J Comp Physiol B (1995) 165: 18–28

ORIGINAL PAPER

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Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees

Accepted: 30 November 1994

Abstract Honey bee colonies can respond to changing environmental conditions by showing plasticity in age related division of labor, and these responses are associated with changes in juvenile hormone. The shift from nest tasks to foraging has been especially well characterized; foraging is associated with high juvenile hormone titers and high rates of juvenile hormone biosynthesis, and can be induced prematurely in young bees by juvenile hormone treatment or by a shortage of foragers. However, very few studies have been conducted that study plasticity in division of labor under naturally occurring changes in the environment. To gain further insight into how the environment and juvenile hormone influence foraging behavior, we measured juvenile hormone titers and rates of biosynthesis in workers during times of the year when colony activity in temperate climates is reduced: late fall, winter, and early spring. Juvenile hormone titers and rates of biosynthesis decreased in foragers in the fall as foraging diminished and bees became less active. This demonstration of a natural drop in juvenile hormone confirms and extends previous findings when bees were experimentally induced to revert from foraging to within-hive tasks. In addition, endocrine changes in foragers in the fall are part of a larger seasonally related phenomenon in which juvenile hormone levels in younger, pre-foraging bees also decline in the fall and then increase the following spring as colony activity increases. The seasonal decline in juvenile hormone in foragers was mimicked in summer by placing a honey bee colony in a cold room for 8 days. This suggests that seasonal changes in juvenile hormone are not related to photoperiod changes, but rather to changes in temperature and/or colony social structure that in turn influence endocrine and behavioral development. We also found that active foragers in the late winter and early spring had lower juvenile hormone levels than active foragers in late spring. In light of recent findings of a possible link between juvenile hormone and neuroanatomical plasticity in the bee brain, these results suggest that bees can forage with low juvenile hormone, after previous exposure to some threshold level of juvenile hormone leads to changes in brain structure.

Key words Corpora allata · Seasonal variation · Juvenile hormone · Social insects · Honey bee, *Apis mellifera*

Abbreviations CA corpora allata · dpm decays per minute · HPLC high performance liquid chromatography · JH Juvenile hormone · RIA radioimmunoassay

Introduction

Division of labor in honey bee (*Apis mellifera*) colonies is based, in part, on "age polyethism", a form of behavioral development in which adult worker bees change jobs as they age [reviews: Winston (1987); Moritz and Southwick (1992); Robinson (1992)]. Bees 1- to 3-weeks of age typically perform tasks within the hive such as queen care, brood rearing ("nursing"), and comb building, while 4- to 7-week old bees perform tasks outside the hive such as foraging for nectar or pollen and colony defense. Bees also can respond to changing environmental (colony) conditions by showing plasticity in age polyethism. Three forms of such plasticity have been identified: accelerated behavioral development (premature foraging) in response to a shortage of

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old bees; retarded behavioral development (overage nursing) in response to a shortage of young bees; and behavioral reversion (from foraging to nursing) in response to a severe shortage of young bees (Rösch 1930; Milojévic 1940; Page et al. 1992; Robinson et al. 1992; Huang and Robinson 1992; Robinson 1992).

JH regulates age polyethism in adult worker honey bees (Jaycox et al. 1974; Jaycox 1976; Robinson 1985, 1987a, b; Robinson 1992). Under normal colony conditions, hemolymph titers of JH increase with age; low titers are associated with the performance of tasks in the hive and a higher titer is associated with foraging (Fluri et al. 1982; Robinson et al. 1987, 1989). Changes in JH titers appear to be regulated primarily by changes in rates of JH biosynthesis, as the two are correlated in bees of different ages performing different tasks (Huang et al. 1991). 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 foraging at a younger age. In addition, plasticity in age polyethism is mediated by changes in JH levels that occur in response to changing environmental conditions (Robinson et al. 1989), especially colony age demography (Huang and Robinson 1992). Accelerated development is associated with a premature rise in JH titers, retarded behavioral development with a prolonged period of low JH titers (Robinson et al. 1989), and behavioral reversion with a drop in JH titers (Robinson et al. 1992).

Our understanding of how environmental changes influence JH and division of labor is limited. This is because most studies of endocrine mediated division of labor in honey bees have been performed in the summer, with experimentally induced changes in the colony environment [but see Robinson et al. (1989)]. To gain further insight into how the environment and JH influence division of labor, we measured JH titers and rates of biosynthesis in workers during times of the year when colony activity in temperate climates is reduced: late fall, winter, and early spring. Honey bees are unique among insects inhabiting temperate zones because they thermoregulate as a social unit to survive the winter rather than become dormant (Seeley and Visscher 1985). Most of the overwintering population of a colony is composed of "winter bees" born in the late fall (Fluri et al. 1977). However, some foragers born in the early fall, especially those with limited foraging experience (Neukirch 1982), form part of the overwintering population (Merz et al. 1979), and perhaps engage in thermoregulation and limited brood rearing until the following spring. If these foragers can survive the entire winter, we reasoned that the "reversion" may even persist until the following spring, with the performance of nursing activities prior to the resumption of foraging. We hypothesized that if these changes in worker behavior also are mediated by JH, bees that cease foraging in the fall and remain in the hive should show a decline in JH titers. This would make them similar to winter bees, which maintain a very low level of JH throughout the late fall, winter, and early spring, and do not undergo typical behavioral development (Fluri et al. 1977, 1982). It has been shown that a drop in JH in foragers can be caused experimentally; foragers induced to revert into nurse bees have low JH (Robinson et al. 1992).

The primary objective of this study was to determine whether there is a natural decline in JH in older bees in the fall, and if so, how this relates to the expression of behavioral reversion during the winter and spring months. Second, after demonstrating seasonal declines in JH, we probed possible causal mechanisms by measuring JH levels of workers before and after moving a colony into a cold room.

