

HORMONAL REGULATION OF BEHAVIOURAL DEVELOPMENT IN THE HONEY BEE IS BASED ON CHANGES IN THE RATE OF JUVENILE HORMONE BIOSYNTHESIS

ZHI-YONG HUANG,¹ GENE E. ROBINSON,¹ STEPHEN S. TOBE,² KOICHIRO J. YAGI,²
COLETTE STRAMBI,³ ALAIN STRAMBI³ and BARBARA STAY⁴

¹Department of Entomology, University of Illinois, Urbana, IL 61801, U.S.A., ²Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1, ³Centre National Recherche Scientifique, Laboratoire de Neurobiologie, B.P. 71, 13402 Marseille Cedex 9, France and ⁴Department of Biology, University of Iowa, Iowa City, IA 52242, U.S.A.

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Abstract—In the adult worker honey bee (*Apis mellifera* L.), increases in the haemolymph titre of juvenile hormone underlie behavioural development, from nest duties to foraging. However, the physiological basis of juvenile hormone titre regulation was unknown. Using a radiochemical assay for juvenile hormone biosynthesis *in vitro*, we demonstrate that differences in juvenile hormone titres among bees performing different age-dependent tasks are a consequence of changes in rates of hormone synthesis by the corpora allata. Rates of juvenile hormone biosynthesis were low in newly emerged bees, 7–9-day-old nurse bees, and 14–15-day-old bees collected from the nest periphery, and high in foragers. Rates of biosynthesis were highly correlated with haemolymph titres of juvenile hormone measured in the same individuals. Corpora allata contained mostly methyl farnesoate, the immediate precursor of juvenile hormone, and released principally juvenile hormone III into the incubation medium, indicating no appreciable hormone storage. We also report similarities and differences in parameters of juvenile hormone biosynthesis between nurse bees and foragers that were found during the course of a detailed characterization of the radiochemical assay for adult worker honey bees. These results, coupled with the fact that it is possible to measure rates of juvenile hormone biosynthesis from individual bees, indicate that the radiochemical assay will be useful in further studies of hormonal regulation of bee behaviour.

Key Word Index: *Apis mellifera*; corpora allata; division of labour; honey bee; juvenile hormone; social insects

INTRODUCTION

Division of labour in honey bee colonies is based, in part, on worker age. Bees change jobs as they age, a form of behavioural development called “age polyethism” (reviewed by Winston, 1987). Juvenile hormone is involved in the control of age polyethism in adult worker honey bees (Jaycox *et al.*, 1974; Jaycox, 1976; Robinson, 1985, 1987a, b; reviewed by Robinson, in press). Haemolymph titres of juvenile hormone increase as the bee ages (Fluri *et al.*, 1982; Robinson *et al.*, 1987). Low titres are associated with the performance of tasks in the nest such as brood care during the first 1–3 weeks of the bee’s 4–7-week adult life, whereas a higher titre at about 3 weeks of age is associated with foraging. Treatment with juvenile hormone (Jaycox, 1976), juvenile hormone mimic (Jaycox *et al.*, 1974), or juvenile hormone analogue (Robinson, 1985, 1987a; Sasagawa *et al.*, 1985) induces precocious foraging.

The division of labour in honey bee colonies is also flexible, because workers can respond to changing colony needs by accelerating, retarding, or reversing their behavioural development (e.g. Rösch, 1930; Milojevic, 1940; Robinson *et al.*, unpublished; reviewed by Robinson, in press). Flexibility in age-based division of labour in honey bee colonies is also regulated by juvenile hormone, because the titre of this hormone is modulated by changes in environmental conditions (Robinson *et al.*, 1989, unpublished). These findings are consistent with the well-supported belief that the corpora allata, the glands that produce juvenile hormone, occupy a key position in the neuroendocrine response of insects to environmental factors (e.g. De Wilde *et al.*, 1959; Tobe and Stay, 1985).

It is not known how the juvenile hormone titre is regulated in adult honey bees, and this information is important for two reasons. First, the relationship

between juvenile hormone titre and rate of biosynthesis is not well understood in insects, because measurements of both variables have been made for relatively few species to date (Lanzrein *et al.*, 1978; De Kort *et al.*, 1982; Tobe *et al.*, 1985; Rachinsky and Hartfelder, 1990). Second, if changes in juvenile hormone titre are due to changes in rate of juvenile hormone biosynthesis in honey bees, then analyses of corpora allata activity may be useful in studies of hormonal regulation of bee behaviour. For example, an important question in the study of division of labour is how colony needs are perceived by workers. The recent discovery that plasticity in honey bee age polyethism is mediated by juvenile hormone (Robinson *et al.*, 1989, unpublished) suggests that it may be possible to probe sensitively for the effects of environmental stimuli on division of labour by measuring juvenile hormone biosynthesis. In other insects it is known that juvenile hormone synthesis by the corpora allata is controlled by neuro-hormones from the brain (reviewed by Tobe and Stay, 1985; Feyereisen, 1985a; Khan, 1988).

