

Juvenile Hormone in Adult Eusocial Hymenoptera: Gonadotropin and Behavioral Pacemaker

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Studies on the role of juvenile hormone (JH) in adult social Hymenoptera have focused on the regulation of two fundamental aspects of colony organization: reproductive division of labor between queens and workers and age-related division of labor among workers. JH acts as a gonadotropin in the primitively eusocial wasp and bumble bee species studied, and may also play this role in the advanced eusocial fire ants. However, there is no evidence that JH acts as a traditional gonadotropin in the advanced eusocial honey bee or in the few other ant species that have recently begun to be studied. The role of JH in age-related division of labor has been most thoroughly examined in honey bees. Results of these studies demonstrate that JH acts as a "behavioral pacemaker," influencing how fast a worker grows up and makes the transition from nest activities to foraging. Hypotheses concerning the evolutionary relationship between the two functions of JH in adult eusocial Hymenoptera are discussed. Arch. Insect Biochem. Physiol. 35:559–583, 1997. © 1997 Wiley-Liss, Inc.

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INTRODUCTION

The eusocial Hymenoptera live in some of the most complex animal societies on earth. At the heart of this social organization is a reproductive division of labor. One or a few individuals (queens) in a colony specialize in

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reproduction while the masses (workers) engage in little, if any, personal reproduction and perform all the tasks related to colony growth, development, and defense (reviewed by Wilson, 1971). A second key feature of many hymenopteran societies is an age-related division of labor among the workers, in which workers perform different tasks at different ages (reviewed by Robinson, 1992). Here we review how juvenile hormone (JH) regulates reproductive division of labor between queens and workers and age-related division of labor among workers in adult eusocial Hymenoptera. We conclude with a brief section speculating on the evolutionary relationship between these two functions. JH is also known to influence the development of individuals into either queens or workers in bees and ants, and into morphologically distinct castes of workers in ants. Hormonal control of caste determination has been covered in excellent reviews of insect polyphenism (Nijhout and Wheeler, 1982; Wheeler, 1986, 1991; Nijhout, 1994) and is beyond the scope of this paper.

REPRODUCTIVE DIVISION OF LABOR

There is tremendous variation in the complexity of social organization within the eusocial Hymenoptera. This variation forms a continuum, but it is often useful to classify eusocial species into one of two groups based on degree of social complexity (Michener, 1974; Fletcher and Ross, 1985). In primitively eusocial species, there are little or no morphological differences between queens and workers, colony size is small (typically 20–100 individuals), and colonies have annual life cycles. Included in this group are the eusocial halictids, eusocial carpenter bees, bumble bees, polistine wasp species that initiate new colonies as solitary individuals, eusocial stenogastrine wasps, and *Microstigmus comes*, the only known eusocial sphecid wasp. In advanced eusocial species, there are pronounced morphological differences between queens and workers, colony size is larger (from hundreds to tens of thousands of individuals), and colonies have perennial life cycles. Advanced eusocial Hymenoptera include the ants, honey bees, stingless bees, vespine wasps, and some polistine wasp species that initiate new colonies as swarms.

Primitively and advanced eusocial species also differ markedly in the mechanisms by which reproductive division of labor is established and maintained. Primitively eusocial species, with their small colony size, employ primarily behavioral mechanisms, including pushing, biting, and prevention of egg-laying. Dominant individuals inhibit ovary development and egg-laying behavior in subordinates via overt behavioral interactions. Because any adult member of a colony of a primitively eusocial species apparently is physically capable of reproducing, behavioral domination is an ongoing process. As a consequence, life in primitively eusocial colonies is characterized by a relatively high degree of aggression.

Direct physical aggression among nestmates in colonies of advanced eusocial species is rare. The major proximate mechanisms underlying reproductive division of labor in this group appear to be primer pheromones produced by queens that inhibit ovary development and egg-laying behavior in workers. These pheromones traditionally have been thought to act directly

on workers, as chemical analogs of the physical aggression found in primitively eusocial species. Recently, Keller and Nonacs (1993) suggested that queen pheromones instead signal to workers the presence of a reproductively dominant queen, which then leads them to delay or forego reproductive development. Evidence in favor of either perspective is lacking, in part because only one queen primer pheromone has been identified to date, that of the queen honey bee (reviewed by Winston and Slessor, 1992).

REPRODUCTIVE DIVISION OF LABOR AND JH

JH is the major gonadotropin in female insects, but the magnitude and mechanism of JH action varies tremendously from species to species (reviewed by Koeppe et al., 1985; Nijhout, 1994). The most widespread roles of JH are the regulation of vitellogenin synthesis in the fat body and the incorporation of vitellogenin into developing oocytes in the ovary. In addition, JH influences oviposition behavior, mating behavior, and pheromone production in several species (Nijhout, 1994). Because of the pervasive role of JH in insect reproduction, social insect biologists have looked to this hormone to provide clues to the physiology of reproductive division of labor. Rather than trying to elucidate hormone regulation in social insects for its own sake, the primary goal of this research has been to use endocrine approaches to gain insight into the behavioral and/or pheromonal mechanisms by which the queen's status as dominant reproductive is established and maintained. Otherwise, the methods used to study the gonadotropic role of JH in the social Hymenoptera are the same as those used to study endocrine regulation of reproduction in other insect orders: studies correlating ovary development with either JH titers or rates of biosynthesis, and studies showing the effect of exogenous hormonal treatment on ovary development and egg-laying behavior.

The regulation of reproductive division of labor by JH has thus far been studied most intensively in four taxa, ranging from primitively eusocial to advanced eusocial: *Polistes* wasps, the bumble bee, *Bombus terrestris*, the fire ant, *Solenopsis invicta*, and the honey bee, *Apis mellifera*. As we will point out, the data are not parallel so that only limited comparisons can be made. JH acts as a gonadotropin in the primitively eusocial wasp and bumble bee species studied, and may also play this role in the advanced eusocial fire ants. However, there is no evidence that JH acts as a traditional gonadotropin in the advanced eusocial honey bee or in the few other ant species that recently have begun to be studied.