The third objective of this study was to measure JH in bees foraging in the late fall and early spring, when JH levels were predicted to be low. Foraging, though extremely rare at these times of year in Illinois due to low temperatures and a dearth of flowers, does occasionally occur. Because foraging during the summer is always associated with high JH [review: Robinson (1992); see also Huang et al. (1991, 1994)], hormone analyses of foragers in the late fall and early spring could be illuminating. If the few bees that are active as foragers during the late fall and early spring have levels of JH as high as summer foragers but inactive foragers have low JH, this would suggest that rapid hormone changes act as a mechanism enabling bees to respond adaptively to sudden foraging opportunities. Alternatively, low JH in bees that are active as foragers during the late fall and early spring would indicate that bees do not always require continuously high levels of JH to forage. This alternative is especially intriguing in light of recent results suggesting a link between JH and neuroanatomical plasticity in honey bees. The mushroom bodies, the region of the insect brain most closely associated with learning and memory (Heisenberg et al. 1985; Erber et al. 1987; Davis 1993; Debelle and Heisenberg 1994), exhibit a striking change in organization that is associated with foraging (Withers et al. 1993). Moreover, JH recently has been implicated as a causal agent in the reorganization of the mushroom bodies (Withers et al. 1995). Low JH in active foragers would suggest organizational effects of JH on brain and behavior in honey bees.

Materials and methods

Bees

Honey bee colonies were maintained according to standard techniques at the University of Illinois Bee Research Facility, Urbana, Illinois. Bees were typical of North American populations of *Apis mellifera* [a mix of predominantly European subspecies (Phillips 1915; Pellett 1938)]. Descriptions of colonies used in each experiment are given below.

Measurement of JH biosynthesis

Rates of JH III biosynthesis were measured with a radiochemical assay (Pratt and Tobe 1974; Tobe and Pratt 1974) adapted and validated for adult worker honey bees (Huang et al. 1991). Bees were collected as described below and immobilized on ice for 20 min to 2 h until their corpora allata-corpora cardiaca complex (CA) was removed. The CA from individual bees was removed and incubated for 3 h in 50 µl modified bee medium (Kaatz et al. 1985) containing 60 $\mu mol \cdot l^{-1}$ of L-[^{3}H-methyl]methionine (NEN, 7.4 $GBq \cdot mmol^{-1}$). JH produced *in vitro* was extracted with 250 µl isooctane and quantified by liquid scintillation spectrometry (cocktail: BioSafe II, Research Products International; counter: Packard Tricarb 460C). Rates of JH biosynthesis are significantly correlated with hemolymph JH titers measured by RIA (Huang et al. 1991). JH III is the only JH homolog in honey bees (Hagenguth and Rembold 1978; Huang et al. 1991; Robinson et al. 1991; Huang et al. 1994). For more details of this assay see Huang et al. (1991).

Measurement of JH titers

JH titers were measured for bees with a newly developed chiral-specific RIA (Hunnicutt et al. 1989). Huang et al. (1994) demonstrated the specificity of this assay for adult worker honey bees. Hemolymph (0.36–9.2 µl) was collected from cold-anesthetized workers as in Robinson et al. (1987), mixed with 500 µl acetonitrile, and stored at −20 °C until analyzed. Samples contained either hemolymph from individual bees or pooled hemolymph from 3–8 bees. JH was extracted twice by adding 1 ml 0.9% NaCl and 1 ml hexane. The supernatant hexane phase, containing JH, was removed and dried in a vacuum centrifuge (Savant SC110). Part of the sample (usually one tenth), dissolved in methanol, was then added to a 200 µl mixture of JH antibody (1 : 28 000 dilution) and radiolabeled JH III (8000 dpm, NEN, 629 $Gbq \cdot mmol^{-1}$). After a 2-h incubation at room temperature, unbound radiolabeled JH was separated from bound JH by incubation with dextran-coated charcoal for 2.5 min. Radioactivity in the supernatant (containing radiolabeled JH bound to antiserum) was quantified by liquid scintillation spectrometry (Beckman LS6000IC). Quantification was made with a standard curve created for each RIA run with known amounts of racemic JH III (Sigma). Previous results (Huang et al. 1994) indicate that results from this RIA agree with those obtained with either the Strambi or Goodman JH RIAs, both of which have been validated with gas chromatography/mass spectroscopy (de Kort et al. 1985; Goodman et al. 1990). All solvents used for measurements of JH biosynthesis and titers were HPLC grade, obtained from either EM Science, Fisher Scientific, or J.T. Baxter Chemical Co. Glassware for measurements of JH biosynthesis and titers was baked at 500 °C for 3.5 h prior to use to minimize JH adsorption (Strambi et al. 1981). For more details of this assay see Huang et al. (1994).

Seasonal changes in JH in fall foragers and young bees

Two colonies, each occupying one Langstroth hive body, were moved from a distant apiary into the Bee Research Facility in early October 1990. Each colony had an adult worker population of approximately 20 000 workers (determined by counting the number of frames covered with bees and multiplying by 2000; Burgett and Burika 1985). One colony was placed in a room on the north side of the building ("North" colony) and the other in a room on the south side ("South" colony). Bees from each colony could fly freely through exits in the walls. The rooms were not heated, and both had a window that was kept open so that room temperatures were similar to outdoor temperatures. Placing the colonies indoors facilitated sampling bees during inclement weather.

The following procedures were used to identify foragers and determine whether they showed a drop in JH during fall and winter. Each hive entrance was obstructed temporarily with a piece of 8-mesh hardware cloth so that returning foragers congregated at the entrance rather than immediately entering the hive. Pollen foragers were identified as bees with pollen loads in their corbicula. We sampled only pollen foragers, rather than both nectar and pollen foragers, because pollen foragers are easier to identify in the field. We believe that JH results from pollen foragers can be generalized to nectar foragers, for three reasons: first, pollen foragers are the same age as nectar foragers (Winston 1987). Second, Huang et al. (1994) showed that there were no differences in rates of JH biosynthesis or JH titers between pollen and nectar foragers, in a study employing methods sensitive enough to detect such differences between other groups of worker bees the same age, namely guards, undertakers, and food storage bees. Third, as described below, similar results were obtained in this study for pollen foragers and water foragers.

