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Reproduction in Worker Honey Bees Is Associated with Low Juvenile Hormone Titers and Rates of Biosynthesis

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Three experiments were performed to determine the role of juvenile hormone (JH) in worker reproduction in queenless colonies of honey bees. In Experiment 1, egg-laying workers had low hemolymph titers of JH, as did bees engaged in brood care, while foragers had significantly higher titers. Experiment 2 confirmed these findings by demonstrating that laying workers have significantly lower rates of JH biosynthesis than foragers do. In Experiment 3, ovary development was inhibited slightly by application of the JH analog methoprene to 1-day-old bees, but was not affected by application to older bees, at least some already displaying egg-laying behavior. These results, which are consistent with earlier findings for queen honey bees, are contrary to a common model of insect reproduction, in which elevated JH titers trigger ovary development, which then leads to oviposition. Previous experiments have demonstrated that JH regulates nonreproductive behavior in workers that is associated with colony division of labor; perhaps this function is incompatible with a traditional role for JH in reproduction. © 1992 Academic Press, Inc.

Division of labor between queens and workers is fundamental to the organization of complex insect societies. Queens lay eggs, while workers perform tasks related to colony growth and development and under most conditions engage in little, if any, direct reproduction. The evolution of division of labor between queens and workers has been studied extensively (e.g., Oster and Wilson, 1978; West-Eberhard, 1988). However, the proximal mechanisms underlying the regulation of worker reproduction are known for only a few species of social hymenoptera (Bell, 1973; Röseler *et al.*, 1981, 1984).

Workers in many species of social hymenoptera are able to produce haploid male offspring, a consequence of haplodiploidy. Regulation of worker reproduction appears to be mediated by the corpora allata, the glands that produce juvenile hormone (JH). JH treatment results in ovary development in the sweat bee *Lasioglossum zephyrum* (Bell, 1973). *Polistes gallicus* queen wasps inhibit the ovary development and egg-laying behavior of subordinate workers via behavioral interactions. In *Polistes* these interactions act on the worker endocrine system (Röseler *et al.*, 1984; reviewed by Röseler, 1985), resulting in a low titer of JH. Queen bumble bees inhibit worker reproduction via pheromones (reviewed by Free, 1987) that also depress the JH titer (Röseler *et al.*, 1981). Measurements of JH hemolymph titers (Röseler, 1977) and rates of biosynthesis (Röseler *et al.*, 1981) and experiments in which workers were treated with exogenous JH (Röseler, 1977) demonstrate that an elevated level of this hormone results in ovary development and oviposition in bumble bees. These findings are consistent with the role of JH as a major gonadotropic hormone in insects (reviewed by Hagedorn, 1983; Koeppe *et al.*, 1985; Raabe, 1986).

In the honey bee, *Apis mellifera*, queen

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pheromones, together with brood pheromones (e.g., Jay, 1970), also inhibit ovary development and egg-laying behavior in workers (reviewed by Free, 1987). Workers usually lay eggs in the presence of a queen only at extremely low frequencies (Page and Erickson, 1988; Visscher, 1989). In queenless colonies, however, worker oviposition is more extensive (see Velthuis, 1985; Page and Erickson, 1988; Robinson *et al.*, 1990, and references therein), and may result in the production of thousands of males. The role of JH in worker honey bee reproduction is not clear. A low injected dose (0.1 or 1 μg) of JH III stimulates the synthesis of vitellogenin, a major yolk protein, in adult worker honey bees (Imboden *et al.*, 1976; Rutz and Lüscher, 1974; Rutz *et al.*, 1976) but does not lead to oogenesis or egg-laying behavior (Rutz *et al.*, 1976). However, a possible role of JH in the regulation of worker honey bee reproduction may have been masked in these studies because they were conducted on colonies with a functioning queen ("queenright" colonies). Studies with bumble bees (van Doorn, 1987) have demonstrated that the effects of JH on worker reproduction are more apparent in the absence of a queen.

Although the effects of JH on worker honey bee reproduction are not well understood, this hormone is known to play a central role in the regulation of age polyethism, nonreproductive worker behavior that is associated with colony division of labor (reviewed by Robinson, 1992). Hemolymph levels of JH increase with worker age (Rutz *et al.*, 1976; Fluri *et al.*, 1982; Robinson *et al.*, 1987). Low titers are associated with brood care ("nursing" behavior), typically during the first 1 to 2 weeks of the worker bee's adult life, and a higher titer at about 3 weeks of age induces foraging (Robinson *et al.*, 1989). Treatment with JH (Jaycox, 1976), JH mimic (Jaycox *et al.*, 1974), or JH analog (Robinson, 1985, 1987a; Robinson and Ratnieks, 1987; Robinson *et al.*, 1989; Sasagawa *et al.*, 1986) induces precocious foraging.

