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Effects of social environment and worker mandibular glands on endocrine-mediated behavioral development in honey bees

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Abstract Previous studies suggest that older honey bee workers possess an inhibitory signal that regulates behavioral development in younger bees. To study how this inhibitor is transmitted, bees were reared for 7 days in double-screen cages, single-screen cages, or unrestricted in a typical colony (control bees). Double-screen cages prevented physical contact with colony members while single-screen cages allowed only antennation and food exchange. Bees reared in double-screen cages showed accelerated endocrine and behavioral development; they had significantly higher rates of juvenile hormone biosynthesis and juvenile hormone titers than did control bees and also were more likely to become precocious foragers. Relative to the other two groups, bees reared in single-screen cages showed intermediate juvenile hormone biosynthesis rates and titers, and intermediate rates of behavioral development. These results indicate that physical contact is required for total inhibition. We also began to test the hypothesis that worker mandibular glands are the sources of an inhibitory signal. Old bees with mandibular glands removed were significantly less inhibitory towards young bees than were sham-operated and unoperated bees. These results suggest that an inhibitor is produced by the worker mandibular glands.

Key words *Apis mellifera* · Division of labor · Juvenile hormone · Behavioral development · Primer pheromone

Abbreviations *CA* corpora allata-corpora cardiaca complex · *JH* juvenile hormone · *QMP* queen mandibular pheromone · *RIA* radioimmunoassay

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Introduction

There is an age-related division of labor among workers in honey bee colonies. Bees typically perform tasks within the hive such as brood rearing (nursing) and hive maintenance during the first 2–3 weeks of adulthood and then switch to foraging and colony defense for the final 1–3 weeks of life (reviewed by Winston 1987). Bees also can respond to changes in colony conditions by accelerating, delaying, or even reversing their behavioral development (reviewed by Robinson 1992). This is an example of the high-level coordination among colony members that characterizes many insect societies (Seeley 1995). However, the physiological mechanisms that underlie the integration of activity into a smoothly functioning colony are not well understood.

Juvenile hormone (JH) is involved in the regulation of behavioral development in adult worker honey bees (reviewed by Fahrbach and Robinson 1996; Robinson and Vargo 1997). JH blood titers typically increase with age; they are low in bees that work in the hive and high in bees that forage and engage in colony defense. In addition, precocious foragers have a precociously high JH titer, overage nurses have a low titer, and bees that revert from foraging to nursing show a drop in JH. Treating bees with JH, JH mimic, or JH analog induces precocious foraging. Recently, it has been shown that removal of the corpora allata, the glands that produce JH, delays bees from developing into foragers; the delay is eliminated with JH analog treatment (Sullivan et al. 1996). These results support the idea that JH is involved in adjusting the rate of behavioral development in response to environmental conditions so that it is appropriate for the needs of the colony (Robinson 1987).

Several lines of evidence demonstrate that one environmental factor, colony age demography, plays a key role in regulating honey bee behavioral development (Huang and Robinson 1992, 1996). In an experimental "single-cohort colony," composed entirely of young bees, about 5–10% of the individuals become precocious

foragers at 6–10 days of age (Huang and Robinson 1992). Transplanting a group of foragers into a single-cohort colony inhibits precocious behavioral development by the young resident bees (Huang and Robinson 1992). Inhibition occurred even if transplanted foragers were not allowed to forage, which means that the resident bees likely sensed the foragers directly, rather than some change in the hive environment such as the presence of freshly collected food. Similar results were obtained from colonies with more typical age structures (Huang and Robinson 1996). When a portion of a colony's foragers was removed to simulate predation, young bees developed faster than those in a control colony in which the same number of bees was removed, but evenly across different age classes. Conversely, when foragers were confined to their hive by artificial rain (via a water sprinkler), young bees delayed, instead of accelerated, their development. A significant negative relationship was found between the proportion of old bees in a colony and the proportion of bees developing into foragers: the more old bees present, the fewer the bees that developed into foragers (Huang and Robinson 1996). These results suggest that development of young bees into foragers is regulated by a negative-feedback process. This hypothesis is consistent with findings from experiments in which bees were reared for 7 days in social isolation in the laboratory. These bees had high rates of JH biosynthesis and also foraged precociously when introduced into a colony (Huang and Robinson 1992), again suggesting that young bees develop into foragers precociously in the absence of the inhibitory effects of older bees. The nature of this inhibitory process is unknown, and is the subject of the experiments reported on in this paper.

Since chemical communication is extensive in insect societies, it is reasonable to consider the possibility that it is involved in the inhibition of younger bees by older bees. Chemical communication is well studied in honey bee colonies (Free 1987; Winston 1987). Bees live in a dark nest and have relatively limited auditory capabilities (Towne and Kirchner 1989), but possess keen olfaction. Chemical cues mediate many different activities, including care of the brood (Free and Winder 1983; Huang and Otis 1991; Le Conte et al. 1995) and queen (Winston and Slessor 1992), nestmate recognition (Breed and Stiller 1992), foraging (Winston 1987), and nest defense (Collins 1980).