About 2000 foragers per colony were each marked with a spot of paint (Testor's PLA) on the dorsal surface of the thorax on an almost daily basis from mid-October to mid-November. Marked pollen foragers ($n = 10$) were collected on 25 October and 15 November at the hive entrance ("fall foragers"). Bees ($n = 7$–10) that were previously marked as pollen foragers also were sampled inside the hive on the same two dates and again on 12 December when foraging ceased for the year. These bees were sampled randomly with respect to behavior and location in the hive. They are later referred to as "inactive foragers". This designation is more robust on 12 December when no foraging at all was observed; on 25 October and 15 November there was some foraging, so inactive foragers could have been foraging prior to our sampling. Rates of JH biosynthesis were determined for bees individually. We intended to continue monthly measurements of JH biosynthesis and initiate observations of behavior the following spring but we could find no more marked fall foragers after 12 December 1990 in either colony.

Seasonal changes in rates of JH biosynthesis in "young" bees also were examined. One-day-old adult bees (< 24 h post emergence) were obtained by placing a frame containing old pupae from each colony into an incubator (33 °C). A total of 350 and 420 1-day-old bees were marked on 6–9 November and reintroduced to the South and North colonies, respectively. In addition, a partial cross-fostering design was used to probe for possible genetic and environmental determinants of seasonal variation in JH levels. One hundred and twenty 1-day-old bees from the North colony were marked with a distinctive paint color and introduced into the South colony. Young bees ($n = 10$) were sampled in the hive once a month, from November to March, as were the inactive fall foragers. North colony bees cross-fostered in the South colony were sampled on 21–22 March ($n = 10$ per day).

This experiment was performed two additional times with some variation. The reason for performing the experiment in the fall of 1991 was to confirm the seasonal decline in JH biosynthesis in fall foragers observed in 1990. Rates of JH biosynthesis were determined for pollen foragers ($n = 10$) collected on 2 and 7 October 1991 from two different colonies (50 and 85) than those used in 1990 (each in two Langstroth hive bodies, with populations of about 40 000 bees). About 2400 pollen foragers from each colony were then marked from 2–13 October. Marked bees ($n = 10$) were collected from inside each hive on 13 November, a cool day when there was no foraging, and rates of JH biosynthesis determined.

The primary reason for performing the experiment in the fall of 1992 was to determine whether JH titers also drop in fall foragers, as do rates of JH biosynthesis. Rates of JH biosynthesis were determined for pollen foragers ($N = 10$) collected on 30 September and 2 October 1992 from two different colonies (54 and 82) than those used previously (each in two Langstroth hive bodies, with populations of about 40 000 bees). About 500 pollen foragers from each colony were then marked on 3–4 October, and the colonies moved into the Bee Research Facility as in 1990. Marked bees ($n = 10$) were then collected from inside each hive on 4 November, a cool day

when there was no foraging, and both rates of JH biosynthesis and JH titers determined.

Rates of JH biosynthesis and JH titers were determined for each individual bee. Hemolymph was obtained first without damaging the head and then the CA were dissected immediately. Preliminary experiments indicated that rates of JH biosynthesis are not affected by prior hemolymph sampling.

In the fall of 1992 we also attempted to increase the survival of fall foragers over the winter so that behavioral analyses on them would be possible the following spring. To improve recovery of fall foragers in the spring, a total of 2200–2500 pollen foragers were marked for each of two colonies on 3–7 October. The two colonies were especially strong, with populations of about 60000 bees. They were not disturbed again until March, when a search was made for marked fall foragers. This was done because of concern that the high mortality of fall foragers in the 1990 experiment might have been due to our disturbing the colonies monthly in the fall to obtain samples for hormone analyses.

Experimental induction of changes in JH in summer foragers and young bees

As described below, we detected a decline in JH in foragers and young bees under natural conditions in the fall. The following experiment therefore was performed to determine whether exposure to low temperatures in the summer can also cause the JH drop. On 31 July 1992, 1800 pollen foragers and 600 1-day-old bees were marked (as described above) from a typical colony with a population of about 40000 bees. Five days later pollen foragers and "young" bees (now 5 days old) were collected for hormone analyses ($n = 10$). On the next day, 5 August, the colony was moved into an environmental chamber with a temperature of 4 °C in constant darkness; 8 days later foragers and young bees were sampled again. Rates of JH biosynthesis and JH titers again were determined for each individual bee on both sampling dates.

JH levels in spring foragers

Hormone analyses were performed on two groups of unmarked bees identified as foragers in early spring 1992 ("spring foragers"). Returning pollen foragers ($n = 26$–30) were collected from Colony 34 on two unseasonably warm days, 22 February and 3 March (daily high temperatures were 17 and 25 °C, respectively). Additional ($n = 100$) pollen foragers were marked on 3 March and collected inside the hive on 16 and 23 March after a return to more seasonable temperatures (average daily high temperature from 10–23 March was 5.5 °C). On 16 March we could find only seven inactive, marked, spring pollen foragers, so we included three marked bees without pollen loads caught while attempting to enter the hive and three additional unmarked flying bees. Unmarked returning pollen foragers also were collected on 7 and 15 May. Another group of spring foragers used for hormone analyses were water foragers from unknown colonies, sampled at an outdoor water feeder adjacent to the Bee Research Facility. Water foragers were collected on the same dates as the pollen foragers.

Again, both rates of JH biosynthesis and JH titers were determined. Unlike in previous experiments, different bees were used for each assay, but rates of biosynthesis were determined on the same day that the hemolymph samples were taken (except for bees sampled on 23 and 24 March). Hemolymph was pooled from 3–8 individual workers because at the time we had not determined that the RIA was sensitive enough for individual bees. Only two samples of pooled hemolymph ($n = 7$–8 bees per sample) were obtained for pollen foragers and water foragers on 23 February because a limited number of foragers were present.

