Behav Ecol Sociobiol (1996) 39 : 147–158 © Springer-Verlag 1996

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Regulation of honey bee division of labor by colony age demography

Received: 8 November 1995/Accepted after revision: 10 May 1996

Abstract The age at which worker honey bees begin foraging varies under different colony conditions. Previous studies have shown that juvenile hormone (JH) mediates this behavioral plasticity, and that worker-worker interactions influence both JH titers and age at first foraging. These results also indicated that the age at first foraging is delayed in the presence of foragers, suggesting that colony age demography directly influences temporal division of labor. We tested this hypothesis by determining whether behavioral or physiological development can be accelerated, delayed, or reversed by altering colony age structure. In three out of three trials, earlier onset of foraging was induced in colonies depleted of foragers compared to colonies depleted of an equal number of bees across all age classes. In two out of three trials, delayed onset of foraging was induced in colonies in which foragers were confined compared to colonies with free-flying foragers. Finally, in three out of three trials, both endocrine and exocrine changes associated with reversion from foraging to brood care were induced in colonies composed of all old bees and devoid of brood; JH titers decreased and hypopharyngeal glands regenerated. These results demonstrate that plasticity in age-related division of labor in honey bee colonies is at least partially controlled by social factors. The implications of these results are discussed for the recently developed "activator-inhibitor" model for honey bee behavioral development.

Key words Age demography · Behavioral development · Behavioral plasticity · Juvenile hormone · Temporal polyethism

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Introduction

Insect societies must cope with changes in colony population size and age structure that are associated with colony development, time of year, food availability, predation pressure, and climatic conditions. Not surprisingly, age-related systems of division of labor in colonies of social Hymenoptera can be highly flexible (reviewed by Robinson 1992). Despite the typical pattern of "temporal polyethism", in which young workers perform tasks in the nest while older workers forage, individuals can accelerate, delay, and even reverse their behavioral development in response to changes in their colony's internal and external environment. The adaptive significance of plasticity in temporal polyethism is that it enables a colony to grow, develop, and reproduce despite changing conditions.

The regulation of plasticity in age-related division of labor is a puzzle, particularly in species with larger colonies, because it is unlikely that individual workers can monitor the needs of the whole colony and then adjust behaviors accordingly. There also is no evidence for centralized control, in which some individuals perceive all or most of the colony's requirements and cause other colony members to switch tasks (Wilson and Hölldobler 1988). In social insects with small colonies, the queen does perceive colony needs and modulate worker activity levels (Gamboa et al. 1990), but it is not known whether she can specifically direct the activities of workers from one task to another.

An approach that integrates behavioral biology, endocrinology, genetics, and developmental biology has been developed to explore the regulation of plasticity in age-related division of labor in honey bee colonies (reviewed by Robinson 1992; see also Robinson et al. 1989; Huang and Robinson 1992; Giray and Robinson 1994). The goal is to understand the mechanisms that regulate plasticity in honey bee behavioral development in general, but past and current research focuses on the age at which foraging begins because

this is the most pronounced transition in a honey bee's life, and thus can be studied most easily.

Juvenile hormone (JH) is involved in the regulation of behavioral development in adult worker honey bees (reviewed by Robinson 1992; see O'Donnell and Jeanne 1993 for evidence of JH-mediated temporal polyethism in a polybiine wasp). JH blood titers typically increase with age; they are low in bees that work in the hive performing brood care ("nursing") 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 and Robinson 1995). Treating bees with JH, JH mimic, or JH analog induces precocious foraging (Jaycox 1976; Jaycox et al. 1974; Robinson 1985, 1987; Robinson and Ratnieks 1987; Robinson et al. 1989; Sasagawa et al. 1989). Plasticity in behavioral development also appears to be mediated by JH. 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 titer (Robinson et al. 1989, 1992). These results support the idea that changes in colony conditions act on the endocrine system to cause changes in temporal polyethism (Robinson 1987).

