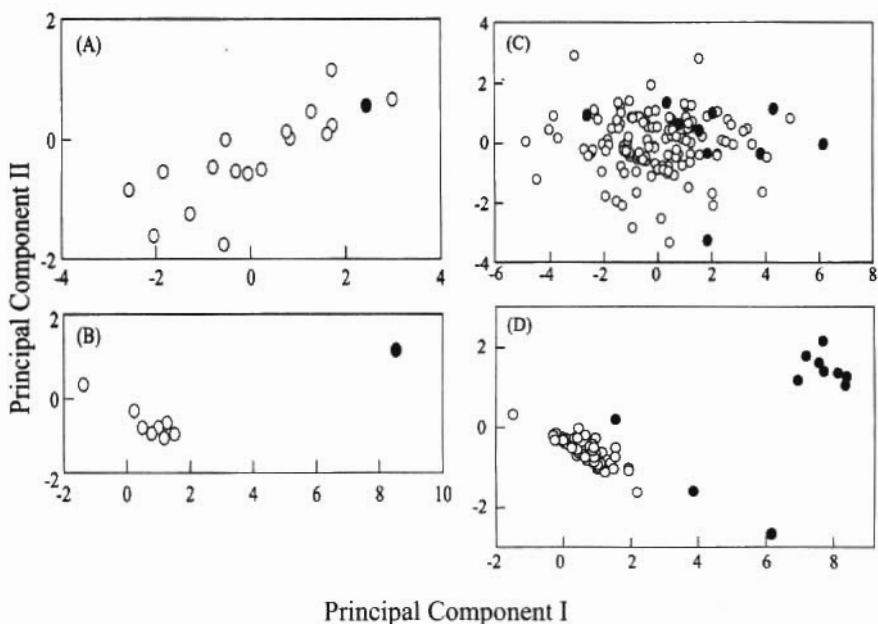


Fig. 2. A and B, Relative positions of queen and workers, with reference to their body size (A) and ovarian condition (B) in a typical colony. Data on body size and ovarian condition are subjected to principal component analysis and the relative positions of the queens and workers are plotted in the space of the first two principal components. C and D: The relative positions of queens and workers from 11 colonies, derived from a similar analysis. Note that in body size (C) queens and workers are not separated from each other, but that in their ovarian condition (D) queens and workers are well separated from each other. (Reprinted from Gadagkar, 2001b)

The situation with ovarian development is quite different the unique position of the queen is obvious (Fig. 2B). Fig. 2C and 2D depict the results of a similar analysis except that data from 11 different colonies are analysed simultaneously. When variation across colonies is thus taken into consideration, a new result comes to the fore - while most workers in most colonies have poorly developed ovaries compared to the queens of most colonies (Fig. 2D), many workers in many colonies are larger in body size compared to the queens of many other colonies (Fig. 2C). Even if the queen of a colony is sometimes larger in body size than all her nestmates, the fact that workers of some colonies can be larger in body size than queens of other colonies is striking proof of the lack of morphological caste differentiation between queens and workers. If queens and workers are morphologically distinct, the process of caste differentiation must necessarily take place in one of the pre-imaginal stages because no morphological changes are possible in the adult stage. But, in the absence of morphological differentiation between queens and workers the process of caste differentiation can also take place in the adult stage.

Another line of evidence for post-imaginal regulation of reproductive caste differentiation in *R. marginata* comes from experiments in which hundreds of female wasps were isolated at eclosion and provided suitable conditions for them to build nests and lay eggs, much as single foundresses do in nature (Gadagkar *et al.*, 1988; 1990). The clear-cut results of several replicate experiments was that about 50% of the wasps under these conditions built nests and laid eggs while the remaining 50% died without doing so, in spite of living longer on average than the time taken by those laying eggs to begin to do so. At the time these experiments were performed, the exciting result was that 50% of the wasps did not build nests and lay eggs. In the present context however, the fact that 50% of the wasps in a population can potentially build nests and lay eggs is the relevant result. Since there is always one and only one individual per colony who lays eggs, only a small fraction of out of the wasps who are potentially capable of laying eggs actually end up having the opportunity to do so and this is what we mean by post-imaginal regulation of reproductive caste differentiation.