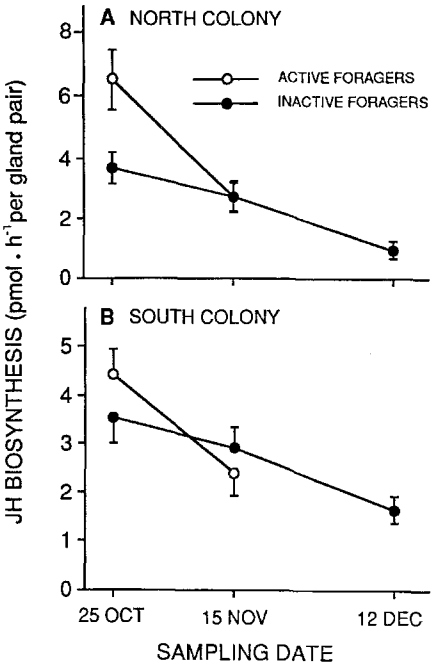

Fig. 1A, B Seasonal declines in mean (±SE) rates of JH biosynthesis in active fall foragers and previously marked foragers that were sampled inside the hive when inactive. $n = 6$–10 for each point [North colony (**A**): $n = 10$ except inactive foragers on 12 Dec, $n = 7$. South colony (**B**) active foragers, 25 Oct and 15 Nov, $n = 10$; inactive foragers, 25 Oct and 15 Nov, $N = 9$; inactive foragers, 12 Dec, $n = 6$]. No active foragers were present in either colony on 12 Dec

Statistical analyses

Differences in rates of JH biosynthesis and JH hemolymph titers were determined by t-tests modified for unequal variance when necessary (Steel and Torrie 1980). Two-way analysis of variance also was performed (on log-transformed values if there was heterogeneity in variances or lack of normality). Regression analyses determined whether there was an increase in JH over the course of the spring (with sample dates converted to Julian dates). The relationship between rates of JH biosynthesis and JH titers also was determined with correlation and regression analyses on the data from the cold room experiment. Means ± SE are given throughout this paper.

Results

Seasonal changes in JH in fall foragers and young bees

Two-way analysis of variance revealed significant differences in rates of JH biosynthesis between the two colonies ($F = 15.44$, $P < 0.001$). There were, however, several patterns common to both colonies. Two-way analysis of variance revealed significant differences in rates of JH biosynthesis over time ($F = 7.61$, $P < 0.001$). Rates of JH biosynthesis decreased significantly in fall foragers from mid- to late fall in both the South and North colonies ($F = 18.35$, $P < 0.0001$), but the colony X time interaction was not significant ($F = 1.16$, $P = 0.28$) (Fig. 1). There was a significant difference in rates of JH biosynthesis between active

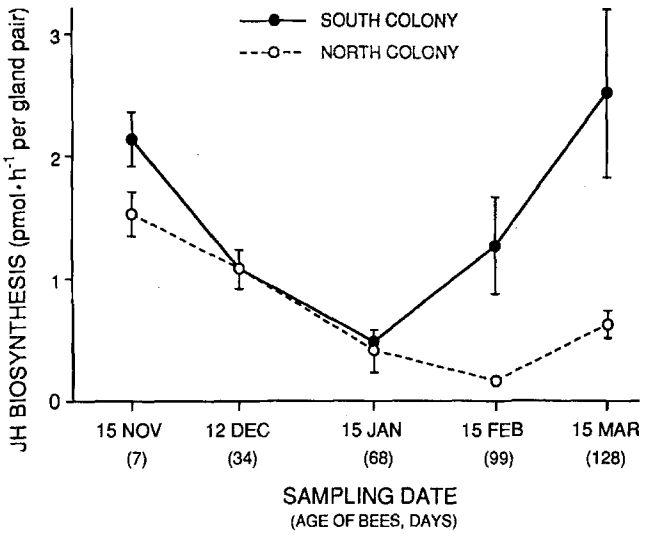

Fig. 2 Seasonal changes in mean (±SE) rates of JH biosynthesis in "young" bees from the South and North colonies ($n = 10$)

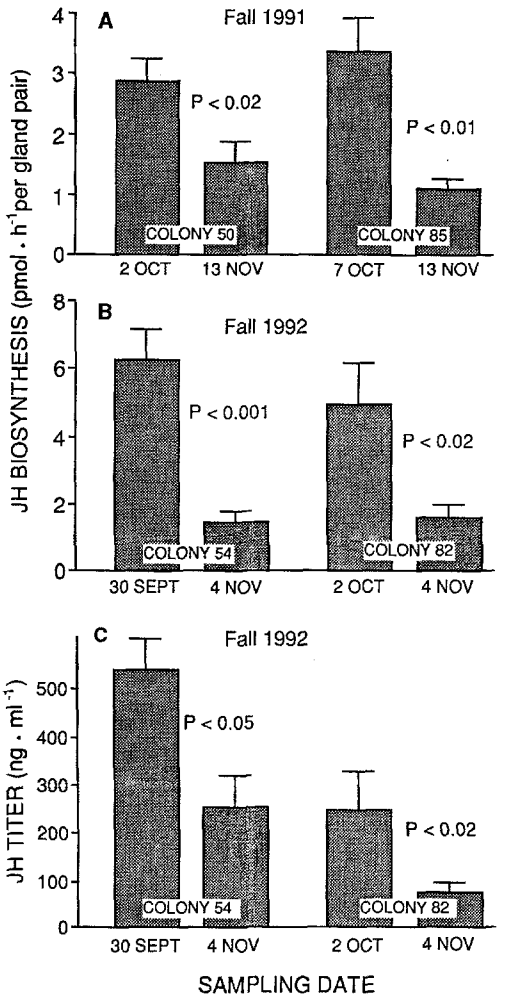

Fig. 3A–C Seasonal declines in mean (±SE) rates of JH synthesis (**A** and **B**) and JH titers (**C**) in fall foragers ($n = 10$)

and inactive foragers ($F = 4.49$, $P < 0.03$, t-test), due mainly to the relatively low biosynthesis rates for inactive foragers on 25 October in the North colony. Inactive fall foragers sampled on 12 December had very low rates of JH biosynthesis, no different than young bees sampled on that day (Fig. 1; $P > 0.1$ for both colonies, t-tests).