Polistes Wasps

As a primitively eusocial genus, queens of *Polistes* (subfamily Polistinae) employ various behavioral means to inhibit the ovary development and egg-laying behavior of nestmates (reviewed by Reeve, 1991; Röseler, 1991); pheromones are not known to be involved. There are two contexts in which *Polistes* queens dominate their nestmates. First, during colony foundation, when female foundresses join together to initiate a new nest, one foundress becomes dominant and monopolizes egg-laying. Later, after the nest is established and

worker offspring emerge, the queen dominates the workers, preventing them from developing their ovaries and laying eggs.

Female *Polistes* often jointly establish new colonies (reviewed by Reeve, 1991). These "co-foundresses" engage in aggressive social interactions leading to the establishment of a dominance hierarchy. The first experimental evidence for a gonadotropic role for JH in *Polistes* came from Bohm's (1972) study in which Ayerst JH (a synthetic mixture of eight isomers of *Cecropia* JH) was applied topically to diapausing females of *P. metricus*. The result was increased ovary development. Subsequently, most endocrinological work on *Polistes* used the European species, *P. dominulus* (formerly *P. gallicus*), focusing on the dominance hierarchy established among co-foundresses during colony initiation. Röseler et al. (1980) found that the dominant female in foundress associations of *P. dominulus* typically has larger corpora allata with higher rates of JH biosynthesis than those of subordinate females. Consistent with a gonadotropic function in this wasp, Röseler et al. (1980) also showed a positive correlation between ovary development and JH titer (using the semi-quantitative *Galleria* assay). In a later study, Röseler et al. (1984) demonstrated that after a dominance hierarchy has been established endocrine activity in subordinate foundresses (as reflected by corpora allata volume) is inhibited by the dominant foundress.

JH, and ecdysteroids, are also implicated in the regulation of dominance behavior in *P. dominulus*. Röseler et al. (1984) reported that injection of JH-I and 20-hydroxyecdysone, together or separately, significantly increased the chance of females becoming dominant. Röseler et al. (1985) found that dominance in ovariectomized females was closely correlated with corpora allata volume, whereas intact individuals showed no such correlation. Based on the assumption that ecdysteroids are produced by the ovary in adults, as they are in other insect orders (Nijhout, 1994), Röseler et al. (1985) suggested that JH and ecdysteroids normally interact to determine dominance, but in the absence of ecdysteroids only those individuals with relatively high JH titers can achieve dominance.

Individuals in established colonies of some species of *Polistes* form dominance hierarchies; the queen occupies the α -position as the principal aggressor and egg-layer (reviewed by Reeve, 1991). Little work has been done on the endocrine correlates of queen dominance over workers in *Polistes*. In the one study published to date, Barth et al. (1975) found that topical treatment of *P. annularis* workers with JH-III resulted in increased ovary development in workers and elevated levels of dominance behavior.

These results are consistent with the notion of JH as a gonadotropin, and also suggest a role for this hormone in the control of aggressive behavior. In addition to regulating ovary development, JH interacts with ecdysteroids to influence dominance behavior. The connection between endocrine regulation of ovary development and behavior serves to coordinate reproductive status with behavioral status (Röseler, 1991), which is a common theme in behavioral endocrinology. The wasp with the highest endocrine activity in the colony can achieve both behavioral and reproductive dominance. JH titers in *Polistes* must be sensitive to the effects of social interactions, but precisely how behavioral dominance inhibits the endocrine system of subordinates is not known.

Bumble bees

Bumble bees (Apidae) appear to use both primitive (behavioral) and advanced (pheromonal) means of regulating reproductive division of labor (Michener, 1974; Fletcher and Ross, 1985). Bumble bees are primarily adapted to the temperate zone, where individual queens initiate annual colonies that break up in the fall. The following generalized account of reproductive division of labor in bumble bees comes primarily from studies on the European species, *Bombus terrestris*.

In young colonies of *B. terrestris*, aggressive interactions are rare, suggesting that early in colony ontogeny the queen is able to suppress ovary development in workers, presumably by means of pheromones (van Honk et al., 1981; van Doorn and Heringa, 1986). As colony size increases, the ovaries of some workers develop to an increasing extent and they begin to lay eggs. The onset of worker reproduction is not solely a function of changes in queen control (Bloch et al., 1996). The onset of worker reproduction may also be related to a decrease in the inhibition of young workers by older workers, either by behavioral and/or chemical means (Bloch et al., 1996). When workers begin to lay eggs, the queen becomes overtly hostile toward them. But eventually the queen is driven from the nest or killed (van Honk and Hogeweg, 1981; van Honk et al., 1981) and dominant workers produce large numbers of males (Michener, 1974; van Honk et al., 1981; Owen and Plowright, 1982). A dominance hierarchy of workers also develops when the queen is experimentally removed; workers rapidly develop their ovaries, become aggressive toward one another, and eventually a single dominant worker lays most of the eggs (van Doorn, 1989).

Nearly all endocrine work on bumble bees to date has been done on *B. terrestris*, centering on queen regulation of JH and reproduction in workers. Röseler (1977) and van Doorn (1989) found that injection of JH-I increased ovary development in workers. In addition, the degree of ovary development in queenless workers has been shown to be associated with JH titer, as measured by the *Galleria* assay (Röseler, 1977). This is consistent with the report of a strong positive correlation between ovary development and the rate of JH biosynthesis in queenless workers (Larrere and Couillaud, 1993). Bloch et al. (1996) confirmed this correlation, after validating for the first time for this species the radiochemical assay for JH biosynthesis in vitro (Pratt and Tobe, 1974; Tobe and Pratt, 1974). Moreover, Bloch et al. (1996) reported a sharp rise in rates of JH biosynthesis three days before the increase in ovary development (Fig. 1). These results suggest that the rapid ovary development observed in queenless workers is stimulated by an increase in JH.

Some workers living with a queen begin developing their ovaries early in the colony cycle, but much more slowly than do queenless workers (Röseler, 1974, 1977). That ovary development occurs at all in queenright workers, despite their low rates of JH biosynthesis (Larrere and Couillaud, 1993), indicates that other factors besides JH are involved. But a role for this hormone under queenright conditions is nevertheless suggested by results showing that workers in the presence of their queen also undergo rapid ovary development when injected with relatively high doses (~50 µg) of synthetic JH-I (Röseler, 1977).