Huang and Robinson (1992) tested two hypotheses to explain how worker bees acquire information on changing colony conditions that results in changes in behavioral development. The "worker-worker" hypothesis predicts that this information is acquired while adult workers interact with one another, perhaps via trophallaxis (Ribbands 1952; Free 1965). The "worker-nest" hypothesis predicts that this information is acquired while interacting with the nest and its contents, such as the colony's food stores and brood, perhaps during bouts of "patrolling" behavior (Lindauer 1952). Evidence for worker-worker effects was obtained in two independent tests (Huang and Robinson 1992). In a series of laboratory experiments, bees reared for 7 days in complete isolation exhibited precociously high rates of JH biosynthesis and precocious foraging, while bees reared in small groups showed more normal age-related patterns. In a set of field experiments, transplants of groups of foragers from a typical colony to "single-cohort colonies", initially composed of all 1-day-old resident bees, inhibited precocious foraging in the residents. This inhibitory effect occurred even if the nest entrance was closed for several days (prior to behavioral observations), thus denying the foragers the opportunity to change the nest environment by bringing in fresh nectar and pollen. This inhibitory effect was also detected when soldiers (older bees involved in colony defense; see Breed et al. 1990), rather than foragers, were transplanted. Results of the transplant experiments are consistent with the laboratory experiments and indicate that younger bees are inhibited from developing in the presence of older bees. These findings do not exclude the possibility that honey bee behavioral development also is influenced by as yet undetected worker-nest effects, but they clearly implicate colony age demography as a key factor in this process.

Huang and Robinson (1992) proposed an "activator-inhibitor" model to explain how colony age demography can influence temporal division of labor via worker-worker interactions. According to this model, JH is thought to be an intrinsic "activator" that promotes behavioral development. An "inhibitor", an as yet unidentified factor(s) transferred among workers, suppresses JH and behavioral development. They further proposed that the activator and inhibitor were coupled, such that older bees, with high JH titers, either produce or transfer more inhibitor than younger workers.

These empirical and theoretical analyses highlight the potential importance of colony age demography in the control of temporal division of labor in honey bee colonies. In this study we test this hypothesis. We predicted that worker behavioral or physiological development can be accelerated, delayed, or reversed, solely by manipulating colony age structure.

In experiment 1 old bees were depleted from colonies, and precocious foraging was predicted. In experiment 2 all bees were confined to their colonies, thus changing the "effective" colony age structure. We predicted that this manipulation would cause delayed foraging, because younger bees would be more likely to encounter the (confined) old bees. In experiment 3 all young bees were removed from colonies and hormonal reversion was predicted. Previous studies already have shown that similar manipulations of the adult bee population result in such reversion (Page et al. 1992; Robinson et al. 1992 and references therein). However, these studies were conducted with colonies that contained brood, suggesting that reversion occurs in response to exposure to larvae that need to be fed. We looked for evidence of reversion in colonies without brood, because we wished to determine whether reversion can also occur solely in response to a dramatic alteration of colony age demography. Since the need to use broodless colonies precluded direct sampling of nursing bees, we tested this prediction by exploiting previously known tight linkages between behavioral development, exocrine gland status, and JH levels in honey bees (reviewed by Winston 1987; Robinson 1992).

Materials and methods

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).

Triple-cohort colonies

Each trial of experiments 1 and 2 was performed with a pair of "triple-cohort colonies" (Giray and Robinson 1994). Triple-cohort colonies were composed of known numbers of "young", "middle-age", and "old" bees (see below). Each colony contained less than 2,000 workers, which is smaller than typical colonies with 15–40,000 bees (Seeley 1985). Workers in such small colonies, however, can show normal ontogeny of behavior (Giray and Robinson 1994). Using these colonies, we could precisely vary colony age demography while keeping constant other potentially important variables, such as colony population size and genetic structure.