Almost all marked fall foragers died in the late fall. We could find only 12 fall foragers from the South colony and 6 foragers from the North colony during the 12 December collection. No marked fall foragers were found during the 19 March 1991 collection.

Rates of JH biosynthesis also decreased in young bees from November to January, and increased after January (South colony) or February (North colony) (Fig. 2). The increase is more apparent in the South colony, but statistical analyses reveal that there was a significant increase in each colony. A quadratic model best describes the changes in JH biosynthesis rates over time in both colonies ($F = 9.31$ and 26.8 for South and North colonies, respectively, $P < 0.001$ for both colonies). This result indicates that young bees showed a decline in JH followed by an increase in both the South and North colonies.

Young bees from the North colony that were cross-fostered in the South colony had rates of JH biosynthesis that were more similar to their parental type. The mean rate of JH biosynthesis for North bees in the South colony on 20–21 March 1991 was 0.94 ± 0.15, which was significantly different than the rate for South bees in the South colony, 1.36 ± 0.19, but not significantly different than the rate for North bees in the North colony, 0.62 ± 0.08 ($n = 20$ for each group, Tukey's test, $P < 0.05$). These results suggest that there is a genetic component to seasonal changes in JH. Differences between the values for 20–21 March and those for 15 March (Fig. 2) are probably because the 20–21 March assays were inadvertently performed with an older aliquot of methionine that we suspect had a lower specific activity.

Results from the experiments that began in the fall of 1991 or 1992 confirmed the seasonal decline in JH biosynthesis in fall foragers observed in 1990. There again were significant drops in rates of JH biosynthesis, in colonies 50 and 85 in the fall of 1991 (Fig. 3A), and colonies 54 and 82 in the fall of 1992 (Fig. 3B). RIA analyses also were performed for bees from colonies 54 and 82 and a significant drop in JH titers was detected (Fig. 3C). Results from 1992 also revealed colony variation in the timing of the drop in JH in fall foragers. Foragers from two other colonies located in the same apiary as colonies 54 and 82 were sampled on 30 September and 2 October 1992, at the same time as the first samples of foragers were taken from colonies 54 and 82. Foragers from these two other colonies already had significantly lower, "nurse-like", rates of JH biosynthesis (2.30 ± 0.38 and 1.55 ± 0.31; $n = 10$ for both

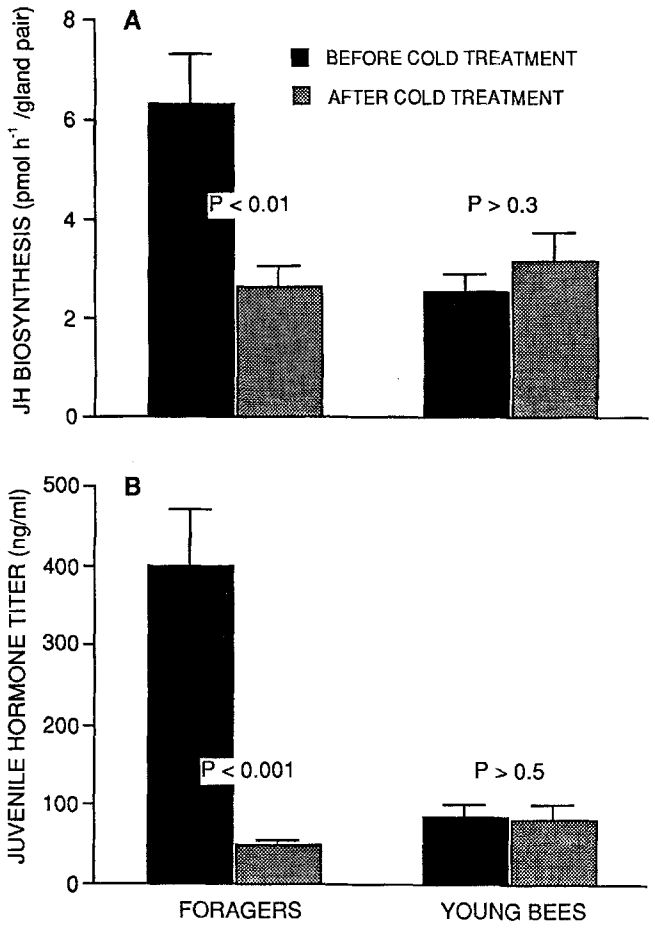

Fig. 4A, B Mean (±SE) rates of JH biosynthesis (**A**) and JH titers (**B**) in summer foragers and young bees, 1 day before and 8 days after, their colony was moved to a cold room at 4 °C ($n = 10$)