The role of JH in the regulation of age polyethism is novel, given that the primary, though not exclusive, function of this hormone in adult insects is to regulate reproductive physiology and behavior (reviewed by Truman and Riddiford, 1974; Koeppe *et al.*, 1985; see also Rankin, 1978). In addition, age polyethism itself is considered to be a derived trait and is usually associated with insect societies that have relatively large populations and the most pronounced division of labor between queens and workers (Wilson, 1971; Michener, 1974). To begin to infer the possible evolution of this unique function for JH, it is important to know whether JH regulates both reproduction and age polyethism in worker honey bees.

Three experiments were conducted to determine the role of JH in worker honey bee reproduction. In Experiment 1, hemolymph titers of JH were measured for egg-laying workers, nurse bees, and foragers from queenless colonies. Among queenless workers, foragers are least likely to have developed ovaries and to engage in egg-laying behavior (Velthuis, 1985). JH titers that are higher for laying workers than for foragers would imply that a high level of JH is involved in worker reproduction in honey bees, as in other social and nonsocial insects. A titer for laying workers similar to that for nurse bees, and lower than that for foragers, would suggest that JH does not play this common role in worker honey bees. In Experiment 2, rates of JH biosynthesis for laying workers and foragers were measured to confirm the results of the first experiment. In Experiment 3, the effect of exogenous hormone treatment on ovary development was determined to help interpret the correlative results of the first two experiments.

METHODS

Experimental colonies. In Experiment 1, queens were removed from three source colonies that were each divided into two queenless colonies, each composed of ca. 15,000 workers, four combs of brood, two

combs of honey and pollen, and two empty combs of worker-sized cells (for food storage). One queenless colony from each source colony was used; these are designated Colonies 1–3 (the other queenless colonies were not used in any experiment). One empty comb of mostly drone-sized cells was added to each queenless colony to facilitate sampling of laying workers. Workers of European subspecies lay only haploid drone eggs and preferentially oviposit in drone-sized cells (Page and Erickson, 1988).

In Experiment 2, a queenless colony ("Colony 4") was established by removing ca. 5000 workers, two combs of honey and pollen, and one empty comb from a typical colony in the apiary. One empty comb of mostly drone-sized cells was then added to this unit. Establishment of a queenless colony without brood resulted in an earlier onset of worker ovary development and oviposition than in colonies with brood but had no other observable effect on behavior.

In Experiment 3, queens were removed from two source colonies. Each colony was divided into three queenless colonies (designated Colonies 5A,-B, and -C and 6A,-B, and -C), each composed of ca. 5000 workers, two combs of brood, one comb of honey and pollen, and one empty comb of mostly drone-sized cells. Colonies 5A and 6C were used in Experiment 3a and Colonies 5B, 5C, and 6A in Experiment 3b. Colony 6B was not used because its population was too small.

In each experiment, queenless colonies were transported to a distant apiary and arranged to minimize the possibility of workers entering the wrong hive and becoming members of a foreign colony. About 1 week after establishing the queenless colonies, we destroyed all immature queens that the workers reared to replace the loss of their original queen. All frames in each colony were then inspected every 3 or 4 days until eggs were observed. Colonies used in Experiment 1 were located at The Ohio State University Bee Laboratory, in Columbus, Ohio. Colonies used in Experiments 2 and 3 were located at the University of Illinois Bee Research Facility, in Urbana, Illinois. Bees at both localities are typical of North American populations of *Apis mellifera* [a mix of predominantly European subspecies (Phillips, 1915; Pellett, 1938)].

Behavioral classification. Laying workers were collected on the comb containing mostly drone-sized cells. Laying workers were unambiguously identified on the drone comb because oviposition is the sole behavior that involves backing into the comb's cells. Only individuals taken from cells containing one or more eggs were sampled because workers sometimes fail to deposit eggs in the cells they have backed into (Sakagami, 1958). This procedure helped ensure that samples contained bona fide laying workers. High levels of ovary development in most laying workers collected (see Results) confirmed the validity of this sampling procedure.