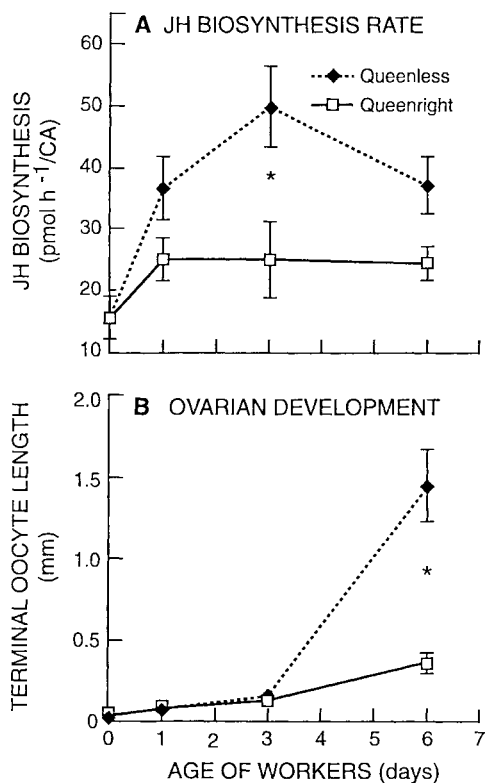


Fig. 1. Rates of JH biosynthesis (a) and ovary development (b) in the bumblebee, *Bombus terrestris* (n = 8–24 individuals for each data point). Asterisks denote significant differences between queenless and queenright workers. (From Bloch et al., 1996).

Ovary development and egg-laying are not necessarily regulated in tandem in *B. terrestris*. As stated above, some workers can develop their ovaries in the presence of the queen, but the queen can still prevent workers with mature oocytes from ovipositing (Larrere and Couillaud, 1993). It is not known whether JH also plays a role in the regulation of egg-laying behavior in *B. terrestris*. Larrere and Couillaud (1993) showed that queens emerging from diapause lay eggs despite exhibiting low rates of JH biosynthesis, suggesting that other factors also influence oviposition, such as nutritional status (West-Eberhard, 1996).

JH has also been implicated in the control of dominance behavior in *B. terrestris*, as in *Polistes*. Larrere and Couillaud (1993) reported that dominant workers have higher rates of JH biosynthesis than do subordinate workers. In addition, workers in queenless groups injected with synthetic JH-I were more likely to become dominant (van Doorn, 1989).

There is suggestive evidence of a queen pheromone in *B. terrestris*. Van Honk et al. (1980) showed that *B. terrestris* workers exhibited greater ovary development in the presence of queens whose mandibular glands had been removed than in the presence of queens with intact glands. Röseler et al.

(1981) reported that extracts of queen mandibular glands suppressed rates of JH biosynthesis in young workers. But the values reported for control workers were far lower than those of Bloch et al. (1996), suggesting atypical *corpora allata* function due to suboptimal culturing conditions. No active components of this pheromone have yet been isolated or identified.

In summary, results from *B. terrestris* are consistent with those from *Polistes* and again provide evidence for JH as a gonadotropin that mediates reproductive division of labor. Though JH plays a role in ovary development in bumble bees, it is not yet clear what, if any, effect JH has on egg-laying behavior in either workers or queens.

Fire Ants

Reproductive division of labor in ants has been intensively studied from behavioral and evolutionary perspectives (Hölldobler and Wilson, 1990), but surprisingly little is known about endocrine control of reproduction in this group. Workers of the fire ant *Solenopsis invicta* (subfamily Myrmicinae) lack functional ovaries and are completely sterile (Hölldobler and Wilson, 1990). However, winged virgin queens, which normally fly from the mound on mating flights before laying eggs, can shed their wings (dealate), develop their ovaries, and lay eggs in the nest in the absence of a functional queen (Fletcher and Blum, 1981a). These reproductive virgins do not mate in the nest, and therefore produce only male-destined, haploid eggs.

Results of several studies indicate that dealation and ovary development in fire ants are controlled by JH. Kearny et al. (1977) found that treatment with synthetic JH-I, -II, or -III induced both wing-shedding and egg-laying in virgin queens isolated from their queen. In addition, removal of the *corpora allata* (allatectomy) prevented dealation and egg-laying in isolated virgin queens, and topical treatment of Ayerst JH restored these responses (Barker, 1978, 1979).

As expected in a highly eusocial species, the proximate mechanism that normally prevents virgin queens from dealating and initiating reproductive maturation in fire ants appears to be a primer pheromone produced by the reigning queen. Indirect evidence for such a pheromone was obtained by Fletcher and Blum (1981a,b), who developed a bioassay based on their discovery that corpses of functional queens were effective in inhibiting virgin queen dealation and ovary development. Recent studies employing glandular extracts in this bioassay suggest that the pheromone is produced by the poison gland (Vargo and Slessor, unpublished data). Efforts are currently underway to isolate and identify the active components.

The putative fire ant queen pheromone seems to act by suppressing JH titers (Vargo and Laurel, 1994), as in *B. terrestris*. Topical treatment with the JH analog methoprene induced virgin fire ant queens to develop their ovaries, even in the presence of a fully functional (i.e., pheromone-producing) queen (Fig. 2). Results of other experiments by Vargo and Laurel (1994) further suggest that the pheromone prevents ovary development by blocking vitellogenin uptake by the ovaries rather than preventing vitellogenin synthesis; high levels of vitellogenin are present, yet the ovaries remain undeveloped. One hypothesis to explain the action of the putative fire ant queen

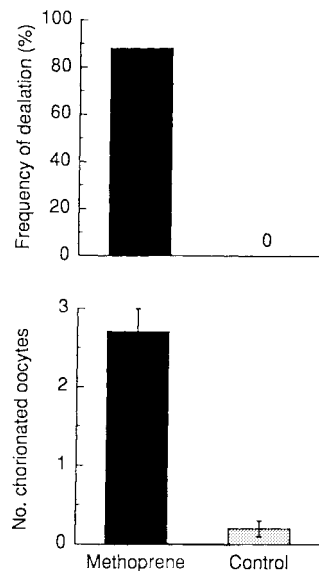


Fig. 2. Effect of the JH analog methoprene on wing shedding (dealation) and number of chorionated eggs (mean \pm SE) in fire ants, in the presence of a queen (dose: 0.5 μ g racemic methoprene in 0.5 μ l acetone, applied topically; n = 50; controls, n = 45, were treated with acetone alone). (From Vargo and Laurel 1994).

pheromone is that it depresses JH production so that JH titers are below the threshold needed to stimulate vitellogenin uptake by the ovaries, but still above the level required to induce vitellogenin synthesis by the fat body. Alternatively, different hormones may control vitellogenin synthesis and vitellogenesis (see Nijhout, 1994). Oviposition activity is highly correlated with ovary development in virgin queens (Fletcher and Blum, 1983; Vargo and Fletcher, 1989), indicating that egg-laying behavior occurs once mature eggs are present in the ovarioles.