Using a radiochemical assay of juvenile hormone biosynthesis (Tobe and Pratt, 1974; Pratt and Tobe, 1974) that is widely used in studies of insect neuro-endocrinology (see Tobe and Stay, 1985), we report that there is a strong correlation between rates of juvenile hormone biosynthesis and haemolymph titres of juvenile hormone in adult worker honey bees, suggesting that titres are regulated primarily through changes in biosynthesis. We also describe similarities and differences in parameters of juvenile hormone biosynthesis between bees displaying two different age-dependent behaviours, brood care or foraging. These findings are supported by the results of a detailed characterization of the radiochemical assay for adult worker honey bees, also presented here, that is based on an established protocol for using this assay on other species (Feyereisen, 1985b).

MATERIALS AND METHODS

Bees

Bees were obtained from colonies maintained according to standard techniques at the University of Illinois Bee Research Facility, Urbana, Ill. They were typical of North American populations of *Apis mellifera* [a mix of predominantly European subspecies (Phillips, 1915; Pellett, 1938)].

Bees of known age were obtained by removing combs containing developing pupae from a colony and placing them in an incubator (33°C). Emerging adults ($N = 800$) were marked on the abdominal dorsum with a spot of paint (Testor's PLA) and introduced to a different colony with a population of about 40,000 workers, occupying two Langstroth hive bodies.

Individuals engaged in either brood care ("nurse" bees) or foraging were sampled, as in previous studies of hormonal regulation of division of labour

(Robinson *et al.*, 1989). Focusing on these two tasks simplifies physiological analyses of division of labour because they are associated with easily detectable differences in worker age, behaviour, and juvenile hormone titre. A nurse bee was identified as a worker with her head in a cell containing a larva (e.g. Sakagami, 1953; Robinson, 1987a). Foragers were obtained by temporarily obstructing the hive entrance with a piece of 8-mesh hardware cloth. Only returning bees with pollen loads in their corbiculae were collected. In one experiment we also sampled 1-day-old bees upon emergence from combs in the incubator and 14–15-day-old workers from the nest periphery. Workers were collected and stored in vials without food for 30–60 min at room temperature. They were then immobilized on ice for 5 min to 3 h until dissected.

Incubation medium

A medium specially formulated for honey bee tissue maintenance and culture (Kaatz *et al.*, 1985) was modified as follows: methionine and bovine serum albumin were omitted, and 1 mM leucine and Ficoll (20 mg/ml) added. The pH of the medium was adjusted to 7.0 (with a mixture of 1.5 NaOH:1 KOH, v:v) and the osmolarity adjusted to 520 mOsmol/l (with sucrose) to make it similar to honey bee haemolymph (Florkin and Jeuniaux, 1974). The medium was sterilized by filtration (Millipore, 0.22 μ m). Preliminary studies indicated that use of this medium ("bee medium") resulted in significantly higher rates of juvenile hormone biosynthesis than either Grace's insect medium (Gibco), or bee medium with 5% bovine serum albumin ($F = 7.77$, $P < 0.01$, $N = 36$). Rachinsky and Hartfelder (1990) also reported that their modified bee medium is superior to other media for juvenile hormone biosynthesis in honey bee larvae, presumably because it has a Na:K ratio that is more similar to that of honey bee haemolymph.

Dissection of corpora allata

After removing the head, an incision was made posteriorly and the corpora allata–corpora cardiaca complex removed. The complex was then placed in a disposable culture tube (Kimble 10 \times 50 mm, pre-baked for 3 h at 500°C) containing 25 μ l non-radioactive bee medium. Corpora allata–corpora cardiaca were incubated together in all experiments reported here; a pilot study revealed that corpora allata–corpora cardiaca complexes showed similar rates of juvenile hormone biosynthesis to corpora allata incubated without corpora cardiaca ($t = 0.31$, $P > 0.3$, $N = 12$) (corpora allata–corpora cardiaca complexes are henceforth referred to as "corpora allata"). Dissections were conducted in a dissection saline with a Na:K ratio similar to that of the bee medium (55 mM NaCl, 35 mM KCl, 3 mM CaCl₂·2 H₂O, 10 mM MgCl₂·6 H₂O, 55.5 mM glucose, 55.5 mM fructose, 14.6 mM sucrose). Rates of juvenile hormone biosynthesis for glands dissected in

this saline were not significantly different than for glands dissected with bee medium ($t = 0.30$, $P > 0.3$, $N = 12$). The size of one corpus allatum per bee was measured immediately after dissection with an ocular micrometer. Corpus allatum volume was calculated with the formula $V = \pi \cdot (L \times W^2)/6$, where L is the larger diameter and W the smaller diameter, measured when the corpora allata were oriented side by side.