A nurse bee was identified as a worker with her head in a cell containing a larva. This criterion has been used widely in studies of division of labor (e.g., Sakagami, 1953; Seeley, 1982; Robinson, 1987a). Moreover, results from queenless colonies with allozyme genetic markers (Robinson *et al.*, 1990) have revealed genotypic differences between laying workers and nurse bees identified in this way. These results suggest that nurses and laying workers are distinct groups of workers in queenless colonies.

Foragers were obtained by temporarily obstructing the hive entrance with a piece of 8-mesh hardware cloth. We collected returning bees that alighted on the screen with either pollen loads in their corbiculae or distended abdomens, presumably full of nectar or water.

All workers sampled for hormone analyses were older than 19 days of age, including laying workers and nurses, presumably because they were among the youngest bees in these queenless (and eventually broodless) colonies.

Determination of ovary development. Ovaries were classified as either undeveloped or developed. "Undeveloped" ovaries had no enlargement of oocytes (Stage "1," according to the descriptive scheme of Velthuis, 1970) whereas "developed" ovaries had at least one, partially developed, round, or bean-shaped egg (Stage "2"), or at least one mature, sausage-shaped egg (Stage "3"). We classified ovaries as undeveloped or developed rather than use a finer classification system for the following reason. Honey bees develop and lay eggs continuously, rather than in batches (see Engels and Imperatriz-Fonseca, 1990); a bee that happened to have been sampled just after ovipositing, with only partially developed eggs present, may thus be in a similar reproductive state to a bee that happened to have been sampled with one or more fully developed eggs present. Ovaries were not examined for signs of oviposition.

We determined the degree of ovary development for bees from Colonies 1 and 4 that were designated as laying workers or foragers. This was done to validate the criteria used for behavioral classification and to determine whether measurements of JH could be related to state of ovary development as well as to egg-laying behavior. Effects of exogenous hormone treatment on ovary development were examined for bees from Colonies 5A, 5B, 5C, 6A, and 6C. Dissections were performed on specimens stored at −20°.

Determination of JH titers. Laying workers, nurses, and foragers (n = 120, 68, and 215, respectively) were collected for JH analysis, in groups of 5–11. Bees in each group were chilled (0° for 5 min) and immobilized with plasticine in a dissecting dish. Hemolymph (2.5–15 μl) was removed from each bee; a total of 42–53 μl was collected from each group and pooled for analysis because the JH assay is not sensitive enough to determine titers from individual bees. More foragers were

used for these analyses than laying workers or nurses because they yielded relatively smaller samples of hemolymph. Hemolymph samples were placed in 150 μl methanol (all solvents were HPLC grade, from Fisher Scientific). The methanol–hemolymph mixture was partitioned against hexane three times and the hexane phase, containing the JH, removed. Samples were stored at −20°.

JH titers were determined by radioimmunoassay (RIA) in France (Strambi *et al.*, 1984). This assay has been shown to provide consistent results that agree with titer determinations made by gas chromatography/mass spectrometry (GC/MS) (de Kort *et al.*, 1985). In addition, results from honey bees (Robinson *et al.*, 1987, 1989) agree with measurements made with the *Galleria* bioassay (Fluri *et al.*, 1982) and a GC/MS assay (Hagenguth and Rembold, 1978). All of these techniques have demonstrated that honey bees have only JH III in their hemolymph; hormone titers in this paper thus refer only to this homolog. All samples were coded, for blind analyses.

Measurement of JH biosynthesis. Rates of biosynthesis for the corpora allata were determined *in vitro* with a radiochemical assay (Tobe and Pratt, 1974; Pratt and Tobe, 1974; modified by Feyereisen and Tobe, 1981). Huang *et al.* (1991) recently adapted and validated this assay for adult worker honey bees and, in addition, demonstrated that rates of JH biosynthesis are highly correlated with JH hemolymph titers in worker bees. To perform this assay, corpora allata corpora cardiaca complexes were dissected in sterile saline. Each gland pair was transferred to a disposable tissue-culture tube (baked for 6 hr at 500°C) that contained 25 μl "bee medium" (modified from Kaatz *et al.*, 1985) and was preincubated for 20–60 min until all glands were dissected. Incubations began with the addition of 25 μl bee medium containing 100 μ*M* L-[*methyl*-^{3}H]methionine (sp act 200 mCi/mmol; New England Nuclear, Wilmington, DE). The glands were incubated in the dark at 35° for 3 hr. Medium was then collected and extracted with 250 μl iso-octane. Iso-octane extract (200 μl) was added to 5 ml scintillation cocktail (Biosafe II, RPI Corp.) and assayed for radioactivity by liquid scintillation spectrometry. The values obtained were corrected by a blank incubation for each assay. Gland pairs from each individual bee were cultured and assayed individually (see Huang *et al.*, 1991, for additional details).