The regulation of egg production and oviposition activity in mature, mated fire ant queens may also be subject to pheromonal control. Whereas in most populations of this species colonies are monogynous, with a single laying queen, some populations have colonies with many laying queens (reviewed by Ross and Keller, 1995). Queens in such "polygynous" colonies produce fewer eggs, on a per capita basis, than do single queens in monogynous colonies (Vargo and Fletcher, 1989). Using an assay based on the inhibitory potency of queen corpses, Vargo (1992) obtained evidence suggesting that the reduced fecundity of polygynous queens results, at least in part, from mutual pheromonal inhibition. He hypothesized that the pheromone acts by suppressing titers of endogenous JH, but the mode of action of the pheromone remains to be elucidated.

Vargo and Laurel (1994) demonstrated that topical application with methoprene also induced virgin fire ant queens to dealate (Fig. 2). The mechanism linking JH with dealation is not known, but Vargo and Laurel (1994) proposed that JH may act directly on the nervous system to elicit this behav-

ior. Evidence for direct JH action on the nervous system is exceedingly rare, as discussed later in this paper (see "age-related division of labor and JH").

JH thus acts as a gonadotropin in fire ants, with evidence for involvement in ovary development, dealation, and egg-laying behavior. But recent studies suggest that JH does not act as a gonadotropin in all ant species. Sommer et al. (1993) investigated the relationship between JH titers and reproductive dominance in *Diacamma* sp. (subfamily Ponerinae), measuring JH with the gas chromatography/mass spectroscopy (GC/MS) method of Rembold and Lackner (1985). This ant lacks a true queen caste; colonies are headed instead by a mated, reproductively active, worker "gamergate." In the absence of a gamergate, other workers engage in aggressive interactions leading to the establishment of a dominance hierarchy. Sommer et al. (1993) found that neither gamergates nor α -workers had detectable levels of JH-III. In contrast, JH titers were positively correlated with age in nonreproductive workers, a situation very similar to that found in worker honey bees (see "Age-related division of labor and honey bees", below). In another study, Sommer and Hölldobler (1995) reported that topical application of JH-III to founding queens of *Lasius niger* (subfamily Formicinae) reduced egg production, which is contrary to what has been found for *Polistes*, *B. terrestris*, and fire ants. More species need to be studied to gain a better understanding of the hormonal basis of reproductive division of labor in ants.

Honey bees

The European honey bee, *Apis mellifera* (Apidae), is one of the best-studied insects. Despite a vast literature on its ecology, social behavior, and social physiology (see Winston, 1987; Moritz and Southwick, 1992; Seeley, 1995), the endocrinology of reproduction in this species is not well understood.

Studies published 20 years ago already cast doubt on the assumed gonadotropic role of JH in honey bees. Vitellogenin synthesis and vitellogenesis in honey bee queens are only slightly reduced by allatectomy, even when performed as early as the pupal stage (Engels and Ramamurty, 1976; Ramamurty and Engels, 1977; Daerr, 1978; see Engels and Imperatriz-Fonseca, 1990). In addition, Ramamurty and Engels (1977) reported that topical application of JH-I only weakly stimulated ovary development in allatectomized laying queens. Engels (1978) found that JH-I, -II, or -III applied topically to unoperated queens failed to stimulate vitellogenesis or ovary development. More recent work also has failed to establish a role for JH in queen honey bee reproductive development. For example, mated laying queens, with well-developed ovaries, have JH titers much lower than young virgin queens, whose ovaries are far less developed (Fluri et al., 1981; Robinson et al., 1991; Fahrbach et al., 1995).

There also is no solid evidence implicating JH in the reproductive development of worker honey bees. Inconsistent with a gonadotropic role for JH, workers in queenright colonies show an inverse relationship between vitellogenin titer and JH titer. Although JH titers increase with worker age (Fluri et al., 1982; Robinson et al., 1987; Huang et al., 1991), vitellogenin titers reach a peak within the first few days of adult life and then gradually decrease; by 20 days of age there is little if any detectable vitellogenin (Engels, 1974). The

relevance of these results can be questioned because in the presence of a queen, honey bee workers only infrequently lay eggs in the nest (Page and Erickson, 1988; Visscher, 1989, 1996; Ratnieks, 1993). However, results consistent with these have also been obtained for workers that do engage in reproduction. Some workers develop their ovaries and lay (haploid) male eggs if a colony loses its queen and does not have young larvae from which to rear a replacement queen (reviewed by Bourke, 1988). Laying workers have low JH titers and low rates of JH biosynthesis (Robinson et al., 1992), as do queens. Furthermore, methoprene treatment did not cause increased ovary development in workers in queenless, broodless colonies (Robinson et al., 1992).

Recent results suggest that it is premature to conclude that JH does not play some role in regulating reproductive development in honey bees. Kaatz et al. (1992) demonstrated that queens inhibit JH biosynthesis in workers. They also showed that this effect is mediated by one of the major components of the queen mandibular pheromone, 9-keto-2(*E*)-decenoic acid. Queen suppression of JH in young workers has recently been shown in the field (Pankiw et al., submitted).