Studies of mammals have revealed that the regulation of reproductive development often involves chemical signals that either advance or delay maturation (Stern and McClintock 1998). For example, age of puberty onset in the female house mouse is influenced by the social environment, via an interplay of chemical activators and inhibitors (reviewed by Vandenbergh 1983; Vandenbergh and Coppola 1986). Androgen-dependent chemical activators accelerate the onset of puberty, while adrenal-gland-dependent inhibitors delay it. These signals are thought to be perceived by the vomeronasal organ, which sends projections to the accessory olfactory bulb. Information is transmitted from the accessory olfactory bulb to higher brain centers that ultimately control the release of ovarian luteinizing hormone (Lomas and Keverne 1982; Darney et al. 1992). Chemical communication thus enables a female to precisely time reproductive development in response to changes in group density and sexual composition (Darney et al. 1992).

Recent results already implicate chemical communication in the control of worker honey bee behavioral development. A contact pheromone produced by the mandibular glands of the queen (''QMP''), already known to mediate several processes in the honey bee colony (Winston and Slessor 1992), was shown also to inhibit rates of worker JH biosynthesis (Kaatz et al. 1992), JH blood titers, and rate of behavioral development (Pankiw et al. 1998). Because a major component of the worker mandibular gland secretion, 10-hydroxy-(E)-decenoic acid (10-HDA), shows structural similarity to the major component of QMP, 9-oxo-(E)-2-decenoic acid (9-ODA), it is possible that mandibular glands of workers are involved in the regulation of worker behavioral development.

Experiments 1 and 2 were designed to gain more insight into the modality by which a signal might be transmitted from older bees to inhibit the behavioral development of younger bees. We were particularly interested in whether physical contact is required for social inhibition. If social inhibition of behavioral development in honey bee colonies has a chemical component, and if it can occur without physical contact, this would suggest the presence of a volatile pheromone inhibitor. If social inhibition requires social contact, this would suggest either the presence of a contact pheromone inhibitor, or specific inhibitory behaviors, or both. Experiment 3 was designed to test the hypothesis that the worker mandibular glands are a source of an inhibitor of behavioral development.

Materials and methods

Focal bees

Bees were from colonies maintained according to standard techniques at the University of Illinois Bee Research Facility, Urbana, Illinois. They were typical of current North American populations of *Apis mellifera* in this area (a mix of predominantly European subspecies; Phillips 1915; Pellett 1938). In some cases (detailed below), adult bees were marked on the dorsal surface of the thorax with a spot of paint (Testor's PLA) when 0–24 h (1 day) old. One-day-old bees were obtained by removing frames of pupae from colonies and placing them in an incubator (34 °C and 80% RH).

Measurement of JH biosynthesis

Rates of JH 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). Collected bees were immobilized on ice for ca. 20–120 min and then their corpora allata-corpora cardiaca complex (CA) was isolated. The CA from individual bees was incubated for 3 h in 50 µl bee medium (Kaatz et al. 1985, modified by Huang et al. 1991) containing 60 $\mu mol \cdot l^{-1}$

of L-[^{3}H-methyl] methionine (NEN, 7.4 GBq mmol·l^{-1}). Radiolabelled (^{3}H) 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 blood JH titers measured by radioimmunoassay (RIA) (Huang et al. 1991). JH III is the only JH homolog detected in honey bees (Hagenguth and Rembold 1978; Huang et al. 1991; Robinson et al. 1991; Huang et al. 1994). For more detailed methods see Huang et al. (1991).

Measurement of JH titer

After collection, bees were immobilized on ice for ca. 10–30 min and then blood was taken. Blood (0.6–6.5 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 analyzed. The capillary tube and other glassware that may contact JH was baked at 500 °C for 3.5 h prior to use to minimize JH adsorption (Strambi et al. 1981). All solvents were HPLC grade, obtained from either EM Science, Fisher Scientific, or J.T. Baxter Chemical.

A chiral-specific RIA (Hunnicutt et al. 1989) was used to measure the JH III titer. This assay has been validated for adult worker honey bees by Huang et al. (1994). Previous results (Goodman et al. 1993; Huang et al. 1994; Huang and Robinson 1995) indicate that values from this RIA agree with two other RIAs, both of which have been validated with gas chromatography/mass spectroscopy (de Kort et al. 1985; Goodman et al. 1990).

The sensitivity of the RIA is about 5 pg R(-) JH III per sample. Typical inter- and intrassay variation for JH determinations was 9.2% and 10.6%, respectively ($n = 10$). Detailed description of the RIA can be found in previous studies on honey bees (Huang et al. 1994; Huang and Robinson 1995, 1996).

Behavioral observations

Bees were identified as foragers according to standard criteria: returning to the hive with distended abdomens (reflecting nectar or water foraging) or with pollen on their corbiculae (pollen foraging). They were given a second paint mark (a color different from their age marking) when entering the hive entrance. The size of the hive entrance was reduced to 0.7 cm × 1.1 cm to facilitate paint marking. The second paint mark allowed us to determine the total number of foragers in a colony; simply counting foragers was not accurate because focal bees were not individually marked and could make variable numbers of foraging trips each day. Each colony was observed 1 h in the morning and 1 h in the afternoon, weather permitting. After the experiment was over, each colony was anesthetized by CO_2 and killed by freezing. Censuses were performed to determine whether there was differential mortality among colonies.

Experiment 1: effects of social environment on age-related changes in JH biosynthesis rates and blood titers

Treatments

Bees that emerged over a 24-h period in the incubator were reared for 7 days in a typical colony in one of three ways: individually in cages with single-screens that allowed only antennation and food exchange with colony members; individually in plastic cages with double screens that prevented physical contact with colony members; or with unrestricted access to colony members (control bees). Control bees also differed from the two caged experimental groups in having unimpeded movement as well as access to food in the honeycombs and probably other stimuli in the hive. However, it has previously been shown that bees reared for 7 days in groups in the laboratory show normal rates of JH biosynthesis, while those reared in social isolation show precociously high rates of JH biosynthesis and a precocious onset of foraging when placed in a colony (Huang and Robinson 1992). These results suggest that any differences between control and caged bees in the present experiment are related to differences in social interactions rather than other factors.