Effect of methoprene on ovary development. In Experiment 3a, we determined whether artificially elevated hormone titers affect the development of worker ovaries by topically treating 450 one-day-old bees with 200 μg (*RS*) methoprene, a JH analog, dissolved in 5 μl acetone. To obtain bees of known age, sealed brood was removed from Colonies 5A and 6C at the time of dequeening and placed in an incubator (33°). Emerging bees were marked on the thorax with a spot of paint (Testor's PLA), treated, and reintroduced to their own colony. Control bees (n = 450) were treated with 5 μl acetone alone. A total of 150 treated and 150 control bees was introduced together each day over a 3-day period for each colony. Previous experiments indicate that simultaneously introducing methoprene-treated and acetone-treated bees apparently does not result in transfer of methoprene from treated to control bees (Robinson, 1985, 1987a). Marked bees, 19–21 days of age, were collected with a portable vacuum cleaner 7 days after eggs were first observed in each colony, when many eggs in each colony were present. Marked bees were collected randomly with respect to behavior from all four combs in each hive.

In Experiment 3b, we determined whether artificially elevated hormone titers affect older bees that are assumed to already possess developed ovaries. We marked and treated workers (with either methoprene or acetone, as in Experiment 3a) that were found on the drone comb in Colonies 5B, 5C, and 6A, at least 7 days after eggs were first observed in each colony (n = 150 treated and 150 control bees per colony). Because workers preferentially oviposit in drone-sized cells (Page and Erickson, 1988), collecting workers only from the drone comb increased the likelihood of treating bees with developed ovaries. We attempted to mark and treat as many workers as possible that actually displayed oviposition behavior during this process, which amounted to about 50 for all three colonies. Marked and treated bees were of unknown ages, but were at least 13 days old, based on observations of when the last cohort of adults emerged following queen removal in each colony. Bees were collected 7–10 days after treatment, in the same manner as in Experiment 3a. All marked bees were at least 20 days of age when collected.

The efficacy of methoprene as a JH analog in honey bees is well established (Robinson, 1985, 1987a,b; Robinson and Ratnieks, 1987; Robinson *et al.*, 1989; Sasagawa *et al.*, 1986; Sasagawa, 1989). Although some evidence (Prestwich, 1987) suggests that there may be different receptor sites for JH homologs and analogs in the tissue of at least one insect species (*Manduca sexta*), methoprene has demonstrated JH-like activity in many species, at the molecular (Wyatt *et al.*, 1987; Osir and Riddiford, 1988), physiological, and behavioral levels (Staal, 1975). The dose of 200 μg was chosen because it is known to exert strong effects on the physiology and behavior of worker honey bees in queenright colonies (Robinson, 1987a,b; Robinson *et al.*, 1989).

RESULTS

Experiment 1: JH Titers in Laying Workers, Nurses, and Foragers

Laying workers from queenless colonies had low hemolymph titers of JH, as did

nurse bees. Foragers had a significantly higher level of JH than both nurses and laying workers (Table 1).

The ovaries of laying workers were significantly more developed than those of foragers [laying workers: 29% undeveloped and 71% developed (n = 31); foragers: 82% undeveloped and 18% developed (n = 51); $P < 0.001$, G test for heterogeneity (Sokal and Rohlf, 1981)]. This result indicates that the behavioral sampling methods for laying workers and foragers result in a high proportion of bees with developed and undeveloped ovaries, respectively.

FIG. 1. Mean levels of JH biosynthesis ± SE for laying workers and foragers from a queenless colony of honey bees (n = 10 bees for each group). All bees were at least 20 days old when sampled. P value based on t test.

Experiment 2: Rates of JH Biosynthesis in Laying Workers and Foragers

Laying workers from queenless colonies had significantly lower rates of JH biosynthesis than did foragers (Fig. 1), confirming the results of Experiment 1. Results from Experiments 1 and 2 demonstrate that low levels of JH are associated with developed ovaries and egg-laying behavior in worker honey bees.

