Juvenile hormone and division of labour in *Apis cerana*

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ABSTRACT

Apis cerana juvenile hormone (JH) titres were measured at various stages of behavioural development (newly emerged, nurse and returning foragers). A chiral-specific radioimmunoassay was used to measure JH titres. In a second experiment, the bees were treated with methoprene to see if behavioural development was accelerated. *A. cerana* had similar JH titres to *A. mellifera,* and returning foragers had the highest JH titres. The proportion of methoprene treated bees that became foragers was higher than the controls although the difference was not significant in *A. cerana*.

INTRODUCTION

Division of labour in the western honey bee (*Apis mellifera*) has been extensively studied, especially during the last 30 years (reviewed in Winston, 1987; Seeley, 1995). Workers typically change their jobs as they age in a colony with stable age demography, progressing from cell cleaning and brood rearing during the first two weeks, to nectar processing and comb building in the third week and, finally, to foraging when they are about 25–30 days old (Seeley, 1982). This progression of tasks, a form of behavioural development, is correlated with a major insect developmental hormone, juvenile hormone (JH). While JH plays a vital role in metamorphosis in most insects, including honey bees, its role in adult western honey bees is 'maturing' rather than 'juvenilizing'. JH titres in blood typically increase with age; they are low in bees that perform in-hive tasks such as nursing, comb building and other activities, and high in foragers (Rutz *et al.*, 1976; Fluri *et al.*, 1982; Robinson *et al.*, 1987, 1989; Huang *et al.*, 1994; Huang & Robinson, 1995). When corpora allata, the organs that produce JH, are removed from workers and incubated *in vitro* and measured for rates of JH biosynthesis, they also show the same upward change with age: low in young bees and high in foragers (Huang *et al.*, 1991). Applying JH, JH analog or JH mimic to bees causes workers to forage earlier (Jaycox, 1976; Jaycox *et al.*, 1974; Robinson, 1985, 1987; Robinson & Ratnieks, 1987; Robinson *et al.*, 1989; Sasagawa *et al.*, 1989). These results suggest that not only is JH correlated with behavioural development in western honey bees, but it also plays a key role in modulating the pace of behavioural transition in workers. In fact it was thought that high JH would 'activate' foraging behaviour and without it bees will not perform this behaviour (Huang & Robinson, 1992).

More recent studies indicate that JH is not essential for the nurse to forager transition. Foragers were observed to have JH levels (both JH titre and JH biosynthesis) comparable to summer nurses (< 100 ng/ml or < 2 pmol/h) during early spring and late autumn, suggesting that bees are capable of foraging with low JH levels (Huang & Robinson, 1995). Despite of their lower JH levels (compared to foragers in summer), foragers still had a higher JH titre compared to nurses or other in-hive bees sampled on the same day. Foragers almost always have high JH titres in summer, but in one study they were found to have low levels (< 100 ng/ml) one summer in Vancouver, Canada (Pankiw *et al.*, 1998), and one summer in Illinois, USA (Z Y Huang, unpublished data). Furthermore, workers with their corpora allata removed still commenced foraging, although on average about 5–6 days later compared to the control bees (Sullivan *et al.*, 2000). This delay in foraging transition can be reversed by applying methoprene (a JH analog) to the bees on the same day the surgery was performed. These results suggest that JH is not essential for behavioural development to occur, but it does affect the pace of behavioural development. Despite these complications, JH still remains the best indicator for behavioural development: one can randomly sample a bee from the hive, measure its JH titres, and infer what stage it is at in the developmental cycle.

Apis cerana is closely related to *A. mellifera* and they share many common characteristics. *A. cerana* seems to have an age-related division of labour (Z Y Huang, personal observation), although no detailed behavioural observation was ever published. They also show guarding and undertaking behaviours, similar to *A. mellifera* (Z Y Huang, personal observation). This study was conducted to determine whether JH titres are different among *A. cerana* workers performing different tasks, and whether treating them with methoprene would accelerate their behavioural development. Com-

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parative studies such as this will help understand the evolutionary mechanisms underlying social behaviour and the role JH plays in behavioural development in the genus *Apis*.