The cages used in this experiment (40 mm × 25 mm, manufactured by JZ^s BZ^s, California) are commonly used by beekeepers to ship queens. The cages are perforated with openings (3 mm × 4 mm). Bees inside single-screen cages are able to antennate and exchange food with workers outside the cage (Z.-Y. Huang, unpublished observations). Trials conducted in which caged bees were not given their own food supply (see below) confirm that feeding across the cage occurred. Bees reared in single-screen cages were individually placed into cages (35–120 cages), and then the cages were placed in a wooden frame that was placed into the colony.

A second group of cages (35–120 cages), again each containing one bee, was inserted in a wooden frame that itself was enclosed in a metal screen cage (460 mm × 240 mm × 80 mm) before being placed into the same colony. The metal screen cage prevented the bees from physically contacting colony members, because the cages were at least 20 mm away from the screen of the metal cage. Proboscis length is shorter than 7.2 mm (*A. mellifera caucasica*; Ruttner 1975) while antennal length is about 5 mm (Z.-Y. Huang, personal observation). However, bees in "double-screen cages" should have been able to perceive the same volatile odors of the colony as did those in single-screen cages.

Each cage was provisioned with about 400 mg of sugar candy (a mixture of confectionery sugar and honey with a dough-like consistency), more than enough food to sustain a bee for the 6- to 8-day experimental period (average consumption per bee: 11 mg per day; Z.-Y. Huang, unpublished observations). Bees in single-screen cages were provided with food in trial 1 only. Food was eliminated in subsequent trials to encourage more social contacts between caged bees and colony bees; we reasoned that hungry individuals might solicit food (and thus social contact) more actively. A drop of water was given to each caged bee daily, because otherwise the candy became too dry to consume. In trials in which bees in single-screen cages were not fed, they were nevertheless subjected to a sham watering treatment (the frame of cages was removed from the hive, brushed to remove adhering bees, held out of the hive for the same amount of time, and then returned).

Control bees were paint marked at 1 day of age and introduced into the same colony as the caged bees. Control bees were not caged, had the full range of social contacts with colony bees and were thus expected to have normal age-related endocrine status. Since bees were assayed when they were 6–8 days old (see below), control bees were expected to have low rates of JH biosynthesis and low JH titers as they were most likely to be nursing brood.

Five trials of this experiment were conducted in the summers of 1993 and 1994. In each trial a different colony was used to rear the caged and control bees. These colonies were all typical field colonies in our research apiaries, subject to standard management techniques but otherwise unmanipulated. Colonies had adult bee populations of approximately 46 000–70 000, each occupying 3–4 Langstroth hive boxes. Double-screen cages and single-screen cages were placed together on the topmost hive box, surrounded by partially filled honey frames (trials 3–5). In the first two trials there were either two frames of open brood (trial 1) or six frames from which honey was removed in the hive box where cages were located (trial 2).

Assays

Bees were assayed when they were 6–8 days old. Rates of JH biosynthesis were measured in all five trials ($n = 6$–10 bees per treatment group). In trials 1 and 5, JH blood titers also were quantified ($n = 4$–10). Bees from trials 1 and 4 were offspring of the same queen, who was instrumentally inseminated (Laidlaw 1977) with semen from a single drone. Bees from other trials were offspring of different, naturally mated queens.

Experiment 2: effects of social environment on rates of behavioral development

Behavioral analyses were conducted in parallel with trials 3 and 5 of Experiment 1 to determine whether manipulations of the social environment affected behavioral development in a manner consistent with effects on JH. As in Experiment 1, bees were reared for 7 days in double-screen cages, single-screen cages, or as control bees. Fifty of each group were then collected (uncaged, if necessary) and mixed with 1600 other 7-day-old bees to form a small single-cohort colony. The colony contained two frames of honey and pollen, and a caged queen. The queen was caged because in pilot studies queens were killed by bees reared in double-screen cages, possibly due to the fact that they were "aged" precociously and would not accept a new queen. The 1600 bees were obtained by marking 1-day-old bees with a spot of paint (Testors PLA) on the thorax and reintroducing them to their natal colony (different than the one used for the experimental bees). Foraging observations were conducted only on the bees from the 3 experimental groups when they were 9–13 days old. In the second trial, 1300 background bees and 60 bees per treatment group were used. The colony was established when bees were 8 days old and observations were performed when they were 10 and 11 days old.

Experiment 3: are bees with their mandibular glands removed less inhibitory towards younger bees?

Treatment

Mandibular glands from worker bees were removed in a procedure similar to that of Gary (1961) for queen bees. Bees were immobilized on ice for ca. 5–120 min and secured on a dissecting dish with three pieces of plasticine. One piece of plasticine held the head sideways, another secured the head, and a third secured the thorax. Two incisions of the cuticle were made to form a triangle below the eye and above the mandible. The flap of cuticle was lifted, a pair of forceps was inserted to grasp the base of the mandibular gland, and the entire gland removed. The cuticle folded back to its original position, so it was not necessary to seal the incisions. This procedure was repeated for the contralateral gland. Sham operations were performed by making the same incisions and cuticle lifting, but not removing the gland. Intact control bees were not operated upon at all, but were cooled and painted as the operated and sham-operated bees.