The significance of pheromonal suppression of JH production in honey bees is unclear. But given that honey bee queen mandibular pheromone is known to be involved in the maintenance of reproductive division of labor (reviewed by Winston and Slessor, 1992; Robinson, 1996), these results point to a still undiscovered role of JH in honey bee reproduction. Robinson et al. (1992) suggested that JH may play more of a primer role in stimulating ovary development, at least in worker honey bees. These authors hypothesized that removal of the queen and her pheromonal signals may cause a brief "commitment peak" of JH activity that primes the fat body and/or ovaries of young workers to develop in response to some other factors. This is analogous to hormonal control of egg development in young previtellogenic females of the yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae). Post-eclosion exposure to JH makes the ovaries and fat body of this mosquito responsive to other hormones that act on these organs later during ovary development (reviewed by Hagedorn, 1985). If a similar situation exists in honey bees, then high hemolymph titers of JH would not be necessary for vitellogenin synthesis, vitellogenin uptake, or egg-laying behavior, after a brief earlier exposure to the hormone. This hypothesis remains to be tested in honey bees, but there is some evidence against it. As mentioned above, allatectomy of queen pupae (Engels and Imperatriz-Fonseca, 1990) or newly emerged adult queens (Daerr, 1978) does not prevent ovary development in adults, suggesting that, at least in queens, a commitment peak of JH early in adulthood is not necessary for the ovaries to develop.

Much work remains to be done on the hormonal basis of reproduction in honey bees. In addition to elucidating what role, if any, JH plays in ovary development, it is important to identify other agents. There is preliminary evidence for the involvement of a neuroendocrine factor (Kaatz, 1988). In addition, the highest titers of ecdysteroids, primarily 20-hydroxyecdysone and makisterone A, are found in laying workers and queens (Kaatz, 1987; Robinson et al., 1991), suggesting that studies probing the role of ecdysteroids in the regulation of honey bee reproduction would be fruitful.

JH AS A GONDATROPIN IN THE EUSOCIAL HYMENOPTERA

JH does not appear to play a typical role in the regulation of reproduction in all species of eusocial Hymenoptera studied to date. However, as West-Eberhard (1996) points out, most studies on the role of JH in social insects have not adequately controlled for age and nutritional status, and these factors, known to influence reproductive development, may also mediate the action of JH. Similarly, Wheeler (1996) suggested that after the onset of endocrine-mediated oogenesis, subsequent reproductive activity may be regulated by the flow of nourishment from the workers, and not by hormones. If so, there could be very different mechanisms controlling ovary development in young and old individuals. In future work, it will be important to distinguish between the initiation of ovary development in young individuals and the regulation of ovary development in mature, reproductively active individuals.

AGE-RELATED DIVISION OF LABOR AMONG WORKERS

In addition to a division of labor between queens and workers, there is a pronounced division of labor among workers in many species of advanced eusocial Hymenoptera. This is not as evident in primitively eusocial species, although only a few species have been thoroughly studied. Division of labor among workers is associated with differences in worker age and/or morphology. Age-related division of labor is nearly universal, while size-related division of labor is limited to approximately 10% of the known ant genera (Hölldobler and Wilson, 1990) and the non-hymenopteran termites. The evolution of a highly structured worker force is generally seen as an evolutionary consequence of the developmental divergence between queens and workers. According to this scenario, once workers were limited to serve as helpers their characteristics were shaped further by natural selection to increase colony fitness (West-Eberhard, 1975; Oster and Wilson, 1978).

Age-related division of labor in insect societies is based on a form of behavioral development by workers known as age polyethism. The basic pattern of age polyethism is strikingly similar from species to species (Hölldobler and Wilson, 1990). Young workers perform tasks in the nest such as brood care and nest maintenance, and then venture outside to collect food and defend the nest when they get older.

AGE-RELATED DIVISION OF LABOR AND JH

The role of JH in age-related division of labor has been best studied in honey bees. Results of these studies demonstrate that JH acts as a "behavioral pacemaker," influencing how fast a worker grows up and makes the transition from nest activities to foraging.

Worker honey bees show typical age polyethism (reviewed by Winston, 1987; Robinson, 1992; Moritz and Southwick, 1992). They spend the first two to three weeks of adult life working in the hive and the remaining one to three weeks of life as foragers. The first experiments on the role of JH in the

regulation of age polyethism were performed by Jaycox et al. (1974) and Jaycox (1976) more than 20 years ago. Results of these studies suggested that treatment with either JH I or the Law-Williams JH mimic (Law et al., 1966) on the first day of adult life can induce a worker bee to forage precociously. More detailed studies confirmed this result (Robinson, 1985, 1987a; Robinson and Ratnieks, 1987; Sasagawa et al., 1986, 1989), demonstrating that application of methoprene accelerated behavioral development.

Early studies with the *Galleria* assay suggested that older bees had higher levels of JH compared with younger individuals (Rutz et al., 1976; Fluri et al., 1982). More recently, it has been demonstrated that there is a striking correlation between behavioral status and JH titer in honey bees. Radioimmunoassay (RIA) has shown that JH titers are low in bees that work in the hive performing brood care ("nursing") and other activities, and high in foragers (Robinson et al., 1987, 1989; Huang et al., 1991, 1994; Huang and Robinson, 1995). Comparable results have been obtained by analyses of JH titers with GC/MS (Hagenguth and Rembold, 1978). The most recent RIA results (Huang et al., 1994; Huang and Robinson, 1995) were obtained by analyzing hemolymph samples taken from individual bees, thanks to the development of a highly sensitive assay (Hunnicuttt et al., 1989) specifically validated for adult worker honey bees (Huang et al., 1994). Measurements of JH biosynthesis in vitro using the radiochemical assay (Pratt and Tobe, 1974; Tobe and Pratt, 1974) validated for adult worker honey bees (Huang et al., 1991) have shown that differences in JH titer between hive bees and foragers are correlated with differences in corpora allata activity (Fig. 3).