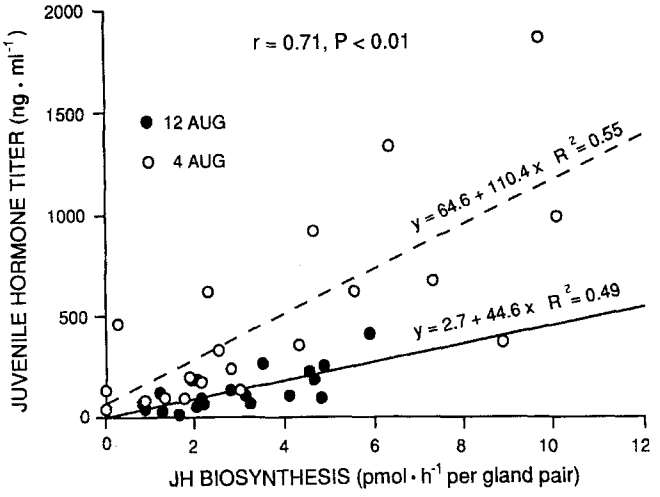

Fig. 5 Correlation and regression analyses of rates of JH biosynthesis and JH titers for data from Fig. 4. Both hormone measurements were performed on the same, individual, bees ($n = 40$). *Solid line* indicates the regression for the 4 August data, obtained one day before the colony was placed in the cold room. *Dashed line* indicates the regression line for the 12 August data, obtained 8 days after the colony was placed in the cold room

colonies) relative to foragers from colonies 54 and 82 (Fig. 3B; $P < 0.05$, t-tests). These results also suggest that there is a genetic component to seasonal changes in JH.

As in the first trial of this experiment, no fall foragers marked from colonies 50 and 85 in the fall 1991 were found alive when surveyed in March 1992, despite the larger numbers of foragers marked (about 2400 bees per colony). Similarly, in March 1993 there were fewer than five marked fall foragers present in both colonies 54 and 82, which were located indoors as in the fall of 1990. Our attempts in the fall of 1992 to increase the survival of fall foragers over the winter in order to perform behavioral analyses on them the following spring also were not successful. Fewer than ten of the original 2200–2500 marked fall foragers were recovered in March from either of the two large colonies left undisturbed.

Experimental induction of changes in JH in summer foragers and young bees

Confinement in a cold room in the summer induced a decline in JH in foragers. On 4 August, 1 day before we moved the colony into the cold room, pollen foragers had both high rates of JH biosynthesis and high JH titers, as is typical. Eight days later, foragers had significantly lower rates of JH biosynthesis and titers (Fig. 4A, B).

Confinement in a cold room in the summer did not induce a decline in JH in young bees. One day before we moved the colony in the cold room, young bees had low rates of JH biosynthesis and low JH titers, as is typical, and there were no significant changes 8 days later. Despite the lack of a decrease in JH in young bees, there may have been an effect on this group that was not apparent due to limitations of our experimental design. Perhaps young bees would have had higher levels of JH had they not been exposed to cold. JH titers or rates of biosynthesis have been shown to increase in workers from 1 to 2 weeks of age (Robinson et al. 1987; Huang et al. 1994).

Measurements of JH biosynthesis and JH titers from the same bees were significantly correlated ($r = 0.71$, $n = 40$, $P < 0.01$). Regressions of JH titers on JH biosynthesis rates also were significant for both sampling dates separately ($P < 0.05$, Fig. 5). However, the slope of the regression (111.1 ± 23.5) for 4 August, i.e., before exposure to cold, was significantly greater than that for 12 August, after exposure to cold (44.6 ± 10.8, $P < 0.05$, t-test).

JH levels in spring foragers

Active fall foragers sampled on 15 November 1990 had low rates of JH biosynthesis relative to those of

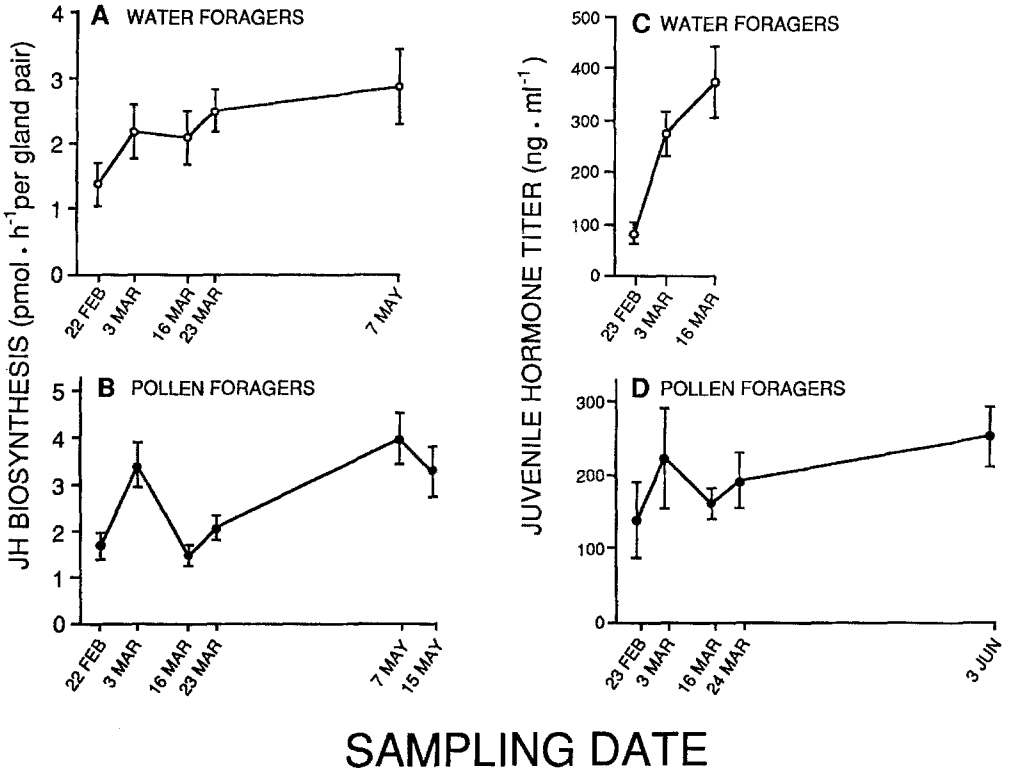

Fig. 6A–B Seasonal changes in mean (±SE) rates of biosynthesis and titers in spring water foragers (**A** and **C**) and pollen foragers (**B** and **D**). Pollen foragers collected on 16, 23, 24 March were inactive and were sampled from inside the hive; water foragers always were collected at the water feeder. Sample sizes indicated on each bar. Temperatures recorded at the Bee Research Facility at the time when bees were collected were: 18° (22 Feb.), 21° (3 Mar.), 4° (16 Mar.), 8° (23 Mar.), and 18°C (7 May)