Radiochemical assay

The assay was performed according to established procedures (Pratt and Tobe, 1974; Tobe and Pratt, 1974; modified by Feyereisen and Tobe, 1981) and is summarized as follows. Incubations began after all dissections were complete (1–3 h) by adding to each tube an additional 25 μ l bee medium that contained L-[3 H-methyl]methionine (NEN, specific activity 7.4 GBq/mmol). The optimal final concentration of methionine was determined to be 100–150 μ M (see Results) and was used in all experiments reported in this paper. Corpora allata were incubated for 3 h in darkness at 35°C. Glands incubated at this temperature exhibited the highest rates of juvenile hormone biosynthesis among the four temperatures tested (20, 30, 35 and 40°C). This temperature is also closest to the temperature maintained in the centre of a honey bee nest during the summer (see Seeley, 1985). Corpora allata were not shaken during incubation because we found that rates of juvenile hormone biosynthesis were not significantly affected by shaking ($t = 0.57$, $P > 0.3$, $N = 12$).

Juvenile hormone was extracted according to the method of Feyereisen and Tobe (1981). Glands were removed following the incubation period, and 250 μ l isooctane added to each tube of medium (isooctane and all other solvents were HPLC grade, from Fisher Scientific). A 200- μ l aliquot of isooctane extract was removed after thorough vortexing and centrifugation (10 min, 1700 g), and radioactivity determined by liquid scintillation spectrometry.

To determine the time course of juvenile hormone biosynthesis, corpora allata from nurses and foragers ($N = 3$ and 4, respectively) were incubated for 9 h, but were transferred to new medium after 1, 2, 4 and 6 h of incubation.

The effect of farnesoic acid (a juvenile hormone precursor) on juvenile hormone biosynthesis was determined by incubating corpora allata from foragers in medium that contained either 0, 20, 40, 80, 160, 320, 640 or 1000 μ M farnesoic acid ($N = 6$ per concentration).

Two experiments were performed to determine the precise rates of juvenile hormone biosynthesis. We measured the ratio of incorporation of the S-methyl moiety of radiolabelled methionine into the carbon skeleton of the juvenile hormone molecule. In the first experiment, the medium was modified by omitting fructose and glucose, and adding [3 H]methionine (36 μ M, 7.4 GBq/mmol, NEN) and

[2- 14 C]acetate (3.6 mM, 0.074 GBq/mmol, NEN). In the second experiment, [14 C]methionine (100 μ M, 2.07 GBq/mmol, NEN) and [3 H]farnesoic acid (160 μ M, 1.01 GBq/mmol, courtesy of G. D. Prestwich) was used in standard bee medium. Analyses were performed by thin-layer chromatography (TLC) and liquid scintillation spectrometry.

Identification of radiolabelled compounds synthesized by the corpora allata

Two experiments were performed with high performance liquid chromatography (HPLC). In Experiment 1, 4–5 pairs of corpora allata from nurse bees or foragers were incubated per incubation tube (100 μ l medium) for 15 h (6 tubes each for nurse bees and foragers). Each sample ($N = 5$) was prepared by pooling isooctane extracts of medium from either two tubes (for foragers), or three tubes (for nurse bees). In Experiment 2, corpora allata from individual bees ($N = 46$ nurse bees and 41 foragers) were incubated separately for 3 h. Each sample ($N = 6$) was prepared by pooling medium extracts from 10–22 individual bees. In Experiment 1, juvenile hormone was extracted from the samples immediately, whereas in Experiment 2 the isooctane-medium mixtures were stored at -70°C for 1–2 days before extraction.

Juvenile hormone was extracted according to Weaver *et al.* (1980), except isooctane was used instead of chloroform. Samples were each passed through a Na_2SO_4 column and loaded onto a pre-washed silica cartridge (SepPak), rinsed with *n*-pentane, and eluted with *n*-pentane:diethyl ether (1:1). They were then evaporated under nitrogen and redissolved in 10 μ l hexane. HPLC analysis was performed on a 5 μ m Brownlee Spheri-5 Silica column (250 cm \times 4.6 mm) with water-saturated redistilled ethyl ether (10%) in hexane as solvent. Flow rate was 1 ml/min at a pressure of 16–18 atm and detector wavelength was set at 219 nm. Fractions were collected at 12-s intervals and radioactivity determined by liquid scintillation spectrometry. Synthetic juvenile hormone III (Chemical Dynamics Corp.) and methyl farnesoate (courtesy of F. C. Baker, Sandoz Crop Protection Corp.) were used as standards.

Thin-layer chromatography

TLC was performed to provide independent identification of radiolabelled products of the corpora allata and to quantify the relationship between the amount of juvenile hormone released into the incubation medium (juvenile hormone “release”) and the total amount of juvenile hormone biosynthesized. This information is necessary to determine whether measuring juvenile hormone release alone provides an accurate estimate of total juvenile hormone biosynthesis (Tobe and Stay, 1985). For this experiment only, juvenile hormone biosynthesis is defined as the amount of juvenile hormone in the medium plus the amount present in the glands at the conclusion

of the incubation period. Juvenile hormone was extracted by first adding methanol and disodium ethylene-diamine-tetraacetate, and then partitioning against chloroform (Weaver *et al.*, 1980). TLC was performed with a solvent system of xylene/ethyl acetate (3:1), with juvenile hormone III (Sigma) and methyl farnesoate as internal markers.