Five trials were performed in 1995 and 1996. In the first two trials, a few crystals of antibiotic (1:1:1 streptomycin, penicillin, and phenylthiourea) were put near the incisions; however, antibiotic treatment did not cause a noticeable decrease in mortality so it was discontinued in later trials. Bees ($n = 10$) sacrificed 3 weeks after surgery and examined under a stereoscope microscope (×64) showed no evidence of gland regrowth.

Our original intent was to remove the mandibular glands of foragers because older bees are hypothesized to produce or transfer more inhibitor than younger bees (Huang and Robinson 1992, 1996). However, forager mortality due to gland removal was too high, so we removed the glands of younger bees, allowed them to age in colonies, and then tested their ability to inhibit the behavioral development of younger (intact) bees. In trials 1, 2, and 3, glands were removed from 1- or 2-day-old bees, and they were used at 14–15 days of age. In the interim they were set up as the oldest age cohort in a double-cohort colony, which results in precocious behavioral development (Page et al. 1992). Workers with their mandibular glands removed were observed to fly and forage normally in these trials.

In trials 4 and 5, glands were removed from 18- to 23-day-old hive bees and they were used 3 days later. In trial 3, we also studied a colony that received no transplant of old bees at all, as a control. The bees used as transplants (mandibular glands removed, sham, or control bees) for each trial were of the same age and genetic origin. The numbers of transplanted bees introduced into each set of two or three colonies were: 47, 93, 56, 78, and 110 workers, for trials 1 to 5, respectively.

Assay

Bees treated as described above were transplanted into a single-cohort colony composed of 1-day-old bees, and observations were performed to determine whether the transplants were able to inhibit precocious foraging by resident bees. This assay was based on transplant experiments with intact older bees (Huang and Robinson 1992), which showed strong inhibition, i.e., very few resident bees showing precocious foraging. Previous studies have shown that the inhibition of precocious foraging is a specific effect of transplanting older bees and not due to adding foreign bees. Young bees transplanted from a foreign colony are not able to inhibit the development of resident bees (Huang and Robinson 1992).

Each trial of the experiment was performed with a set of two or three single-cohort colonies. Single-cohort colonies were made with 1000 1-day-old bees (obtained and marked as described above) according to standard procedures (Robinson et al. 1989). Because we needed to make two or three colonies at the same time, it was necessary to obtain 1-day-old bees from more than one source colony. To ensure that all colonies in each trial had similar genotypic compositions, 1-day-old bees from different source colonies were removed from the incubator, brushed into the same pan, and thoroughly mixed before being marked. Each colony in a trial was headed by a naturally mated, sister queen, less than 1 year old. Queens remained caged during the experiment, so that the amount of brood did not vary among colonies. Each colony also had the same resources: one empty frame and one frame that was one-half filled with honey and one-third filled with pollen. Using single-cohort colonies, we were thus able to minimize intercolony variation in genetic structure, age demography, and resource quantity, all of which can influence worker behavioral development (Robinson et al. 1989; Giray and Robinson 1994; Huang and Robinson 1996; Schulz et al. 1998). Single-cohort colonies were used also because it is more efficient to study the ontogeny of precocious foraging rather than foraging that occurs under more typical conditions, which is 2 weeks later. Observations of foraging were made when resident bees were 7–13 days old, and foragers were identified as described in the general method.

Statistical analyses

In Experiment 1, differences among treatments in rates of JH biosynthesis and JH titers were analyzed by one-way analysis of variance (SAS Institute 1985), with social environment (double-screen cage, single-screen cage, or control) as the independent variable. Data were transformed by square root or log transformation when necessary to obtain a normal distribution for residuals or to stabilize variances among the treatments. In Experiments 2 and 3, differences among treatments in the distribution of foragers and non-foragers were analyzed with 2×2 contingency tables with the *G*-statistic (Sokal and Rohlf 1981); two-tailed tests were conducted in all cases. Means ± SE are given throughout this paper.

Results

Experiment 1: effects of social environment on age-related changes in JH biosynthesis and blood titers

Social environment exerted a strong effect on the endocrine status of worker honey bees. In five out of five trials, bees reared in double-screen cages for 6–8 days showed significantly higher rates of JH biosynthesis than did control bees, which were unrestrained and free to interact with all other colony members (Fig. 1). In two out of two trials, bees reared in double-screen cages also