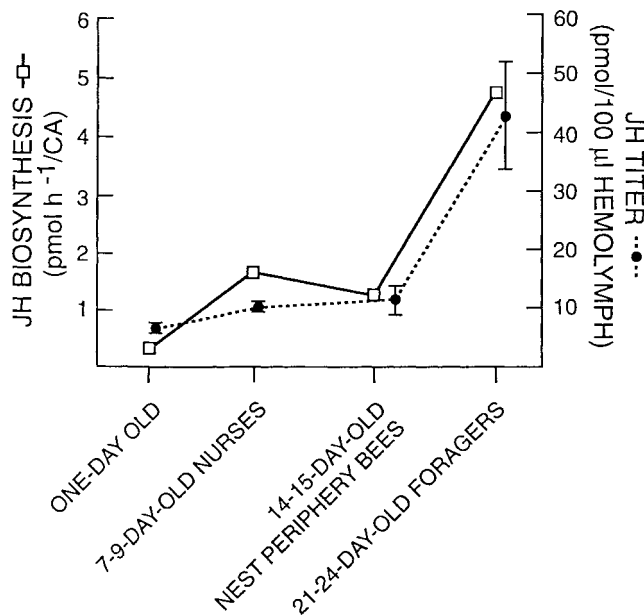


Fig. 3. Rates of JH biosynthesis and hemolymph titers of JH in worker honeybees ($n = 10$ individuals for each biosynthesis data point and 6–9 groups of bees for each titer data point, 5–9 bees per group, including the same bees for which rates of biosynthesis were measured). It is now possible to measure JH titers from individual bees (Huang et al., 1994). (From Huang et al., 1991.)

Age polyethism in honey bees is associated with marked changes in exocrine gland activity (reviewed by Winston, 1987). These changes are also influenced by JH. For example, when workers are young and in the nursing phase of life, their hypopharyngeal glands are largest and produce some of the material that they feed to the brood; these glands degenerate in foragers and shift to produce α -glucosidase, an enzyme involved in the conversion of nectar into honey. Low titers of JH or low rates of JH biosynthesis are typically associated with well-developed hypopharyngeal glands, while JH treatment induces premature hypopharyngeal gland degeneration (Rutz et al., 1974; Beetsma and Ten Houten, 1974; Jaycox et al., 1974; Jaycox, 1976; Sasagawa et al., 1989; Huang et al., 1994). Methoprene treatment similarly induces premature production of the alarm pheromones 2-heptanone and isoamyl acetate, which are also age-related processes (Robinson, 1985). However, results from "soldier" bees indicate that hypopharyngeal gland development is not always associated with age-related behavior and endocrine development; Huang et al. (1994) found that soldiers had larger hypopharyngeal glands than did foragers, despite their similar age and JH level. Nevertheless, it is clear that JH is a major factor in the tight linkage that generally exists between behavior and exocrine gland development in honey bees.

Age-related division of labor in many insect societies is very flexible (reviewed by Robinson, 1992). Worker honey bees are able to respond to changing colony needs by altering their typical patterns of age polyethism. Honey bee behavioral development is highly responsive to environmental and social cues, such as weather, season of the year, colony nutritional status, and especially colony age demography (Robinson, 1992; Huang and Robinson, 1992, 1996). These factors appear to influence behavioral development, at least in part, via their effect on the endocrine system. For example, young bees show precocious foraging in colonies that lack a normal complement of foragers, and precocious foragers have JH titers that are comparable to those of normal-age foragers (Robinson et al., 1989; Huang and Robinson, 1992). Similarly, young bees in colonies lacking a normal-sized force of nurses will continue to tend brood despite their advancing chronological age; these overage nurses continue to have low JH titers, as do normal-age nurses (Robinson et al., 1989). Behavioral reversion, from foraging to nursing, may also occur if there are no other nurses in the colony (Page et al., 1992; Robinson et al., 1992, and references therein), and "reverted nurses" show correspondingly lower JH titers (Robinson et al., 1992; Huang and Robinson, 1996). JH thus appears to act as a behavioral pacemaker, but how colony age demography acts on the endocrine system of the bee is not known (Huang and Robinson, 1992, 1996). Huang and Robinson (1992) have hypothesized that a worker-produced "inhibitor" circulates through the colony, and that older bees either produce more or transfer more of this inhibitor than do younger bees. The inhibitor is hypothesized to depress JH biosynthesis and behavioral development. This inhibitor has not yet been identified, but results consistent with this hypothesis have recently been obtained (Huang and Robinson, 1996).

JH AND AGE-RELATED DIVISION OF LABOR: CURRENT ISSUES

The role of JH in age-related division of labor is better understood than for reproductive division of labor in honey bees, but there are critical gaps in the former as well. The most important unresolved issue is exactly how JH acts to regulate worker honey bee behavioral development. It is hypothesized that JH affects the central nervous system to modulate response thresholds to the stimuli that elicit task performance (Robinson, 1987a,b). This is analogous to the regulation of behavior by steroid hormones in birds and mammals (e.g., Arnold and Breedlove, 1985, and references therein). However, it is not known whether, or how, JH influences the central nervous system of the honey bee.

JH may influence changes in brain structure that are associated with honey bee behavioral development. Withers et al. (1993) found that foragers have a larger neuropil volume in the mushroom bodies than do younger bees. Foraging is probably the most cognitively demanding task performed by honey bees and the mushroom bodies comprise the region of the insect brain most closely associated with learning and memory (see Fahrbach and Robinson, 1996). Withers et al. (1995) showed that bees that were prevented from foraging and treated with methoprene still showed mushroom body neuropil expansion, thus implicating JH as a causal factor in this structural plasticity. Similarly, JH has been shown to regulate neurogenesis in the brain of the cricket, *Acheta domesticus* (Orthoptera: Gryllidae; Cayre et al., 1994). JH may also influence other aspects of brain function, such as the electrophysiological properties of some neurons (Stout et al., 1991) or levels of peptide and biogenic amine neuromodulators. Progress in determining the relationship between JH and the central nervous system is hampered by a poor understanding of JH receptors. It is likely that tissues other than the nervous system (Riddiford et al., 1987; Shemshedini and Wilson, 1993; Engelmann, 1995) will provide the first definitive characterization of a cellular JH receptor (but see King et al., 1994, for evidence of specific binding of radiolabeled JH to cockroach nervous tissue).