Correlation between juvenile hormone titre and rate of biosynthesis

Both variables were measured in the same individuals: 1-day-old bees, 7–9-day-old nurses, 14–15-day-old bees located on the periphery of the nest, and 21–24-day-old foragers ($N = 9$ or 10 per age group). Bees were chilled (0°C for 5 min) and immobilized with plasticine in a dissecting dish. Haemolymph ($2.5\text{--}15\ \mu\text{l}$) was removed from each bee, pooled ($21.9\text{--}41.7\ \mu\text{l}$ for each age group), and placed in $150\ \mu\text{l}$ methanol. The methanol-haemolymph mixture was partitioned against hexane three times and the hexane phase, containing the juvenile hormone, removed. Samples were coded for blind analyses and stored at -20°C . Immediately after haemolymph was taken from a bee, its corpora allata were removed and placed in incubation medium for the radiochemical assay. Additional marked bees from the same colony were collected for titre determinations only, to yield a total of 5–8 measurements per age group of workers.

Juvenile hormone titres were determined by radioimmunoassay (RIA) (Strambi *et al.*, 1984). This technique provides consistent results that agree with analyses made by gas chromatography/mass spectrometry (GC/MS) (De Kort *et al.*, 1985). In addition, juvenile hormone titre determinations for honey bees with this RIA (Robinson *et al.*, 1987, 1989, unpublished) agree with measurements made with the *Galleria* bioassay (Fluri *et al.*, 1982) and a GC/MS assay (Hagenguth and Rembold, 1978).

Statistical analyses

Mean rates of biosynthesis (\pm SEM) were calculated based on measurements of individually incubated corpora allata (i.e. a gland pair). Sample sizes thus refer to the number of individual bees assayed, unless otherwise indicated. Statistical tests used are reported with the results.

RESULTS

Identification of radiolabelled products

HPLC analyses, performed on a total of 17 samples of extracts of incubation medium or glands, demonstrate that juvenile hormone III is the only detectable juvenile hormone homologue produced by the corpora allata of adult worker honey bees. In Experiment 1, the major radioactive compound released into the medium by the corpora allata of foragers was juvenile hormone III ($97.2\% \pm 0.7$, $N = 3$)

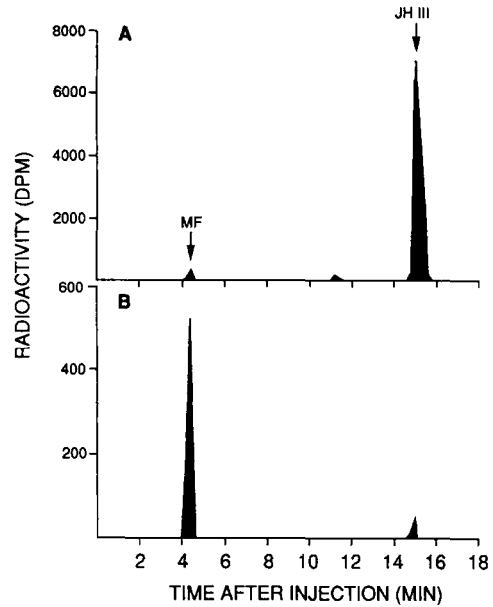


Fig. 1. HPLC radiochromatograms of *in vitro* products from honey bee corpora allata. (A) Compounds released from the corpora allata of foragers. (B) Compounds present in the corpora allata of foragers. Fractions were collected at 12-s intervals. Arrows indicate retention times of synthetic juvenile hormone III and methyl farnesoate.

[Fig. 1(A)]. Traces of methyl farnesoate ($1.5\% \pm 0.7$) were also found [Fig. 1(A)]. Two other unknown components (retention times 8 and 10 min, respectively) were also detected that did not correspond to juvenile hormone I or II; together they constituted on average less than 2% of the total extracted radioactivity [one of them is visible in Fig. 1(A)]. The major radioactive compound released into the medium by the corpora allata of nurse bees was also juvenile hormone III ($84.5\% \pm 5.35$, $N = 2$) and $9.44\% (\pm 5.89, N = 2)$ of the radioactivity was associated with methyl farnesoate.

In Experiment 2, juvenile hormone III also was the major radioactive compound released into the medium by the corpora allata of both foragers and nurse bees, but the percentage of radioactivity associated with juvenile hormone III was lower (foragers: $65.0\% \pm 8.2$, $N = 4$; nurse bees: $59.0\% \pm 0.03$, $N = 2$). A peak of radioactivity (retention time 15.5 min) was detected in medium extracts from foragers (19.5%), but was not present in the extracts from nurse bees ($<1\%$). The compound was not identified, but it was not a juvenile hormone homologue or any known precursor.

The cause of the discrepancy between the two experiments may be related to sample degradation in Experiment 2 (Tobe S. S., unpublished results), because samples were not extracted immediately as in Experiment 1. This contention is supported by the results of TLC analyses, which were more consistent with the results of the HPLC analyses in Experiment 1. They showed that $89\% \pm 1.1$ ($N = 3$) of the radioactivity in iso-octane extracts of incubation medium

comigrated with the unlabelled juvenile hormone III internal standard. Although the percentage of juvenile hormone III produced was lower in Experiment 2 relative to Experiment 1, 87% of the radioactivity in Experiment 2 was still associated with either juvenile hormone or methyl farnesoate, a juvenile hormone precursor. Thus both HPLC and TLC analyses indicate that juvenile hormone III is the principal product of synthesis and release in adult worker honey bees.