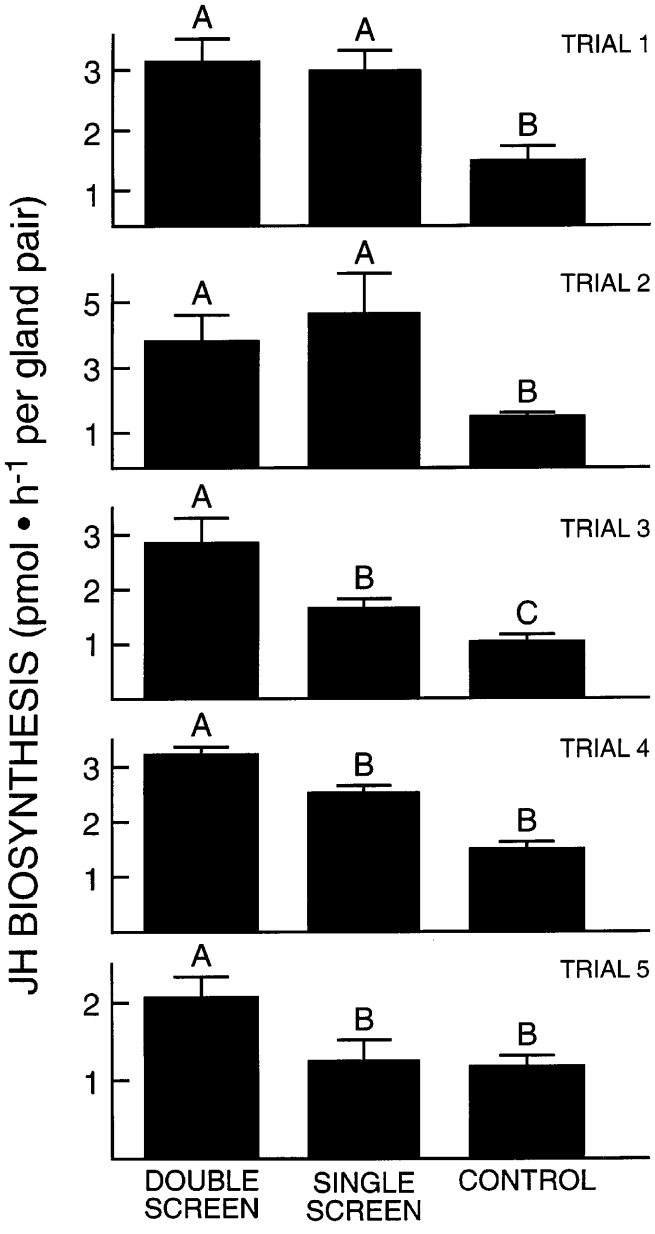

Fig. 1 Effects of social environment on rates of juvenile hormone (JH) biosynthesis by the corpora allata in vitro (mean ± SE). n = trial 1: 10, 10, and 9 individuals for double-screen, single-screen, and control groups, respectively; trial 2: 10, 10, and 6; trial 3: 8, 8, and 8; trial 4: 8, 8, and 8; and trial 5: 10, 10, and 10. *Bars topped with different letters* are significantly different at the 5% level using Tukey's HSD test. Results of one-way ANOVA: $P < 0.01$ or less for all trials

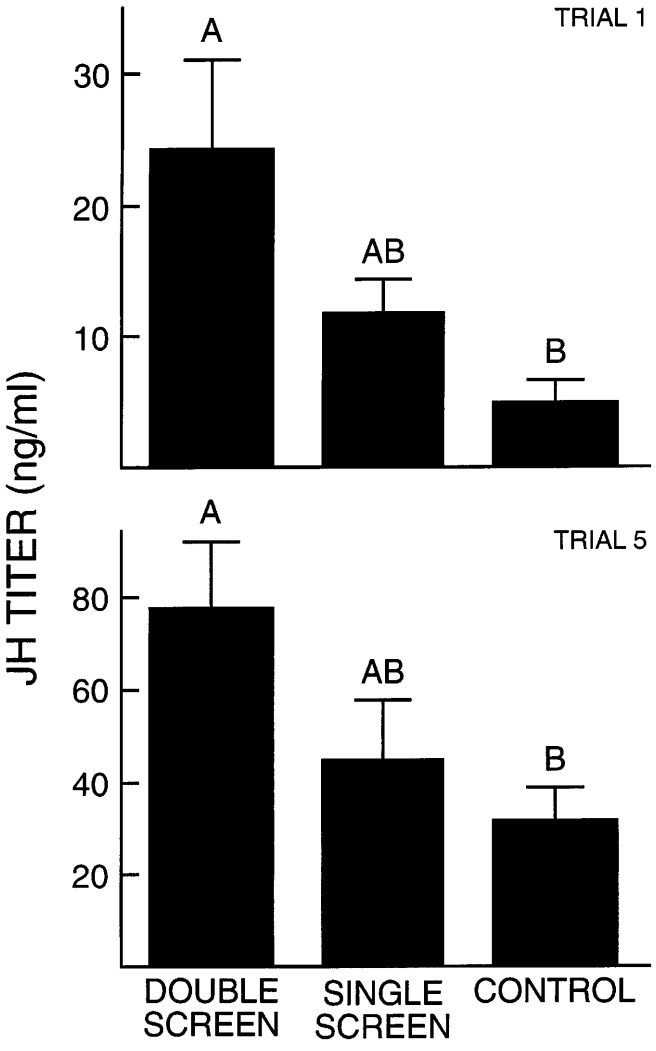

Fig. 2 Effects of social environment on blood titers of JH (mean ± SE). n = 6, 5, and 4 individuals for double-screen, single-screen, and control groups, respectively, in trial 1, and n = 10 for all three groups in trial 2. Notation as in Fig. 1. Results of one-way ANOVA: $P < 0.05$ for both trials. Trial numbers refer to those in Fig. 1

showed significantly higher titers of JH than did control bees (Fig. 2).

In contrast, the effects of being reared in single-screen cages were more variable (Fig. 1). In the first two trials, bees reared in single-screen cages had rates of JH biosynthesis that were not significantly different from those of bees in double-screen cages, but significantly higher than from the control bees. In the third trial, bees in single-screen cages showed intermediate rates of JH biosynthesis, significantly different from rates of bees either in double-screen cages or control bees. In the last two trials, rates of JH biosynthesis of bees in single-screen cages were not significantly different from those of the control bees but significantly lower than those of bees in double-screen cages. JH titers of bees in single-screen cages were not significantly different from those of control bees, nor were they different from that of bees reared in double-screen cages (Fig. 2). Workers in single-screen cages had mortalities of 50%, 77.1%, 47.5%, 44.2%, and 18.6%, for trials 1–5, respectively.