As in Experiment 1, the ovaries of laying workers sampled for Experiment 2 were significantly more developed than those of foragers [laying workers: 100% developed; foragers: 100% undeveloped (n = 10 per group); $P < 0.001$].

Experiment 3: Effect of Methoprene on Ovary Development

Methoprene exerted a slight, but significant, effect on ovary development in both queenless colonies when applied to 1-day-old workers (Table 2, Experiment 3a). However, methoprene did not significantly affect ovary status when applied to bees older than 13 days of age, at least some displaying egg-laying behavior and assumed to have developed ovaries at the time of treatment (Table 2, Experiment 3b).

TABLE 1

MEAN JH TITERS ± SE (pmol JH III PER 100 µl HEMOLYMPH) FOR LAYING WORKERS, NURSES, AND FORAGERS FROM QUEENLESS COLONIES OF HONEY BEES

Colony	Laying workers	Nurses	Foragers	Laying workers vs nurses	Laying workers vs foragers
1	1.6 ± 0.3 (10)	ND	20.4 ± 6.9 (9)	—	$P < 0.01$
2	2.4 ± 0.9 (5)	1.2 ± 0.6 (6)	22.7 ± 5.2 (3)	$P > 0.1$	$P < 0.01$
3	4.2 ± 0.6 (7)	3.2 ± 0.6 (6)	17.9 ± 1.4 (6)	$P > 0.1$	$P < 0.001$

Note. n = number of worker groups, 4–16 workers/group). All bees were at least 19 days old when sampled. P values shown are results of t tests (with unequal variances, where needed). ND, no data; sample not collected.

TABLE 2
EFFECT OF METHOPRENE ON WORKER OVARY DEVELOPMENT IN QUEENLESS COLONIES OF HONEY BEES

Colony	Treatment	Percentage undeveloped	Percentage developed	n	Difference
		Experiment 3a: Effect on newly emerged workers			
5A	Methoprene	85.6	14.4	132	$P < 0.05$
	Acetone	74.4	25.6	117	
6C	Methoprene	68.5	31.5	92	$P < 0.05$
	Acetone	53.3	46.7	90	
		Experiment 3b: Effect on older workers (at least some already exhibiting egg-laying behavior)			
5B	Methoprene	50.7	49.3	75	$P > 0.8$
	Acetone	49.2	50.8	59	
5C	Methoprene	55.3	44.7	38	$P > 0.05$
	Acetone	75.0	25.0	44	
6A	Methoprene	76.0	24.0	75	$P > 0.7$
	Acetone	78.5	21.5	79	

Note. P values are results of G tests for heterogeneity.

DISCUSSION

Reproduction in insects is controlled primarily by ecdysteroids, JH, and neurohormones (reviewed by Hagedorn, 1983; Koeppe *et al.*, 1985; Raabe, 1986), but the relative importance of these hormones varies among species. JH acts as a gonadotropic agent in many species: an increase in JH titer stimulates vitellogenesis and uptake of vitellogenin by oocytes, and then results in the eventual onset of oviposition. In some species high JH titers are not required throughout the reproductive process and drop after vitellogenesis (e.g., Renucci and Strambi, 1983) while in others high titers apparently are required for continued oogenesis (e.g., Cusson and McNeil, 1989; Satyanarayana *et al.*, 1991). High levels of JH are associated with oogenesis and egg-laying behavior in several species of social hymenoptera: *Bombus terrestris* (Röseler, 1977; Röseler *et al.*, 1981), *Polistes* spp. (Bohm, 1972; Barth *et al.*, 1975; Röseler *et al.*, 1984), *Lasioglossum zephyrum* (Bell, 1973), and the fire ant, *Solenopsis invicta* (Barker, 1978). In addition, elevated JH titers appear to be required for vitellogenesis in termites (Lüscher, 1976).

Results of Experiments 1 and 2 demonstrate that JH does not play a typical role in the regulation of reproduction in workers of *Apis mellifera*, especially relative to other social insects. Individuals displaying developed ovaries and egg-laying behavior have low levels of JH. Furthermore, these results cannot be taken to mean that there is a drop in JH after vitellogenesis, because vitellogenesis in honey bees proceeds continuously in reproductively active individuals (see Engels and Imperatriz-Fonseca, 1990). These findings, however, are consistent with those showing that both laying and nonlaying queens have similar titers of JH (Fluri *et al.*, 1981), and lower than those of workers engaged in foraging (Robinson *et al.*, 1992). Queens are able to undergo oogenesis and lay eggs despite the removal of the corpora allata (Hrdy and Slama, 1963; Engels and Ramamurty, 1976; reviewed by Engels and Imperatriz-Fonseca, 1990). Neuroendocrine factors other than JH are implicated in the control of vitellogenesis in queens (Kaatz, 1988).