MATERIALS AND METHODS

Experiment 1. Measurement of JH titres

Experiments were conducted in Beijing, China, September 1993 (colony 1), and Kunming, Yunan, China, November to December, 1999 (colonies 2 and 3). Newly emerged bees, nurses and returning foragers were sampled from *A. cerana* colonies. Newly emerged bees were identified by their appearance and behaviour: abundance of 'fuzzy' hairs, usually damp-looking and matted down, and walking slower than other bees. Nurse bees were identified as bees with their head in a larval cell (colony 1) or randomly collected from the brood comb when bees clustered and showed no natural behaviour (colonies 2 and 3) due to cold weather. Foragers were identified as returning bees with pollen on their pollen baskets or with an extended abdomen. After collection, bees were immobilized on ice for 5–20 min until blood (haemolymph) was taken. Blood (0.9–3.5 µl per bee) was collected with a 5 µl capillary tube, measured to the nearest 0.1 µl, and stored in 0.5 ml acetonitrile at –20°C until brought back to the Apiculture Laboratory at Michigan State University for analysis. Blood from individual bees was analysed for colony 1, for other colonies each sample contained blood from three bees.

A chiral-specific radioimmunoassay (RIA) (Hunnicutt *et al.*, 1989) was used to measure JH titres. This assay has been validated for adult worker honey bees in *Apis mellifera* by Huang *et al.* (1994). Previous results (Huang *et al.*, 1994; Huang & Robinson, 1995) indicate that values from this RIA agree with two other recently developed RIAs, both of which have been validated with gas chromatography/mass spectroscopy (de Kort *et al.*, 1985; Goodman *et al.*, 1990). The RIA was performed according to Huang & Robinson (1996). All solvents were HPLC grade from EM Science. Glassware was baked at 500°C for 3.5 h prior to use to minimize JH adsorption (Strambi *et al.* 1981). The detection limit of the RIA is 5 pg 10 (R) JH per sample. Inter- and intrassay variations for JH determinations were 9.2% and 10.6%, respectively (Huang & Robinson, 1996). JH titre differences were analysed using SAS (SAS Institute, 1985) after appropriate transformations to normalize the data, Tukey's tests were used to compare the means.

Experiment 2. Behavioural acceleration after treatment with methoprene

Newly emerged bees were obtained directly from an *A. cerana* hive. Trial 1 was conducted on 13 November 1999 and trial 2 on 17 November 1999. We also treated *Apis mellifera* as a 'positive control' since it is known that methoprene accelerates worker behavioural development in *A. mellifera*. This was done to ensure that the methoprene we used was effective. In the first trial, both species were treated with 100 or 200 µl methoprene contained in 5 µl acetone. Control bees received 5 µl acetone alone. The two groups of bees were paint-marked on the thorax using different colours (Testor's PLA). Because of the high mortality in *A. cerana* bees in the first trial, we reduced the dosage to 150 µl for these bees in the second trial, and also reduced the volume of acetone to 2.5 µl in both species. When the treated bees started foraging, returning foragers (with pollen or extended abdomen) were collected two hours per day (1 h in the morning and 1 h in the afternoon) from 26 November to 1 December. The data was collected 'blind' to ensure non-bias: the person doing the observation did not know the colours of various treatments. Workers started foraging when they were 13–20 days old (trial 1) or 9–16 days old (trial 2). At the end of the experiments (2 December), all

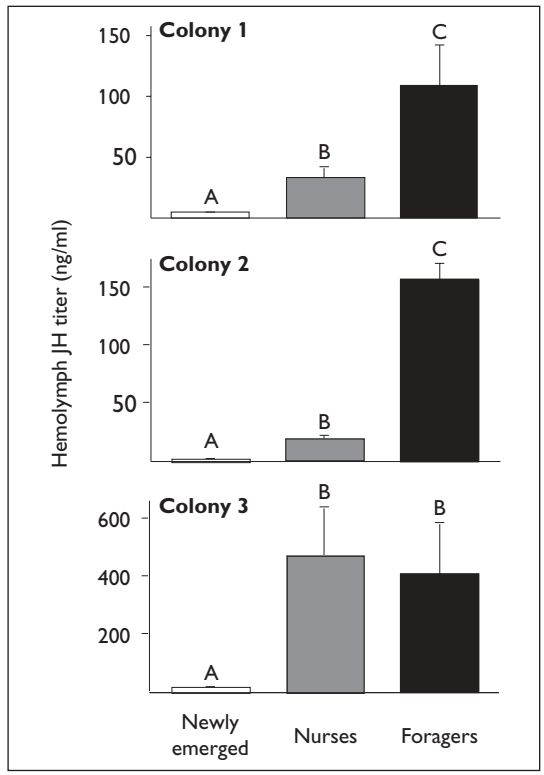

FIG. 1. Juvenile hormone titers (mean ± se) for bees performing different tasks. Sample sizes were 5, 4, and 13 individual workers for newly emerged, nurse, and foragers, respectively, for colony 1, and 4 blood samples (each with blood from 3 workers) for each task for the other two colonies. Means with different letters on top indicate that they are significantly different at 5% level, Tukey's test.

marked bees inside the hive were recovered and assumed to be 'nonforagers'. One way G-tests (Sokal & Rohlf, 1981) were used to analyse the proportion of foragers and non-foragers between the control and treatment group.