Experiment 2: effects of social environment on rates of behavioral development

Social environment also strongly influenced behavioral development in a manner consistent with the effects on

JH. In trial 1, 52% of bees reared in double-screen cages became foragers by 13 days of age, while only 20% of bees in single-screen cages and 1.7% of control bees did so (Fig. 3). Two-way contingency table analysis indicated that all three pair-wise comparisons were highly significant (single-screen versus double-screen: $G = 9.94$, $P < 0.002$, double-screen versus control: $G = 34.8$, $P < 0.0001$, single-screen versus control: $G = 7.98$, $P < 0.005$). Similar results were obtained in the second trial: 57% of bees in double-screen cages became foragers by 11 days of age, while 16% of bees in single-screen cages and 2% of control bees did so (Fig. 3).

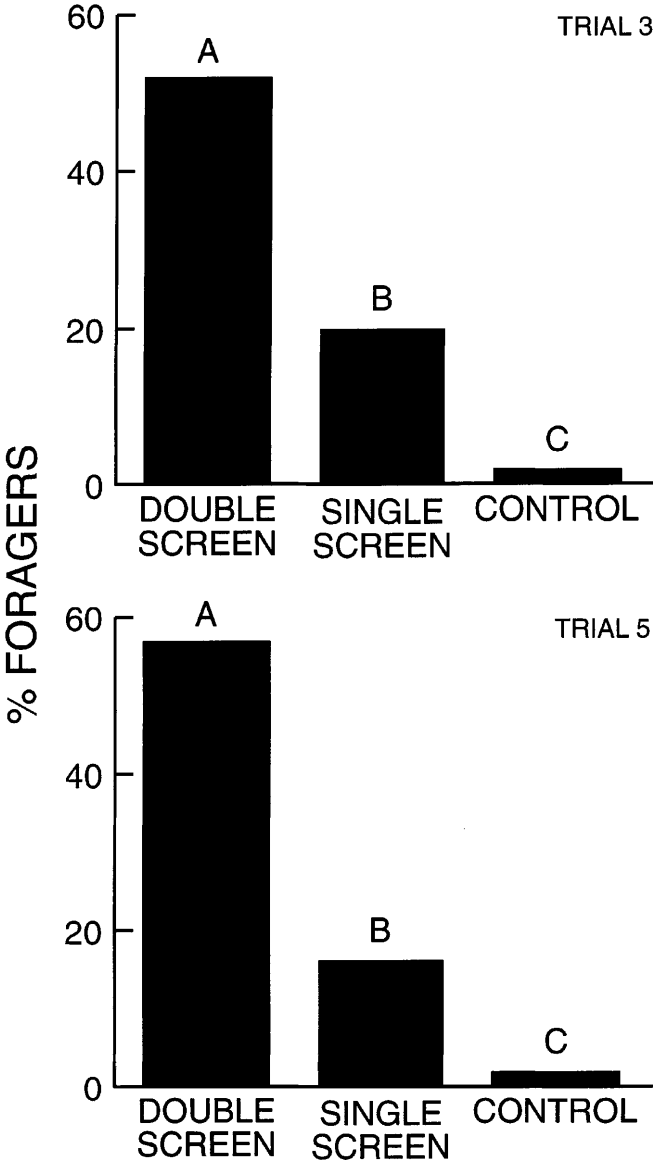

Fig. 3 Effects of social environment on the likelihood of becoming a precocious forager. $n = 50$ (trial 1) or 60 (trial 2) bees per group. *Bars in the same trial topped with different letters* are significantly different from each other (see text, 2-tailed *G*-tests, performed on actual frequencies of foragers and non-foragers). Trial numbers refer to those in Fig. 1

Again, all three pair-wise comparisons were highly significant (single-screen versus double-screen: $G = 9.11$, $P < 0.005$; double-screen versus control: $G = 46.8$, $P < 0.0001$; single-screen versus control: $G = 7.38$, $P < 0.007$).

Experiment 3: are bees with their mandibular glands removed less inhibitory towards younger bees?

Bees with their mandibular glands removed were less able to inhibit the behavioral development of younger nestmates. In four out of five trials, the colony that received transplants of older bees with their mandibular glands removed had a significantly higher proportion of resident bees as precocious foragers than did the colony receiving transplants of control bees (Fig. 4). We have no explanation for the reversed effect in Trial 2.

In two out of two trials in which sham-operated bees were included, the colony receiving transplants of sham-operated bees did not differ in the proportion of precocious foragers relative to the colony receiving control bees (Fig. 4). In addition, in both trials these two colonies had significantly less precocious foragers relative to the colony transplanted with bees with their mandibular glands removed. These results indicate that social inhibition occurred in colonies that received either sham-operated or control bees, but not in those that received bees with their mandibular glands removed.

In one trial we also studied a colony that received no transplant of older bees as another type of control (Fig. 4). This colony produced similar number of precocious foragers to the colony that received bees with their mandibular glands removed, indicating a virtual absence of social inhibition when mandibular glands are removed.