HPLC analyses revealed that extracts of glands from both foragers and nurse bees contained principally methyl farnesoate ($89.3\% \pm 4.9$, $N = 6$), and only $4.9\% \pm 1.9$ juvenile hormone III [Fig. 1(B)]. No other juvenile hormone homologues besides juvenile hormone III were detected from either medium or gland extracts.

Experiments performed with $[2-^{14}\text{C}]$ acetate and $[^3\text{H}]$ methionine revealed that honey bee corpora allata did not incorporate acetate into juvenile hormone, either in the presence or absence of fructose and glucose. When corpora allata from foragers were incubated in the normal medium (i.e. with glucose and fructose), ^{14}C from acetate accounted for only $0.81\% \pm 0.31$ ($N = 4$) of the total radioactivity in the synthesized juvenile hormone. Similarly, when corpora allata were incubated in a medium without glucose and fructose, only $0.15\% \pm 0.11$ ($N = 11$) of the radioactivity was from acetate. We thus failed to observe the predicted molar ratio of 9:1 $^{14}\text{C}:^3\text{H}$ [which is based on the incorporation of nine acetate units from $[2-^{14}\text{C}]$ acetate and one methyl group from L-methionine into each juvenile hormone III molecule (see Feyereisen *et al.*, 1984; Smith *et al.*, 1989)].

Experiments performed with L- $[^{14}\text{C}]$ methionine and $[^3\text{H}]$ farnesoic acid showed that methionine, relative to farnesoic acid, is incorporated into juvenile hormone in a 1:1 molar ratio ($1:1.06 \pm 0.06$ mol L-methionine:farnesoic acid, $N = 22$). A 1:1 molar ratio is expected theoretically because one molecule of farnesoic acid and one methyl group from L-methionine are incorporated into each juvenile hormone III molecule (Tobe and Pratt, 1974; Pratt and Tobe, 1974). This result demonstrates that honey bee corpora allata use methionine as the exclusive methyl donor to produce juvenile hormone III (Tobe and Stay, 1985). Accordingly, we calculated rates of juvenile hormone biosynthesis based on the specific radioactivity of methionine throughout this paper.

Differences in juvenile hormone biosynthesis between nurse bees and foragers

Rates of juvenile hormone biosynthesis for foragers were significantly higher than for nurse bees, at all concentrations of methionine tested except $300 \mu\text{M}$ (Fig. 2) (*t*-test, $P < 0.01$, $N = 8$ per concentration). Rates of juvenile hormone biosynthesis for corpora allata of foragers increased in a dose-dependent manner up to $100\text{--}150 \mu\text{M}$ methionine, whereas rates of juvenile hormone biosynthesis for nurses

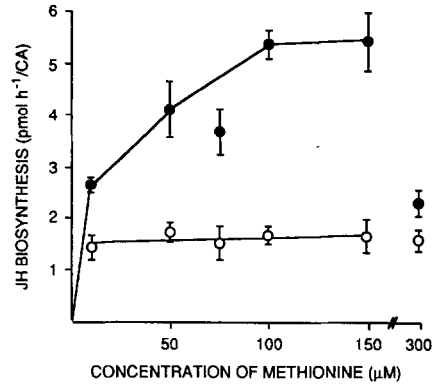


Fig. 2. Effect of methionine concentration on honey bee juvenile hormone biosynthesis. Each point represents the mean (\pm SEM) rate of biosynthesis ($N = 8$). \circ , Nurse bees; \bullet , foragers.

were constant over the range of methionine concentrations tested (Fig. 2). Based on these results, a methionine concentration of $100\text{--}150 \mu\text{M}$ was used in all experiments reported in this paper.

Similarities in juvenile hormone biosynthesis between nurses and foragers

Rates of juvenile hormone biosynthesis over a 9-h incubation period were constant for both nurse bees and foragers (linear correlation: $r = 0.992$, $P < 0.01$, $N = 20$; and $r = 0.997$, $P < 0.01$, $N = 15$, respectively) (Fig. 3). These results demonstrate that the incubation medium is sufficient to support juvenile hormone biosynthesis for at least 9 h. The incubation period for all other experiments reported in this paper was 3 h.