25 October (Fig. 1) and to those reported in previous studies (Huang et al. 1991, 1994). This result suggested that active foraging need not always be associated with high JH. This was confirmed by hormone analyses of bees that were foraging in early spring, 1992 ("spring foragers"). Active water foragers from unspecified colonies and pollen foragers from colony 34 had low, nurse-like rates of JH biosynthesis and titers on 22 February (Fig. 6). Water foragers showed a more gradual increase in rates of JH biosynthesis than did pollen foragers, in part because pollen foragers sampled on 3 March had unusually high rates. However, regression analyses indicated that there was a significant increase in rates of JH biosynthesis for both water and pollen foragers over the course of the spring ($F = 5.47$, $P < 0.05$; and $F = 10.33$, $P < 0.01$, respectively; Figs. 6A, B). There also was a significant increase in JH titers for water foragers ($F = 6.48$, $P < 0.05$; Fig. 6C). JH titers for pollen foragers did not show the same gradual increase as did water foragers, again because of the results for 3 March. However, a significant increase in JH titers for pollen foragers was detected after excluding the data from 3 March ($F = 4.03$, $P < 0.05$, Fig. 6D). Rates of JH biosynthesis and JH titers almost doubled for pollen foragers from late February to May–June (Figs. 6B, D).

Differences in rates of JH biosynthesis between active and inactive foragers were detected again in a limited analysis of spring foragers. The 16 March sample of spring pollen foragers collected from colony 34 included seven inactive marked foragers and six active bees, captured as they flew into their hive (three unmarked and three marked). Active bees had significantly higher rates of biosynthesis than did inactive foragers (2.92 ± 0.55 and 1.21 ± 0.19, respectively; $P < 0.01$, t-test). Rates of JH biosynthesis for inactive foragers were not significantly different from those of bees randomly sampled from within the same hive (0.89 ± 0.19, $n = 8$, $P > 0.2$, t-test).

Discussion

This study demonstrates that natural environmental changes are related to endocrine changes in worker honey bees that are known to mediate plasticity in division of labor. JH titers and rates of biosynthesis declined in foragers under natural conditions, from high levels typically associated with foraging, to low levels associated with tasks performed by young workers. This "hormonal reversion" was shown to occur in the fall in six different colonies over 3 years. Although the other two forms of plasticity in endocrine mediated age polyethism in honey bees, accelerated and retarded behavioral development, have been shown to occur under natural conditions [review: Robinson 1992)], this is the first study to show that hormonal reversion also occurs naturally.

We also showed that endocrine changes in foragers in the fall are part of a larger seasonally related phe-

nomenon in which JH levels in young bees also decline in the fall and then increase the following spring (Fluri et al. 1982). Results from the partial cross fostering experiment in 1990–1991 and the survey of colonies in 1992 suggest that there is genetic variation for sensitivity of the honey bee endocrine system to these seasonal effects but the data are limited. Genetic variation for differences in worker JH and behavior within honey bee colonies has been shown [Robinson et al. (1989); for reviews of the effects of genetic variation on behavioral variation in honey bees see Page and Robinson (1991); Moritz and Southwick (1992)] .

The mechanisms underlying the observed seasonal changes in JH are not clear. Environmental stimuli that affect JH have been particularly well studied in Colorado potato beetles (*Leptinotarsa decemlineata*). In this species seasonal variation in JH titer is influenced by photoperiod, temperature, and food quality (de Wilde et al. 1959; de Kort 1990). It is doubtful that food quality affects honey bees similarly, because their foraging ecology makes it less likely that they experience large variation in food quality. A honey bee colony obtains nectar and pollen from many different species of flowers simultaneously and stores food in its nest for long periods of time. Honey bee colonies do, however, experience large differences in general food availability, but Huang and Robinson (1992) demonstrated that experimentally induced variation in colony food intake does not directly affect JH and behavioral development.

Photoperiod plays a major role in the regulation of seasonal changes in endocrine and behavioral status in other insects (Pener 1992; Rafaeli et al. 1993; Tanaka et al. 1993; Eizaguirre et al. 1994) and in many vertebrates as well (Karp and Powers 1993; Banin et al. 1994). Results from our cold room experiment, however, suggest that photoperiod plays little, if any, role in seasonal hormone changes in honey bees. In the cold room experiment, the seasonal drop in JH in foragers was mimicked by placing a colony into a darkened cold room in the summer for only 8 days, i.e., without gradual changes in photoperiod that would be experienced naturally. Consistent with our findings, other studies have shown that physiological changes associated with winter bees are not induced in response to an artificially shortened photoperiod (Cherednikov 1967; Fluri and Bogdanov 1987). Experiments involving exposure to various light-dark regimes, in addition to complete darkness, could be performed to more rigorously test for an effect of photoperiod on JH in honey bees.

Lower temperatures may lead to decreases in JH in the fall, and higher temperatures to increases in JH in the spring as suggested by Bühler et al. (1983). Results from the cold room experiment are consistent with a temperature effect. Some of the results obtained for spring foragers also are consistent with a temperature effect. There was a striking increase in JH in spring pollen foragers during a period of unseasonably warm weather around 3 March. This was followed by a drop in JH by 16 March when temperatures dropped. However, this suggestion is weakened by the fact that water foragers did not show similar changes at the same time.