It also is not clear whether the effects of JH on behavioral development in honey bees are "activational" or "organizational" (Arnold and Breedlove, 1985). Because foragers were always shown to have high titers of JH, it was assumed that bees require continuous exposure to high levels of the hormone to forage, which would suggest an activational effect. However, foragers with low JH titers have been observed on rare warm days in the winter (Huang and Robinson, 1995), indicating that high titers of JH are not always necessary for foraging. These results, coupled with possible effects of JH on brain structure (Withers et al., 1995), suggest that JH has organizational effects, i.e., effects on brain structure earlier in life that then permit foraging to occur later. This perspective is consistent with the observation that queens (Fahrbach et al., 1995) and drones (Giray and Robinson, 1996) have the highest JH titers early in adult life but are still able to fly later in life with lower titers. But these results then raise the question of the functional significance of the pervasive correlation between high hemolymph JH titers and foraging behavior in the summer (Huang and Robinson, 1995).

The functional significance of the correlation between JH titer and behav-

ior in worker honey bees in general has been questioned by Muller and Hepburn (1994). They reported that allatectomy, corpora allata implantation, or JH-III treatment did not affect the age at which worker honey bees began producing wax or the amount of wax produced. Methoprene treatment affected wax quantity but did not affect the timing of this process. On the basis of these results, Muller and Hepburn (1994) suggested that methoprene treatments caused artifactual results and then questioned whether JH plays any role in the regulation of honey bee division of labor. While distinguishing between physiological and pharmacological effects can be a vexing issue in behavioral endocrinology, the suggestion by Muller and Hepburn (1994) that JH is not involved in honey bee division of labor is not compelling for the following reasons. 1) The experimental results of Muller and Hepburn (1994) are difficult to interpret because measurements of JH were not made to verify the effects of allatectomy and corpora allata implantation. 2) It already has been shown that effects of methoprene treatment on behavior are only evident for tasks that are performed in a strongly age-related manner (Robinson, 1987a), and wax production is a task that is not always as strongly age-related as are others. Although wax production and comb building are generally performed by middle age bees (Seeley, 1995), Muller and Hepburn (1992) themselves showed that bees of a relatively broad age range (3–21 days old) can be involved in wax production, and similarly broad age curves for this task are evident for control bees in Muller and Hepburn (1994). The hypothesis that JH influences age-related division of labor would predict treatment effects on only the more strongly age-related tasks. 3) Whereas in Muller and Hepburn (1994) methoprene affected wax quantity, but not the timing of this process, Robinson (1985) reported that methoprene affected the timing of the production of the alarm pheromones 2-heptanone and iso-amyl acetate, but did not affect the amounts produced. Both pheromones were produced earlier in life, but this did not result in abnormally high amounts. Both pheromones were previously shown to be produced in a strongly age-related manner (reviewed by Winston, 1987). 4) In an attempt to address the issue of whether methoprene is causing physiological or pharmacological effects, Robinson (1987a) compared the effect of methoprene with that of ZR-371, a compound that is structurally very similar to methoprene but has no known hormonal activity. ZR-371 was found to have no effect on honey bee behavioral development when administered at the same dosage as methoprene. And methoprene treatment had no effect on the timing of foraging in *Bombus impatiens* and *B. pennsylvanica* (Cameron and Robinson, 1990), indicating that the material does not always cause individuals to age. 5) The evidence that JH is involved in the regulation of honey bee division of labor is not limited to results of methoprene treatments, but also includes results of treatment with JH-I (Jaycox, 1976), JH-III (Sasagawa et al., 1986, 1989), and hydrophrene (Robinson and Ratnieks, 1987), and extensive correlations between behavior, JH titer, and rates of JH biosynthesis (cited above). 6) Recent results provide stronger evidence for a link between JH and the timing of behavioral maturation; workers allatectomized within 24 hours of adult emergence initiate foraging at an older age relative to control and sham-operated bees, and this delay is eliminated with methoprene (Sullivan et al., 1996).

A more complete understanding of the role of JH in age-related division of labor would be possible if it was known how the production of this hormone is controlled in honey bees. The brain is an important source of allatostatins and allatotropins in other insects (e.g., Woodhead et al., 1989; Donly et al., 1993; Stay et al., 1994), and it is likely that similar mechanisms operate in honey bees, but this has not yet been demonstrated.

Finally, it is of interest to know whether JH is also involved in the regulation of age-related division of labor in other social insect species, particularly in other taxa besides bees. O'Donnell and Jeanne (1993) have recently reported that methoprene treatments affect workers of the tropical wasp, *Polybia occidentalis* (subfamily Polistinae), in the same way they affect honey bee workers. Wasps treated with methoprene on day 1 of adulthood began foraging at younger ages. In contrast, as mentioned above, methoprene treatment did not influence foraging ontogeny in two species of bumble bees, *Bombus impatiens* and *B. pennsylvanica* (Cameron and Robinson, 1990). Perhaps this is because age polyethism in these species of bumble bees is much weaker than in honey bees and *P. occidentalis*, or because other factors are involved besides JH. Studies of other species with strong age polyethism are required to resolve this issue.

FROM GONADOTROPIN TO PACEMAKER: EVOLUTIONARY SCENARIOS

JH plays two distinct and important roles in the regulation of life in a hymenopteran society, gonadotropin and behavioral pacemaker. How can we account for these two functions of this hormone? Because the regulation of reproduction is the main role of JH in adult insects in general (Nijhout, 1994), it is reasonable to assume that this was an ancestral function of this hormone in the social Hymenoptera. Given this assumption, the question is: What is the evolutionary relationship between hormonal regulation of reproduction and hormonal regulation of age-related division of labor?

Two hypotheses have been advanced to explain the evolutionary relationship between the two functions of JH in adult eusocial Hymenoptera. Based on studies of bumble bees and honey bees, Robinson et al. (1992) proposed that regulation of age polyethism in honey bees is a new function of JH that was made possible only after it lost its gonadotropic role. According to this scenario, the role of JH changed in honey bees from regulating reproduction to modulating behavioral development. This hypothesis, which has been termed the "novel-function" hypothesis by West-Eberhard (1996), predicts that one or the other role of JH, but not both, will be found in any given highly social insect species.