Discussion

This study provides new information on social control of division of labor in honey bee colonies. The results of Experiments 1 and 2 suggest that physical contact is required for older bees to inhibit endocrine and behavioral development in younger bees. Bees reared in double-screen cages resembled bees reared in single-cohort colonies (in the absence of older bees) or bees reared in complete isolation in the laboratory (Huang and Robinson 1992), even though they were in typical colonies, with a diverse age structure that presumably included many older bees. They had forager-like rates of JH biosynthesis and JH titers (see Huang et al. 1991, 1994; Huang and Robinson 1992, 1996), and exhibited a high likelihood of foraging precociously. In contrast, control bees in Experiments 1 and 2, reared in the same colonies in which double-screen caged bees were reared, exhibited normal endocrine and behavioral development. They had nurse-like rates of JH biosynthesis and JH titers, and exhibited a low likelihood of foraging precociously.

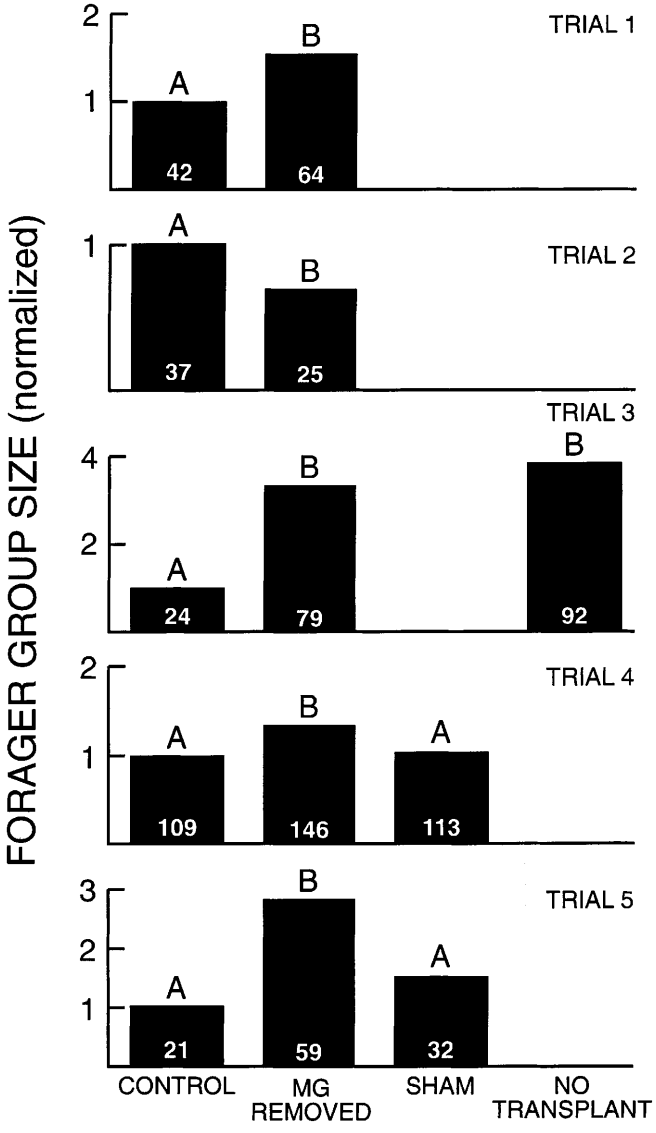

Fig. 4 Effects of mandibular gland removal on inhibitory potency of older bees. For graphical purposes the number of foragers in each colony was normalized by dividing it by the number of foragers in the control colony in each trial. *G*-tests were performed on actual frequencies of foragers (*inside each bar*) and non-foragers. Larger forager group size indicates lower inhibitory potency of the transplanted bees

Results for bees reared in single-screen cages in Experiments 1 and 2 were not as consistent as for those reared in double-screen cages. These results suggest that social inhibition, though possible through a single-screen, depends on the nature and level of interaction that occurs between caged bees and other colony members, and this varied from trial to trial. Increased levels of social interaction between caged bees and colony members, particularly older colony members, would be predicted to result in greater social inhibition. Consistent with this suggestion, when brood frames (trial 1) or honey storage frames (trial 2) were put next to single-screen caged bees, they showed more similar rates of JH biosynthesis to bees in double-screen cages. This physical arrangement may have resulted in more younger bees in the vicinity of the caged bee (Seeley 1982). This may have led to more interactions between caged bees and younger bees, which are thought to be less inhibitory than are older bees. In trials 3–5, no brood or newly extracted honey frames were near the single-screen caged bees and they showed lower rates of JH biosynthesis, presumably due to increased contacts with older bees and more inhibition. In trial 1 workers in single-screen cages also were provided with their own food, which perhaps reduced their interactions with bees outside the cages. However, detailed observations of bees of known age are required to confirm whether the rearing techniques used in this study indeed influenced social interactions in the ways envisioned here.

Because control bees were uncaged in the hive, their lower JH and slower rates of behavioral development relative to the caged bees could be due either to differences in social inhibition as we hypothesized, or to differences in freedom of movement and ability to access other components of the hive. We consider the second possibility less likely for two reasons. First, Huang and Robinson (1992) showed that workers reared in the laboratory in the same enclosures, but in different group sizes, showed different rates of JH biosynthesis. This result suggests that social interactions are potent in influencing endocrine function in the honey bee. Second, in the present study we showed that there were differences between bees reared in single-screen and double-screen cages, even though their movements were restricted similarly. These differences were less marked relative to control bees; nevertheless, differences existed between single-screen and double-screen bees in rates of JH biosynthesis in three out of five trials and in the likelihood of becoming precocious foragers in two out of two trials. There were no differences between these two groups in JH titers, but comparisons with control bees suggest that this assay was less sensitive than the other two, at least with the sample sizes used here.