Young bees ($\leqslant$ 24 h post emergence, designated as 1 day old) were obtained by placing combs of sealed brood in an incubator at 34°C and 80% relative humidity. They were marked with a spot of paint (Testor's PLA) on the dorsal surface of the thorax. Middle-age bees also were marked at 1 day of age (with a different color) and introduced into a "nursery" colony (a typical colony with a population of about 18,000 bees of all ages); they were collected from the nursery colony at 10–12 days of age. Middle-age bees were the focal bees for measurements of behavioral development in experiments 1 and 2.

Old bees were foragers, collected by blocking the entrance of a typical colony with a mesh screen and vacuuming individuals that returned with pollen on their corbiculae. Their ages were not known, but an extensive literature indicates that foragers are the oldest bees in typical colonies (reviewed by Michener 1974; Winston 1987). Foragers were collected from a colony located ~10 km away from where the triple-cohort colony was established to minimize their return to the natal colony.

Middle-age bees and foragers were vacuumed directly into a hive that had a portable vacuum cleaner attached to it (Giray and Robinson 1994). Previous studies (Giray and Robinson 1994) suggest that behavioral development is not affected by vacuuming in this way.

Bees for the young and middle-age cohorts emerged from brood taken from three to five source colonies. To ensure that each member of a triple-cohort colony pair had a similar genetic composition, bees were thoroughly mixed when marked or collected from nursery colonies. Each member of a triple-cohort colony pair was headed by a naturally mated, sister queen, less than one year old. The queen remained caged during the entire experiment, so amount of brood reared would not be a variable. Each member of a triple-cohort colony pair also had the same resources: one empty frame and one frame that was one-half filled with honey and one-third filled with pollen.

Experiment 1: Effects of forager depletion on behavioral development

Foragers were collected from the "forager-depleted" colony for 30 min as they returned to the hive and were then freeze-killed. Bees were determined to be foragers if they returned with pollen loads in their corbiculae or had a distended abdomen (signifying nectar or, less likely, water foraging). Forager depletion occurred for 3–9 days starting 1 day after colony establishment (for 3, 4, and 9 days, in trials 1, 2, and 3, respectively).

A "control" colony in each triple-cohort colony pair was depleted of an equal number of bees as the forager-depleted colony, but the depletion was distributed equally across the three age cohorts. This enabled us to distinguish between possible effects of a reduced population of foragers as opposed to a reduced population of workers in general. We collected foragers at the entrance and bees from the two younger cohorts from within the hive. Both the forager-depleted and control colonies were depleted on the same days, the control colony immediately after the forager-depleted colony so that an equal number of bees could be removed from the paired colonies.

Effects of forager depletion were assessed by determining the proportion of bees from the focal cohort that developed into foragers. Returning foragers were collected at the hive entrance when they were 14–32 days old, during daily 30-min observation sessions (trial 1, 14–25 days old; trial 2, 17–24 days old; trial 3, 26–32 days old). The date of the observations was based on the first appearance of focal bees as foragers. Upon termination of each trial, the colony was freeze-killed (after anesthetization by CO_2), and the number of bees in each cohort was determined (trial 2 and 3). For trial 1, the census was conducted by vacuuming and counting individual bees of each cohort without anesthetization, because most of these bees were then used for trial 2.

The same pairs of triple-cohort colonies were used in both trials 1 and 2 with forager-depleted and control colonies alternated to control for queen effects or unaccounted for colony effects. Colonies in trial 1 were each composed of 500 bees 1–2 days old, 500 bees 10–12 days old, and 500 foragers. A total of 225 foragers was removed from the forager-depleted colony in trial 1 (Due to rain, 100 foragers were collected from inside the hive on 2nd and 3rd day of depletion; they were identified as such by the absence of any paint markings, as the other two cohorts were marked). The colonies in trial 2 were each composed of 500 bees 1 day old, 400 middle-age bees (12–13 day old bees, from the youngest cohort in trial 1), and 270 old bees (22–24 day old, originally from the middle-age cohort in trial 1). A total of 174 bees was removed (either only foragers or bees from all three cohorts, as above) over a 4-day period following colony establishment.