Rates of juvenile hormone biosynthesis for foragers were stimulated by farnesoic acid in a dose-dependent manner, from concentrations of $20\text{--}160 \mu\text{M}$ (ANOVA, $F = 4.2$, $P < 0.005$, $N = 30$) (Fig. 4). Maximum stimulation (3.2 times the control rate) occurred with $160 \mu\text{M}$ farnesoic acid for the corpora allata of foragers. A similar stimulatory

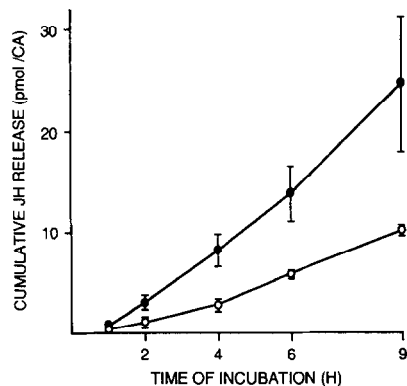


Fig. 3. Time course of honey bee juvenile hormone biosynthesis. Each point represents the mean (\pm SEM) rate of cumulative biosynthesis from either nurse bees or foragers ($N = 3$ and 4, respectively). Total incubation time was 9 h, but corpora allata were transferred to new medium after 1, 2, 4 and 6 h of incubation. \circ , Nurse bees; \bullet , foragers.

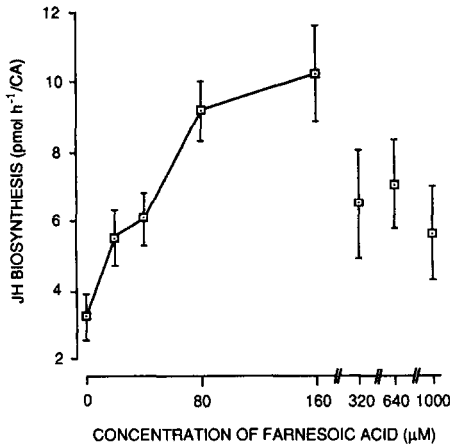


Fig. 4. Effect of farnesoic acid on juvenile hormone biosynthesis by corpora allata of foraging honey bees. Each point represents the mean (\pm SEM) rate of biosynthesis ($N = 6$).

effect occurred for the corpora allata of nurse bees at a farnesoic acid concentration of 160 μM (3.4 vs 1.2 $\text{pmol h}^{-1}/\text{corpora allata}$, $N = 12$, data not shown). Corpora allata of nurse bees and foragers thus showed similar responses to farnesoic acid despite the previously mentioned differences in rates of juvenile hormone biosynthesis.

There is a linear relationship between juvenile hormone release and juvenile hormone biosynthesis for both nurse bees and foragers ($r = 0.99$, $P < 0.001$, $N = 15$; and $r = 0.997$, $P < 0.001$, $N = 32$, respectively) (Fig. 5). Regression analysis of the combined data yielded a slope of 0.95 ± 0.01 ($N = 47$), indicating that 95% of the biosynthesized juvenile hormone was released into the medium. These results are consistent with the results of HPLC analyses presented above. Isooctane extraction of the medium thus provides an accurate estimate of juvenile hormone biosynthesis in adult worker honey bees.

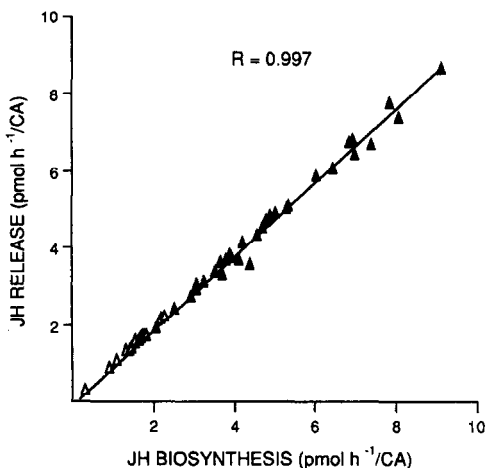


Fig. 5. Relationship between honey bee juvenile hormone biosynthesis and release. For this experiment only, biosynthesis is defined as the total amount of juvenile hormone released into the medium and that present in the corpora allata. Δ , Nurse bees ($N = 15$); \blacktriangle , foragers ($N = 32$).

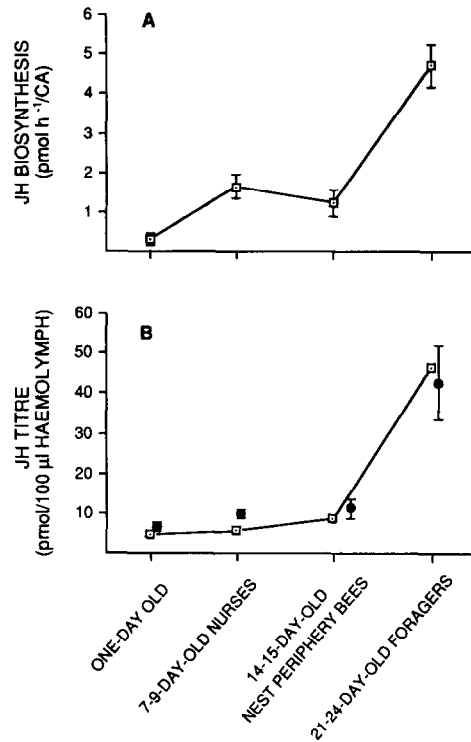


Fig. 6. Correlation between rate of juvenile hormone biosynthesis and haemolymph titre in honey bees. (A) Mean rate of biosynthesis (\pm SEM; $N = 10$); (B) \square , titre determined from pooled haemolymph from the same bees for which rates of biosynthesis were measured; \bullet , mean (\pm SEM) titres from 5–8 groups of bees (5–9 bees per group), including those depicted with \square .