We argued in the Introduction that it is reasonable to hypothesize that social inhibition of behavioral development in honey bee colonies involves a chemical component. The results of Experiments 1 and 2 do not prove or disprove this hypothesis; however, if social inhibition does involve a pheromone, the results of these experiments demonstrate that it is not a volatile pheromone, but rather a contact pheromone. If this is the case, the contact pheromone must be transmitted during food transfer, antennal contact, or licking. This is because these are the only three behavioral interactions possible between bees reared in single-screen cages and colony members. We are certain that bees reared in single-screen cages were fed during their 6- to 8-day confinement period because individually isolated worker bees die without food within 24 h. This means that they also engaged in mutual antennation, because this apparently always is associated with food exchange (Free 1956). Another possibility is that the inhibitory signal is

not chemical, but rather some sort of mechanosensory signal associated with the behaviors of antennal contact, food transfer, or licking.

The results of Experiment 3 demonstrate that removal of mandibular glands renders older bees less inhibitory towards younger bees. This is a specific effect of gland removal because sham-operated bees did not become less inhibitory. The numbers of younger bees that foraged precociously from the control colonies, i.e., those receiving intact older bees as transplants, were higher than in a previous study (Huang and Robinson 1992). This difference may have occurred because some of our "old" transplants were not yet foragers (transplants were selected solely on the basis of age, not behavior), while in previous experiments (Huang and Robinson 1992) bona fide foragers were used. This was necessary because removal of the mandibular glands from foragers caused high mortality, so we operated on younger bees, allowed them to age, and then used them as transplants. Perhaps waiting for all of them to develop into foragers, which would then have allowed us to use intact foragers as controls, would have resulted in the detection of even stronger effects of mandibular gland removal. The apparent difference between results from our control colonies and the study by Huang and Robinson (1992) suggests that there is an increase in inhibitory potency of bees with increased behavioral development, as hypothesized previously (Huang and Robinson 1992).

The results of Experiment 3 suggest that the worker mandibular glands are involved in the inhibition of behavioral development by older individuals in a honey bee colony. The mandibular glands of the queen are already known to produce a contact pheromone that has a number of effects on worker bees, including the inhibition of JH biosynthesis (Kaatz et al. 1992), JH titers, and rate of behavioral development (Pankiw et al. 1998). Moreover, worker mandibular glands contain several compounds that are structurally similar to components of QMP (Plettner et al. 1993). QMP is distributed first by licking, antennation, and perhaps feeding (Naumann 1991); these types of social interaction were implicated in Experiments 1 and 2 as being important in the inhibitory process. But despite this compelling circumstantial evidence, we have only demonstrated that removal of the mandibular glands causes older bees to be less inhibitory. It is possible that removal of the mandibular glands somehow affects an inhibitory behavior or the transmission of inhibitory chemicals produced elsewhere. Experiments that seek to determine whether extracts of the mandibular glands of older workers are inhibitory to younger bees are in progress.

Inhibition of young bees by older nestmates is a central component of a descriptive model proposed by Huang and Robinson (1992) to explain how colony age demography can influence age-related division of labor via social interactions. According to this model, JH is an intrinsic "activator" that promotes behavioral development, and there is a social "inhibitor" that suppresses JH and behavioral development. The activator and inhibitor are hypothesized to be coupled, such that older bees, with high JH titers, either produce or transfer more inhibitor than do younger workers. The results of this study are consistent with the activator-inhibitor model in that they demonstrate that changes in the social environment can influence rate of behavioral development. However, because in Experiments 1 and 2 we did not observe which bees were interacting with the caged bees, our results do not provide confirmation of the hypothesis that older bees are the source of an inhibitor.

The activator-inhibitor model focuses on a single inhibitor produced by workers, but it is becoming increasingly clear that the control of division of labor in honey bee colonies is a complex process, which involves other components. As mentioned above, QMP clearly has been identified as an inhibitor of endocrine and behavioral development (Kaatz et al. 1992; Pankiw et al. 1998). In addition, brood pheromone, a chemically defined mixture of seven esters produced by larval and pupal honey bees, inhibits JH titers and also delays behavioral development (Y. Le Conte and G.E. Robinson, unpublished observations). Future experiments are necessary to determine how these factors interact in an overall control system. At present the activator-inhibitor model provides a heuristic framework for the study of division of labor but more complex models will no doubt be necessary.

Our results suggest the existence of a worker-produced primer pheromone that affects the behavioral development of individuals and thus the organization of the entire honey bee colony. Regulation of endocrine and behavioral development by primer pheromones has been studied extensively in mammals (reviewed by McClintock 1983; Vandenbergh 1983, Vandenbergh and Coppola 1986; Schank and McClintock 1992), but mammalian primer pheromones have not been conclusively identified (Price and Vandenbergh 1992; cf. Novotny et al. 1986). Only two primer pheromones, from any animal species, have been chemically characterized: a steroid sex pheromone that synchronizes reproductive development in goldfish (Dulka et al. 1987), and honey bee QMP (Winston and Slessor 1992). While further experiments are necessary to fully identify and characterize the source of social inhibition in worker honey bees, the possibility of a worker-produced primer pheromone provides a rich opportunity for the study of endocrine-mediated behavioral development and chemical communication in general.

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