The final and most compelling evidence of post-imaginal regulation of reproductive caste differentiation comes from repeated queen-removal experiments. Experimental removal of the queen always results in the emergence of a new queen; one of the workers becomes extremely aggressive and takes over as the colony's next queen. When she starts laying eggs she in turn will become a behaviourally docile queen who nevertheless maintains her reproductive monopoly. We have had the opportunity to witness such queen replacements during experiments designed to understand the nature of future queens (Chandrashekara and Gadagkar, 1992), to compare queens with their successors (Sumana and Gadagkar, 2003), to understand the role of queens in regulating worker activity (Premnath *et al.*, 1995) as well as during studies of natural queen replacements conducted with the aim of constructing pedigrees of queens (Gadagkar *et al.*, 1993). Every observation of queen replacement by a worker wasps proves that individuals potentially capable of functioning as queens are forced to function as workers by processes that bring about reproductive caste differentiation among totipotent adult wasps.

AGE POLYETHISM IN *R. MARGINATA*

Given the primitively eusocial status of *R. marginata*, there is a surprisingly well-developed, remarkably hone bee like- age polyethism. Feeding larvae, building the nest, bringing pulp, and bringing food are four tasks performed sequentially by successively older wasps. More than the absolute age of the wasps, their relative position in the age distribution of the colony appears to strongly influence the task profile of an individual (Naug and Gadagkar, 1998a). As in the case of honey bees, age polyethism in *R. marginata* is flexible. Loss of older individuals results in precocious foragers who forage at abnormally young ages (Naug and Gadagkar, 1998b). Similarly, the reduction in nursing caused by the loss of young individuals is compensated by hard working nurses (Agrahari and Gadagkar, submitted).

R. marginata thus appears to be a unique primitively eusocial species which has both, *Polistes* like reproductive division of labour and honey bee like non-reproductive division of labour, making it an ideal model system to investigate the conundrum of juvenile hormone action and thus understand the evolution of the modulatory function/s of juvenile hormone.

ROLE OF JUVENILE HORMONE IN POST-IMAGINAL REGULATION OF REPRODUCTIVE DIVISION OF LABOUR IN *R. MARGINATA*

To investigate the role of juvenile hormone in post-imaginal regulation of reproductive division of labour, 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 (22cm X 11cm X 11cm) 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 (juvenile hormone treated): 100g of juvenile hormone-III (Sigma Inc. USA) in 2l of acetone, applied on the abdomen on the day of eclosion (sample size = 34 wasps). Treatment 2 (acetone treated): 2l 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).

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. 3, right 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. 3, left panel). Fig. 4 shows the scatter in age of wasps of each group at the time egg laying and clearly brings out the difference between juvenile hormone treated individuals and the acetone treated and control wasps. Thus 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 labour and promotes the production of queens as has been observed in *Polistes* and *Bombus*.

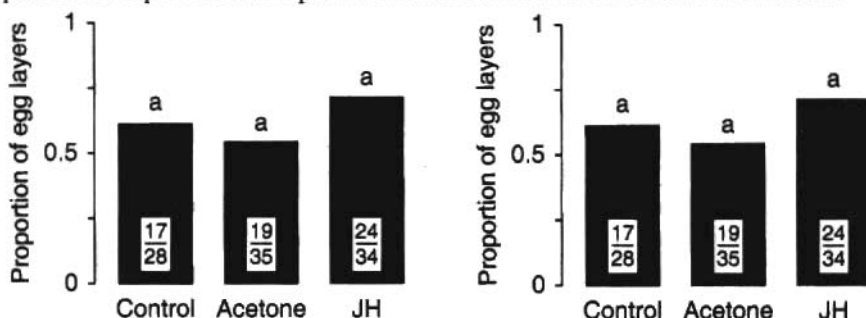


Fig. 3. left panel: Effect of juvenile hormone on egg laying in isolated wasps. The proportion of isolated wasps that lay eggs in the three treatments are not significantly different from each other as indicated by same letter on each bar (Pair-wise G test, $p > 0.05$). The number of egg layers and total number of wasps tested is indicated inside the bars.