A new pair of triple cohort colonies was established for trial 3, from a different set of source colonies than those used for trials 1 and 2. Each colony was composed of 500 bees 1 day old, 600 middle-age bees (16 days old), and 500 foragers. A total of 433 bees was removed from each colony over a 9-day period (either only foragers or bees from all three cohorts, as above). Forager-depleted and control colonies were not reversed and used for a fourth trial because of weather conditions. Furthermore, the results of trials 1 and 2 suggested that this was not warranted.

Experiment 2: Effects of colony confinement on behavioral development

We confined foragers to their hive by simulating rain. This was accomplished by using a timer-controlled lawn sprinkler to sprinkle water on the entrance of the hive (Schneider et al. 1986b). The water was turned on from 0530 to 2000 hours every day, for 3, 4, and 9 days, in trials 1, 2, and 3, respectively. Bees were able to take defecation flights daily from 2000 hours to dusk after the sprinkler was turned off, but casual observations throughout the day indicated that when the sprinkler was on they were otherwise confined to the hive. The control colonies in this experiment were not manipulated.

Experiment 2 was performed simultaneously with experiment 1, and the triple-cohort colonies used in a given trial of experiment 1 and 2 were established at the same time, from the same source colonies, and were identical in population size and age demography. As in experiment 1, the same pair of colonies was used in both trials 1 and 2 but switched between treatment and control. A new pair of triple cohort colonies was established for trial 3. As in experiment 1, the composition of each colony was: 500 bees 1–2 days old, 500 bees 10–12 days old, and 500 foragers in trial 1; 500 bees 1 day old, 400 middle-age bees (12 to 13-day-old bees, from the youngest cohort in trial 1), and 270 old bees (22–24 day old, originally from the middle-age cohort in trial 1) in trial 2; and 500 bees 1 day old, 600 middle-age bees (16 days old), and 500 foragers in trial 3. As in experiment 1, returning foragers were collected at the hive entrance when they were 14–32 days old, during daily 30-min observation sessions (trial 1, 14–25 days old; trial 2, 17–24 days old; trial 3, 26–32 days old). Censuses were performed as in experiment 1.

Experiment 3: effects of removing all young bees on endocrine and exocrine correlates of behavioral development

We removed all young bees from a typical colony, effectively making a new colony composed entirely of foragers or presumed foragers. This was accomplished by collecting 2000 pollen or nectar foragers or bees with worn wings, after temporarily obstructing the hive entrance with a piece of 8-mesh hardware cloth. The bees were vacuumed directly into a small hive containing one comb of honey and pollen, one empty comb, and a mated queen which was caged throughout the experiment. When the 2,000 bees were collected, the parent colony was closed and moved about 10 km away. The "old-bee colony" was moved to the location of its parent colony to minimize the loss of bees to other colonies in the apiary through "drifting". Because some pre-foragers may have joined the old-bee colony as they returned from an orientation flight, only bees with worn wings were sampled. Worn wing is a robust indicator of forager status (Breed et al. 1990).

Two physiological parameters associated with behavioral development were measured: JH blood titers and the size of the brood food-producing hypopharyngeal glands. Associated with behavioral reversion is a drop in JH titers to levels of young nurse bees (Robinson et al. 1992), and a regeneration of the hypopharyngeal glands (Milojévic 1940). Young bees generally have large hypopharyngeal glands (King 1933; Hassanein 1952; Haydak 1957) with high rates of protein synthesis (Brouwers 1982, 1983; Huang 1990), while foragers have smaller, less active, glands that produce invertase (to convert nectar into honey) (Simpson 1960). Furthermore, high JH titers are associated with hypopharyngeal gland degeneration; JH treatments cause a reduction in hypopharyngeal gland size (Rutz et al. 1974; Jaycox et al. 1974; Jaycox 1976).