We suggest that a second mechanism to explain seasonally related changes in JH is seasonally related changes in "effective colony age demography". Huang and Robinson (1992) demonstrated that older bees can inhibit endocrine-mediated behavioral development in younger bees. The observed drop in JH in the fall could be a response to the larger proportion of old bees in the colony due to the seasonal decline in birth rates. In addition, foragers would be more likely to exert inhibitory effects on other bees (and on each other) in the fall because cooler temperatures and the disappearance of flowers lead to reduced foraging, which means that foragers spend most, if not all, of their time in the hive. Results of the cold room experiment are consistent with an effect of colony age demography for the same reasons. Similarly, the increase in JH in spring pollen foragers could be related to a decrease in inhibition caused by the onset of foraging. To determine whether temperature influences the endocrine status of bees independent of its presumed effects on bee behavior and effective colony age demography, additional experiments are required in which foragers are confined to their hive under warm temperature conditions.

The functional significance of the seasonal decline in JH levels in foragers is not clear because they failed to survive the winter. Low JH could theoretically promote the survival of foragers over the winter, perhaps by reducing rates of metabolism (Huang et al. 1994) and therefore consumption of limited stores of honey over the winter. Low JH in winter bees is correlated with higher levels of fat reserves and a much longer life span relative to bees born during the spring and summer (Fluri et al. 1982). The presence of experienced foragers should be adaptive in the early spring when colony food stores are dangerously low, especially if they retain spatial memories that aid them in foraging. Lindauer [cited in von Frisch (1967)] reported anecdotally that a few foragers trained the previous summer to an artificial feeding station returned to the feeding station the following spring; however, we observed almost 100% mortality for fall foragers. This cannot entirely be due to our opening colonies to obtain bees for hormone samples, because high mortality also occurred in two colonies that were left undisturbed. It also is not likely that the high mortality was a consequence of using bees (primarily of Italian origin) that are not adapted to midwest U.S. winters, because Merz et al. (1979) also reported that most fall foragers of *Apis mellifera carnica* die in late fall-early winter in Switzerland, and *A.m. carnica* evolved in northern Europe. Nor can the presence of the parasitic honey bee mites *Acarapis woodi* and *Varroa jacobsoni* explain forager mortality. Careful monitoring in our apiaries indicated

that mites were present in 1991 and 1992 but not in 1990, and fall forager mortality was high in all three years. The adaptive significance, if any, of the observed seasonal decline in JH in fall foragers is unknown.

The finding that bees can forage with low rates of JH biosynthesis and JH titers also is puzzling because summer foragers consistently have high rates of JH biosynthesis and high JH hemolymph titers. Perhaps JH exerts more permanent effects earlier in adult life that permit foraging later on even in the absence of high JH. Little is known about the effects of JH on the nervous system of insects (Stout et al. 1991, 1992; Henley et al. 1992), but an "organizational" effect of JH on the nervous system of the worker honey bee is suggested by recent neuroanatomical findings. There is a striking reorganization of the mushroom bodies of the bee brain that is associated with foraging (Withers et al. 1993), and JH has been implicated as a causal agent in this reorganization (Withers et al. 1995). Bees may be able to forage with low JH after changes in brain structure occur in response to some threshold level of JH. If this hypothesis is correct, the threshold level of JH in the spring would need to be relatively low, because our results suggest that spring foragers did not experience very high titers of JH. This hypothesis then raises the question of why summer foragers always have high JH titers and rates of biosynthesis. Perhaps JH has both organizational and activational effects, as do gonadal steroids in the regulation of bird song [reviews: Arnold and Gorski (1984); Kelly (1986); McEwen et al. (1991); Breedlove (1992)]. For example, in addition to the brain changes discussed above, high levels of JH may be required for the higher levels of foraging activity typical of summer relative to early spring or late fall. Differences in JH between active and inactive foragers in this study, though based on limited data, support this hypothesis.

Another explanation for the finding that bees can forage with low JH is that there is seasonal variation in sensitivity to JH. Our results indicate that even during late fall and early spring, bees that are foraging have higher levels than other bees in the colony, just as in summer. Workers in late fall, winter, and early spring might be more sensitive to JH than bees in summer and would therefore forage with a relatively lower titer (but still higher than that of non-foraging bees). A lower threshold of response might facilitate a rapid, opportunistic response to sudden and brief periods of favorable weather for foraging during the non-summer months. Hormone treatment experiments or preferably measurements of JH receptor densities at different times of year might shed light on this mystery. Unfortunately, the cellular mode of JH action on any tissue including the central nervous system is unclear, but a putative nuclear receptor apparently has been identified in *Manduca sexta* (Palli et al. 1994).

Two results from the cold room experiment suggest that JH titers in worker honey bees may not be regulated solely by changes in rates of JH biosynthesis under all environmental conditions. First, there was a significant correlation between rates of JH biosynthesis and JH titers, but changes in JH biosynthesis explain only 50% of the variation in JH titers ($r^2 = 0.50$). In contrast, Huang et al. (1991) previously reported an r^2 of 0.92 under typical summer conditions. The second result is that JH titers appeared to be more sensitive to environmental change than were rates of JH biosynthesis; JH titers changed more rapidly than did rates of JH biosynthesis. It is possible that quick changes in JH titer require changes in other processes besides hormone biosynthesis, such as degradation and tissue uptake (De Kort and Granger 1981).

In summary, there are dramatic seasonal changes in JH in adult worker honey bees that are associated with colony division of labor, but elucidation of the mechanisms and adaptive significance of these changes require further study. Foragers with low JH and changes in JH titers that apparently occur more rapidly than changes in rates of JH biosynthesis also raise intriguing new questions about the hormonal regulation of bee behavior.

Acknowledgements We thank J.C. Kuehn for expert help in maintaining the bees; D.W. Borst for graciously providing juvenile hormone antiserum; S.E. Fahrbach, T. Giray, D.P. Toma, S.P. Trumbo, C. Wagener-Hulme, M.L. Winston, and two anonymous reviewers for improving the manuscript; and W.A. Robinson, Department of Atmospheric Sciences, University of Illinois, for providing temperature data. Supported by grants from NIMH (MH42274-01) and USDA (92-37302-7856) to GER.

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Communicated by L.C.-H. Wang