Right panel: The mean age at egg laying of juvenile hormone 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. (Reprinted from Agrahari and Gadagkar, 2003, Copyright: Elsevier)

ROLE OF JUVENILE HORMONE IN AGE POLYETHISM IN *R. MARGINATA*

To investigate the possible role of juvenile hormone in regulating age polyethism in *R. marginata*, behavioural observations were conducted on 3 naturally occurring *R. marginata* nests. Observations consisted of, recording every occurrence of feed larva, build, bring pulp and bring food (see Gadagkar, 2001b, for methods of observation). Adults were uniquely marked and randomly assigned to one of two treatments *viz.* juvenile hormone or acetone within 24 hours after eclosion. Subsequently the treated individuals were returned to their natal nests. Because there was no difference between acetone treated wasps and control wasps in the isolation experiments described above, here we did not have control wasps with neither treatment.

We found 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 (2-way ANOVA followed by Tukey-Kramer test, $p > 0.05$; Fig. 5). 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, 1998a).

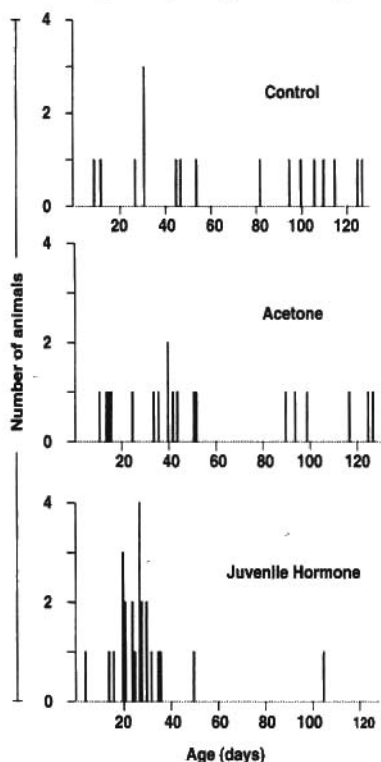


Fig. 4. Number of wasps that initiate egg laying at different ages in the three treatments

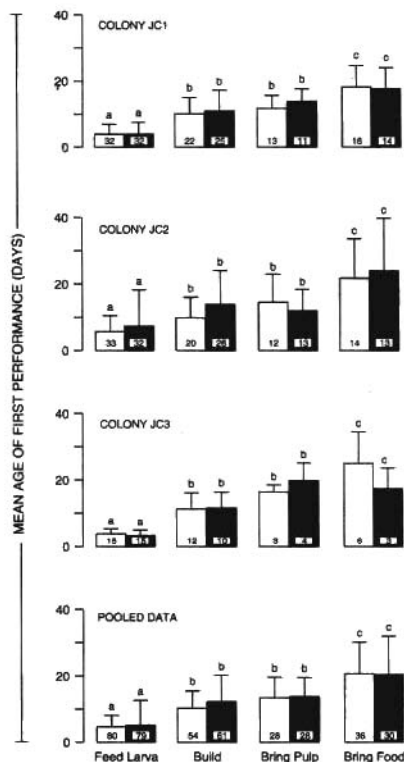


Fig. 5. Mean and Standard deviation of age at first performance of different behaviours in three colonies and in the pooled data. In each panel, bars carrying the same letter are not significantly different from each other. There is no significant difference between juvenile hormone treated wasps (open bars) and acetone treated wasps (filled bars) in all cases (2-Way ANOVA followed by Tukey-Kramer test $p > 0.05$). Number of wasps tested are indicated inside the bars. (Reprinted from Agrahari and Gadagkar, 2003, Copyright: Elsevier)

Of all the primitively eusocial species studied so far, *R. marginata* has the best developed, honey bee like, age polyethism (Naug and Gadagkar, 1998a,b). 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. We conclude therefore that in *R. marginata*, which exhibits both post-imaginal regulation of reproductive division of labour as well as age polyethism, juvenile hormone modulates the former but not the latter.

CONCLUSIONS

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). Neither primitively eusocial species such as *P. dominulus*, where there is a clear evidence for post-imaginal regulation of reproductive division of labour but no evidence of age polyethism, nor species such as honey bees where there is no post-imaginal regulation of reproductive division of labour 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 labour 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 phenomenon, 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 labour is modulated by juvenile hormone in *R. marginata*, we conclude that the novel or single function hypothesis is supported in the present study. Why has juvenile hormone not acquired a "split function" of modulating both ovarian development and age polyethism? 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, 2001b).

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