Correlation between juvenile hormone biosynthesis and juvenile hormone titre

There is a significant correlation between juvenile hormone biosynthesis and juvenile hormone titre, based on measurements of both variables in the same individuals ($r = 0.96$, $P < 0.05$) (Fig. 6). In foragers, juvenile hormone biosynthesis rates and haemolymph titre were significantly higher than 1-day old bees, 7–9-day-old nurse bees and 14–15-day-old nest periphery bees (ANOVA: biosynthesis: $P < 0.001$, $N = 38$; titre: $P < 0.001$, $N = 26$).

Juvenile hormone biosynthesis and corpora allatum volume

Corpora allatum volumes ranged from 0.27 to $1.16 \cdot 10^6 \mu\text{m}^3$. There is a significant correlation between juvenile hormone biosynthesis and corpora allata volume (Fig. 7). However, only 13% of the variation in juvenile hormone biosynthesis can be explained on the basis of volume alone ($r^2 = 0.13$). Separate analyses of the data for nurse bees and foragers revealed that a significant correlation exists only for the former, but not for the latter. In addition, the range of corpora allatum volumes is smaller than the range of rates of juvenile hormone biosynthesis. Mean volumes for nurse bees and foragers were 0.62 and $0.82 \cdot 10^6 \mu\text{m}^3$, respectively, whereas

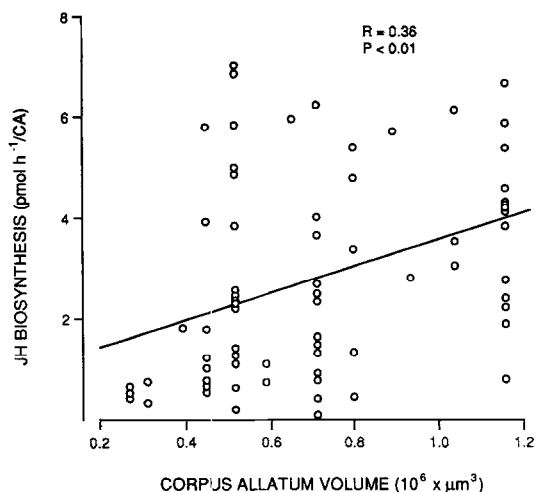


Fig. 7. Relationship between rates of juvenile hormone biosynthesis and corpus allatum volume in honey bees ($N = 70$).

mean rates of juvenile hormone biosynthesis were 1.23 and 4.37 pmol h⁻¹/corpora allata, respectively ($N = 70$).

DISCUSSION

Differences in juvenile hormone titres between adult worker honey bees performing different age-dependent tasks appear to be regulated primarily by changes in rates of biosynthesis rather than by changes in rates of hormone release or degradation. This conclusion is supported by two lines of evidence. First, almost all juvenile hormone in the corpora allata is released upon synthesis. Second, there is a high correlation between juvenile hormone biosynthesis and juvenile hormone titre. There are only two previous studies in which measurements were made of both juvenile hormone titres and rates of biosynthesis from the same individuals, and in both the two were highly correlated (see Tobe *et al.*, 1985), as in our study. Our results are consistent with an apparent absence of juvenile hormone-specific esterases in the haemolymph of adult worker bees (Strambi C. *et al.*, unpublished results). Rachinsky and Hartfelder (1990) reported that juvenile hormone titres in honey bee queen and worker larvae also are regulated by changes in rates of juvenile hormone biosynthesis (see also Tobe *et al.*, 1985). Only very small amounts of juvenile hormone III esterase are found in honey bee larvae, about 1000-fold less than in Colorado potato beetle (De Kort *et al.*, 1977), and its specificity is uncertain. Degradation of juvenile hormone nevertheless is probably important in regulating the haemolymph titre in worker honey bees, because the rate of biosynthesis *in vitro* is relatively high compared with the haemolymph titre. However, the high correlation of titre and rate of biosynthesis in both nurses and foragers suggests that degradation does not appear to play a major role in regulating

differences in titres that are associated with different age-dependent behavioural states.

The *in vitro* rates of juvenile hormone biosynthesis reported here are similar to those reported by Hildebrandt and Kaatz (1990) for adult worker bees. They are much higher than those reported by Bühler *et al.* (1983) (<0.5 pmol h⁻¹/corpora allata) perhaps because in that study glands were incubated in a suboptimal medium, as a medium formulated specifically for honey bees (Kaatz *et al.*, 1985) was not yet available. Our detection of only juvenile hormone III and our measurements of juvenile hormone titres are consistent with previous analyses, using the *Galleria* bioassay (Fluri *et al.*, 1982), a GC/MS assay (Hagenguth and Rembold, 1978), and RIA (Robinson *et al.*, 1987, 1989). High titres in foragers and low titres in nurse bees also agree with experiments demonstrating that foraging behaviour can be introduced by treatment with juvenile hormone (Jaycox, 1976), juvenile hormone mimic (Jaycox *et al.*, 1974), or juvenile hormone analogue (Robinson, 1985, 1987a; Sasagawa *et al.*, 1985).