This experiment was performed three times. Blood samples of foragers ($n = 10$) were obtained from each parent colony just before the old-bee colonies were established. Then foragers and "reverted bees" (see below) were sampled 1, 2, and 4 days after young bee removal (for trial 2, days 1, 3, and 4).

Because the colony lacked brood, bees were sampled by placing a comb containing mostly 3- to 5-day-old larvae in the old-bee colony for 5 min; workers with their heads and thoraces in a cell containing a larva were collected. We call them "reverted bees", rather than "reverted nurses" as in previous studies (Page et al. 1992; Robinson et al. 1992), because it is likely that their brief exposure to brood would preclude most, if not all, real nursing behavior. The brood comb was removed immediately after the reverted bees were collected.

To test that the brood did not attract a group of old bees that for some reason already had unusually low JH titers (unlikely for foragers in the summer; see Huang and Robinson 1995), the first sample of reverted bees was collected 2.5 h after young bees were removed in trial 1. This test was made more stringent in trial 3 by collecting this sample of bees just 10 min after colony establishment. A comparable sample was not collected in trial 2.

Trial 3 differed from trials 1 and 2 in two other ways. First, the old-bee colony in trial 3 was composed of 4,000, rather than 2,000 foragers. Second, the brood comb inserted to identify reverted bees was taken from the parent colony, rather than from a foreign colony, to eliminate the likelihood that the 5-min presentation of brood attracted bees with low JH titers because it was foreign.

Hypopharyngeal gland size was measured for both foragers and reverted bees. Bees were collected 7 and 22 days after colony establishment, in trials 1 and 2, respectively, and 0, 1, 2, 4, and 9 days after colony establishment in trial 3. In trial 3 the same bees were used for both hypopharyngeal gland and JH measurement. We predicted a regeneration of the hypopharyngeal glands, even though there was no brood for workers to feed.

Measurement of JH titer

After collection, bees were immobilized on ice for 10–30 min until blood (hemolymph) was taken. Blood (0.7–7.2 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.

A chiral-specific radioimmunoassay (RIA) (Hunnicutt et al. 1989) was used to measure the JH III titer. JH III is the only JH homolog found in honey bees (Hagenguth and Rembold 1978; Robinson et al. 1991; Huang et al. 1994). This assay has been validated for adult worker honey bees by Huang et al. (1994). Previous results (Huang et al. 1994; Huang and 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 et al. (1994) and Huang and Robinson (1995), but the data from the standard curve were fitted to a five-parameter model (Prentice 1976):

$$\text{DPM bound} = \frac{A - D}{\left[1 + \left(\frac{\log (JH)}{C}\right)^{B}\right]^{E}} + D$$

A–E are the five parameters to be fitted, "DPM bound" is the radioactivity bound to antiserum (dependent variable) and "JH" is the JH amount (independent variable), respectively. Curve fitting was done with a non-linear regression (KaleidaGraph, Synergy Software). JH titers were then calculated by reversing the above formula, using the five parameters in an Excel spreadsheet (Microsoft Corp.). All solvents were HPLC grade, obtained from either EM Science, Fisher Scientific, or J.T. Baxter Chemical Co. Glassware was baked at 500°C for 3.5 h prior to use to minimize JH adsorption (Strambi et al. 1981). The sensitivity of the RIA is about 10 pg (racemic) JH III per sample. Inter- and intra-assay variation for JH determinations was 9.2% and 10.6%, respectively ($n = 10$).