West-Eberhard (1996) presented an alternative hypothesis, the "split-function" hypothesis, which proposes that the ancestral functions of JH included not only physiological maturation, but also behavioral development associated with nest construction, brood care, aggressive defense of young, and foraging. These behavior patterns are also exhibited by solitary species of the aculeate Hymenoptera, which include lineages thought to be ancestral to the eusocial Hymenoptera. West-Eberhard (1996) suggests that in solitary spe-

cies that progressively provision the brood, relatively high JH titers may be associated with foraging and brood care, if: 1) these activities are performed by females that have relatively depleted ovaries, having recently laid an egg, and 2) ovaries with mature eggs inhibit the production of JH. According to this hypothesis, the hormonal mechanism regulating age polyethism is rooted in the mechanism that regulates the physiological and behavioral maturation of solitary ancestors, and the two functions became decoupled in workers and queens of the same species. Thus, rather than constituting a new function, regulation of age polyethism in workers of honey bees and perhaps other species (such as *P. occidentalis*) is seen as a modification of the JH regulatory process underlying maturation.

The split-function hypothesis also suggests that JH has different roles in different members of the colony, according to their nutritional status. According to this hypothesis, queens are relatively well-nourished during larval development and adulthood, which allows for complete ovary development; ovaries in this state would be responsive to JH, allowing for the regulation of oogenesis and egg-laying. In contrast, workers are relatively poorly nourished during larval development and adulthood, which results in less developed ovaries; ovaries in this state are not competent to respond to JH (but JH may still mediate behavioral maturation). Once the functions of JH became decoupled in the two castes, disruptive selection could result in the hormone becoming increasingly important in modulating behavioral development in workers.

The two hypotheses yield different predictions regarding the occurrence of the two functions within a single species. The novel-function hypothesis predicts that in a species where JH regulates age polyethism, it does not regulate ovary development. The split-function hypothesis predicts that both functions of JH can exist in the same species, with regulation of ovary development being the main role in queens and modulation of behavioral development (e.g., age polyethism) the main role in workers. Existing data are too sparse to permit a clear choice between the two hypotheses.

Supporting the split-function hypothesis, JH does have a number of behavioral effects in *Polistes*, *Bombus*, and fire ant reproductives—all species in which JH also regulates ovary development. But the split-function hypothesis predicts that JH acts primarily as a gonadotropin in queens and as a behavioral modifier in workers in the same species, and this has not yet been found. Important caveats are that only a few species have been studied and little attention has been paid to the age or nutritional state of individuals.

Supporting the novel-function hypothesis, JH in honey bees plays a key role in age polyethism but no gonadotropic role of JH has been found, either in queens or laying workers. In the two *Bombus* species that have been studied, JH acts like a gonadotropin but does not influence behavioral development (Cameron and Robinson, 1990). In the primitive ant, *Diacamma* sp., JH titers in workers are positively correlated with age but not with reproductive activity (Sommer et al., 1993).

Recent results provide indirect support for the split-function hypothesis. Giray and Robinson (1996) found that behavioral development in male (drone) honey bees is regulated by mechanisms common to workers, even though drones do not participate in colony division of labor and their only known

function is to mate. In one experiment, drones treated with methoprene started flying at significantly younger ages than did control drones, as is the case for workers (Fig. 4). In a second experiment, there was an age-related increase in JH associated with the onset of drone flight, as in workers. A similar pattern of change in rates of JH biosynthesis by drones has been reported by Tozetto et al. (1995). In a third experiment, drones were derived from egg-laying workers from genotypes that result in either fast or slow worker behavioral development. Sons of rapidly maturing workers started flying at younger ages than sons of slower maturing workers. The occurrence of common mechanisms in the two honey bee sexes with different functions is consistent with the split-function hypothesis. However, the connection between the onset of flight behavior and reproductive maturation in drones has not been examined.

More direct tests of the split-function hypothesis involve determining whether solitary species of Hymenoptera possess endocrine mechanisms regulating behavioral and reproductive development that are similar to what is found in eusocial species. Finding common mechanisms regulating behav-

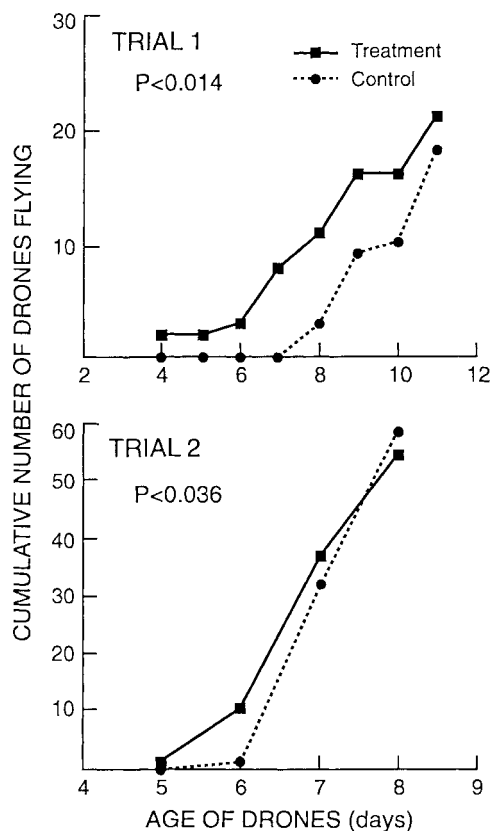


Fig. 4. Effect of the JH analog methoprene on rate of behavioral development in drone honeybees (dose: 500 μ g racemic methoprene in 5 μ l acetone, applied topically). P values based on results of Mann-Whitney U-tests. In trial 1, 21 out of 50 and 18 out of 50 drones were observed flying in the treatment and control groups, respectively; in trial 2, 54 out of 100 and 58 out of 100, respectively. (From Giray and Robinson, 1996.)

ioral development in solitary and eusocial species of Hymenoptera would provide powerful tools to study how reproductive division of labor and age polyethism evolved.

CONCLUSIONS

Studying the role of JH in reproductive division of labor in social insects provides a focal point for blending endocrinology, pheromone biology, and behavior. This synthesis is still in a formative stage but promises to yield important, paradigmatic, results. Studying JH as a behavioral pacemaker in division of labor among workers offers an opportunity to study hormone regulation of brain and behavior. Since division of labor defines the essence of insect sociality, further exploring JH function in the social Hymenoptera will provide new insights into the evolution and functioning of insect colonies.

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