Juvenile hormone titres and rates of juvenile hormone biosynthesis for 14–15-day-old bees from the nest periphery were similar to those for 7–9-day-old nurse bees. Bees from the nest periphery are in a behavioural phase that is in transition from nursing to foraging, and they are involved in nest maintenance and food storage tasks (see Seeley, 1982; and Winston, 1987). Our results suggest that there are discrete states of corpora allata activity associated with nursing and foraging, but not with the intervening behavioural phase(s). Robinson *et al.* (1987) reported no differences in juvenile hormone titres between bees aged 5, 10 or 15 days in one experiment, which is consistent with our findings. They did, however, detect an age-dependent increase in juvenile hormone titres in a second experiment, which suggests continuous changes in corpora allata activity throughout behavioural development. It is clear that low rates of juvenile hormone biosynthesis and low juvenile hormone titres are associated with nursing behaviour and high rates and titres with foraging, but additional studies are needed to determine the relationship between juvenile hormone and other worker behaviours.

Measurements of corpus allatum volumes, which agreed with those in previous studies (Gast, 1967; Breed, 1983; Bühler *et al.*, 1983), indicate that it is not possible to use such measurements to estimate accurately levels of corpora allata activity in honey bees. Similar results have been obtained for other insect species (see Tobe and Pratt, 1975). For example, the volume of a corpus allatum in the wasp *Polistes gallicus* was correlated with juvenile hormone biosynthesis in females undergoing egg maturation, but not in overwintering or parasitized females (Röseler *et al.*, 1980).

Our results reveal two intriguing differences in parameters of juvenile hormone biosynthesis between

nurse bees and foragers, aside from the large differences in rates. Rates of juvenile hormone biosynthesis apparently reached maximal levels in nurse bees at a concentration $\leq 10 \mu\text{M}$ methionine, but increased steadily in foragers up to a concentration of $100 \mu\text{M}$. This difference may reflect differences between nurse bees and foragers in transport of methionine into the corpora allata and/or differences in the formation of S-adenosyl methionine. Insect species show a wide range of optimal L-methionine concentrations for juvenile hormone biosynthesis, from as low as $5 \mu\text{M}$ in termites (Greenberg and Tobe, 1985) to $200\text{--}300 \mu\text{M}$ in locusts (Tobe and Pratt, 1974).

The second difference between nurse bees and foragers is that the rate of juvenile hormone biosynthesis, when stimulated with farnesoic acid, is much lower for nurse bees than for foragers. This suggests that there also may be differences between nurse bees and foragers in the activity of some rate-limiting enzyme(s) prior to the formation of farnesoic acid (see Tobe and Stay, 1985). For both nurse bees and foragers, the degree of stimulation by farnesoic acid we observed (three times the spontaneous rate) is near the lower end of the range reported for other species [1.2–10 times the spontaneous rate (see Tobe and Stay, 1985)]. Relatively low stimulation by farnesoic acid in our results suggests that corpora allata were maintained in relatively optimal conditions for juvenile hormone biosynthesis in our experiments (Tobe and Stay, 1985). Alternatively, low stimulation by farnesoic acid may be a consequence of the inactivation or saturation of *o*-methyl transferase, the enzyme responsible for formation of methyl farnesoate from farnesoic acid, as in last instar *Diploptera* (Yagi *et al.*, in press).

Our results also suggest one difference in juvenile hormone biosynthesis between honey bees and other species. Corpora allata from honey bees apparently do not use acetate as a substrate for juvenile hormone biosynthesis, in contrast to all other insects studied to date (see Tobe and Stay, 1985). Incorporation of [^{14}C]acetate and [^3H]methionine into adult bee corpora allata did not exhibit the expected 9:1 ^{14}C : ^3H incorporation ratio. Corpora allata from honey bee larvae also fail to show the expected ratio (Rachinsky A. and Tobe S. S., unpublished results). Alternatively, perhaps corpora allata from honey bees do use acetate as a substrate for juvenile hormone biosynthesis, but the sucrose present in the incubation medium may have been broken down to simple sugars and used as a carbon source instead of acetate.

The strong correlation between juvenile hormone biosynthesis and titre for nurse bees and foragers indicates that changes in biosynthetic rates appear to accurately presage changes in haemolymph titres that have been determined to mediate behavioural ontogeny in worker honey bees (Robinson *et al.*, 1989, unpublished). The radiochemical assay of juvenile hormone biosynthesis provides a reliable and

sensitive method for the study of hormonal regulation of honey bee behavioural development. It is possible to measure rates of juvenile hormone biosynthesis in individual bees, whereas measurements of juvenile hormone titres (Robinson *et al.*, 1987, 1989) require pooled samples from at least several individuals. The ability to measure rates of juvenile hormone biosynthesis for individual bees will increase the precision of studies relating juvenile hormone and behaviour.

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