RESULTS AND DISCUSSION

Experiment 1. Measurement of JH titres

Juvenile hormone titres changed as workers performed different tasks (fig. 1). In three out of three colonies, newly emerged bees showed the lowest JH titres among the three groups sampled. Nurses (colony 1) or in-hive bees randomly sampled from the brood comb (colonies 2 & 3) showed intermediate JH titres in two out of three colonies. Returning foragers showed the highest JH titres, except in colony 3 where in-hive bees showed similar JH titres as the foragers. JH levels in three behavioural groups in this study were similar to published data in *Apis mellifera* (Huang *et al.*, 1994; Huang & Robinson, 1995). Foragers in colony 3 had JH titres two-times higher compared to that of colonies 1 or 2, suggesting that large colony to colony variations in forager JH titres also exist in *A. cerana*, similar to *Apis mellifera* (Huang & Robinson, 1995).

Experiment 2. Behavioural acceleration after treatment with methoprene

We observed high mortality for both *Apis mellifera* and *Apis cerana* in the first trial (table 1). This is probably a result of bees exposed to very low ambient temperatures as we were picking newly emerged bees from the colonies (8–10°C), and somehow bees became more sensitive to methoperene treatment. This is supported by the observation that *A. mellifera* did not show the same level of mortality with the same dosage in the second trial. Nonetheless, *A. cerana* seems to be much more sensitive to methoprene treatment compared to *A. mellifera*, with mortality higher than *A. mellifera* in both treatment doses and the control. It is not clear whether *A. cerana* is more sensitive to methoprene because of their slightly smaller size, or because of other physiological differences.

TABLE 1. Differential mortality with two *Apis* species when treated with various dosages of methoprene. Only the difference caused by the highest dosage is significantly different (G = 2 1.9, *P* < 0.001).

Dosage	*A. cerana*	*A. mellifera*
0 µg	8.3%	1.7%
100 µg	25.0%	11.7%
200 µg	68.3%	23.3%

The batch of methoprene we used clearly worked as expected, because in both trials *A. mellifera* showed a significantly higher proportion of foragers in treated bees compared to control bees (table 2). Results for *A. cerana* are not as strong, but the G-value (3.6) is almost significant (critical value for *P* = 0.05 is 3.84) for trial 2. The behavioural data here also support the idea that *A. cerana* workers go through similar behavioural development as *A. mellifera*, because workers in both species started foraging when they were 9–16 days old in trial 2.

CONCLUSIONS

This is the first study to show that adult workers in *A. cerana* have similar JH profiles as *A. mellifera* among workers performing different tasks. One earlier study showed no major difference between the two species in timing or peak value of JH between the capped brood stage of *A. cerana indica* and *A. mellifera* (Rosenkranz *et al.*, 1993). Of course, one has to be cautious about interpreting data from radioimmunoassays: any other chemicals that interfere with the binding between JH and its antibody can be misinterpreted as extra JH molecules. More validation steps are needed and are underway. The proportion of methoprene-

TABLE 2. Proportion of bees becoming foragers after treatment with various dosages of methoprene upon emergence. No data in trial 1 was obtained for *Apis cerana* due to high mortality. Probabilities are based on two-tailed tests.

Species	Treatment	% of foragers	Statistic	Probability
Apis mellifera				
Trial 1	control	48.0%		
	200 µg	72.0%	G = 9.3	< 0.01
Trial 2	control	18.2%		
	200 µg	77.8%	G = 25.7	< 0.001
Apis cerana				
Trial 2	control	53.01%		
	150 µg	78.9%	G = 3.6	< 0.1

treated bees that became foragers was not significantly higher compared to that of the control, partly because of the small numbers per treatment group (50 bees per group). These results are preliminary but suggest that JH treatment would accelerate behavioural development in *A. cerana* similar to *A. mellifera*. We are planning to conduct more trials to test the hypothesis.

In conclusion, although our data is preliminary, *A. cerana* and *A. mellifera* seem to show very similar physiological attributes: workers performing different tasks have different levels of JH and older bees (foragers) tend to have the highest JH titres. JH treatment also accelerates behavioural development in workers, in that treated bees show earlier foraging behaviour. *Apis cerana* seems to be more sensitive to methoprene treatment in causing mortality, especially when bees were treated with cold temperatures prior to methoprene treatment.

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