In Experiment 3, ovary development was inhibited by application of the JH analog methoprene to 1-day-old bees. JH treat-

ment also inhibited egg-laying in *Camponotus aethiops* worker ants (Suzzoni *et al.*, 1986). The inhibitory effect in our experiment, however, was not dramatic; 10 to 15% fewer methoprene-treated individuals had developed ovaries, and ovary development still occurred in ca. 14 and 32% of the treated workers from Colonies 5A and 6C, respectively. Methoprene had no effect when applied to older bees, at least some assumed to have already developed ovaries at the time of treatment. These results, coupled with the results of Experiments 1 and 2, suggest the following three possibilities. First, high levels of JH may partially inhibit ovary development only in young workers. Second, high levels of JH may inhibit ovary development in workers of all ages, but older workers may be less sensitive to JH (and require a higher dose of methoprene). The third possibility is that JH may not play a direct role in the regulation of worker honey bee reproduction. It is not possible to determine which interpretation is more likely but the important point is that our results contrast sharply with the stimulatory effects of exogenous JH on ovary development reported for other social insects (Bell, 1973; Röseler, 1977; Röseler *et al.*, 1984).

Our results suggest that the queen does not exert long-term effects on the JH titers of workers. Both nurses and foragers from queenless colonies had JH titers (see Table 1) and rates of JH biosynthesis (see Fig. 1) similar to those of nurses and foragers from queenright colonies (Robinson *et al.*, 1989; Huang *et al.*, 1991, respectively). Hildebrandt and Kaatz (1990), however, reported that the queen can influence worker JH titers: small groups of workers in the laboratory had lower rates of JH biosynthesis when caged for 8 days with a queen (or an artificial source of mandibular gland pheromone) than without a queen. The influence of this queen effect on worker JH and on other aspects of worker physiology, including ovary development, is not known. Willis *et al.* (1990) reported that queen mandibular gland pheromone does not affect worker ovary development. In addition, in queenright colonies JH treatment induces vitellogenesis in workers (Imboden *et al.*, 1976; Rutz and Lüscher, 1974; Rutz *et al.*, 1976) but does not affect worker ovary development (Rutz *et al.*, 1976). It is thus not known whether queen pheromones, worker JH titers, and vitellogenesis are functionally related in bees. The results of Hildebrand and Kaatz (1990) suggest that there is a "commitment peak" of JH activity occurring relatively soon after a queen is removed from a colony that initiates worker reproductive development in some workers, analogous to the peak of ecdysteroids that induces the commitment of epidermis cells during metamorphosis in *Manduca sexta* (Riddiford, 1978). Such a transient change in JH may be detectable 8 days after queen removal (as in Hildebrandt and Kaatz, 1990), but not 2 to 3 weeks after queen removal (as in our experiments), when individuals had already developed into laying workers. Monitoring JH activity throughout worker reproductive development from the time of queen removal to the onset of egg-laying behavior would provide important information on this issue.

Previous experiments with honey bees have demonstrated that JH regulates worker age polyethism (reviewed by Robinson, 1992). Age polyethism is considered to be a derived trait that occurs in the largest and most complex insect societies (Wilson, 1971). Perhaps this relatively novel function for JH is incompatible with a more traditional role in the regulation of reproduction. In bumble bees, JH regulates worker reproduction (Röseler, 1977; Röseler *et al.*, 1981; Cameron and Robinson, 1990) but does not appear to be involved in division of labor among workers (van Doorn, 1987; Cameron and Robinson, 1990). However, bumble bees exhibit only weak age polyethism (Cameron, 1989), and studies of other species that display both worker age polyethism and worker reproduction are needed to evaluate the hypoth-

esis that JH does not play a major role in both processes in the same organism. It is intriguing that JH does not appear to play a typical role in honey bee reproduction, in either workers or queens. If the partial dissociation of JH from reproduction represents the ancestral state for honey bees, then this hormone may have been "available" to regulate age polyethism. However, hormonal regulation of reproduction has been studied in a very few bee species to date, and it is not known how or why this hormone would become dissociated from reproduction.

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