Measurement of hypopharyngeal glands

Glands were dissected from the head in bee saline (see Huang et al. 1994) after obtaining hemolymph samples. The diameter of one representative gland acinus per bee was measured at × 60 magnification. A previous study using this method (Huang and Robinson 1995) yielded results comparable to those of Crailsheim and Stolberg (1989), in which ten acini were averaged. Acinus diameter is used routinely as an index of hypopharyngeal gland size (King 1933; Hassanein 1952; Fergusson and Winston 1988; Crailsheim and Stolberg 1989; Huang et al. 1994), because the peculiar shape of the gland, with hundreds of acini attached to a central duct, precludes simple measurement of total gland size. When brood is present in the colony, larger glands generally indicate higher protein secretory activity (Knecht and Kaatz 1990).

Statistical analyses

In experiments 1 and 2, differences between treatment and control colonies in the distribution of foragers and non-foragers from the focal cohort were analyzed with one-way *G*-tests (Sokal and Rohlf 1981). Correlation and regression analyses were used to determine the relationship between the proportion of old bees in a colony and the proportion of bees in the focal cohort that initiated foraging for all colonies used in experiments 1 and 2 ($n = 12$). In experiment 3, differences in JH titers and hypopharyngeal gland size were analyzed with two-tailed *t*-tests (with unequal variance, when necessary; see Steel and Torrie 1980). Differences in hypopharyngeal gland sizes in trial 3 were analyzed by two-way ANOVA using SAS

(SAS Institute 1985), with behavioral status and time since removal of young bees as independent variables. Regression analysis also was used to assess changes in hypopharyngeal gland size over time in trial 3. Means ± SE are given throughout this paper.

Results

Experiment 1: effects of forager depletion on behavioral development

Depleting a colony of foragers resulted in accelerated behavioral development. In three of three trials, a significantly higher proportion of bees from the focal (middle-age) cohort initiated foraging in the forager-depleted colony compared to the control colony (Fig. 1, experiment. 1).

Experiment 2: effects of colony confinement on behavioral development

Confining foragers to colonies by simulated rain resulted in delayed behavioral development. In two of three trials, a significantly lower proportion of bees from the focal cohort initiated foraging in the forager-confined colony compared to the control colony Fig. 1, experiment. 2).

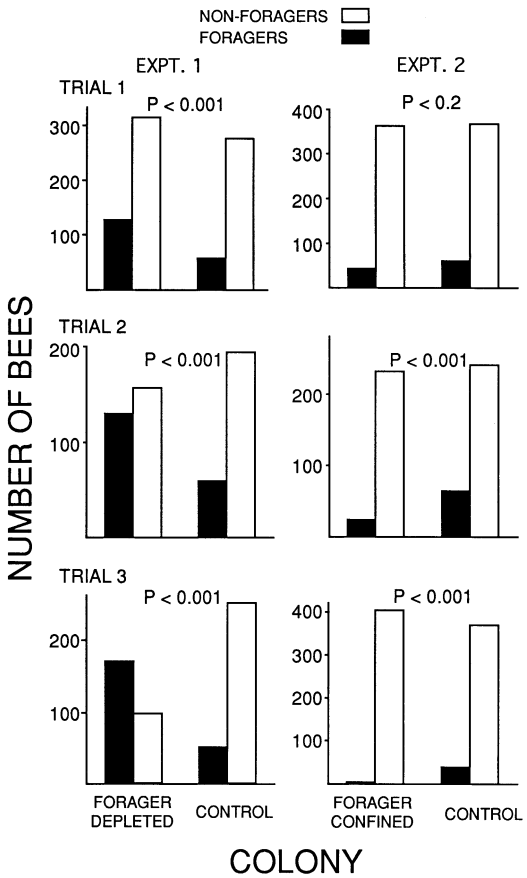

Fig. 1 Number of bees from the middle age cohort in triple cohort colonies that did or did not initiate foraging in response to alterations in colony age demography. Experiment 1, forager depletion. Equal numbers of bees were taken from control colonies, but distributed equally across all three age cohorts. Experiment 2, colony confined with simulated rain. Control colonies were unmanipulated

Relationship between colony age demography and behavioral development

To gain further insight into the relationship between colony age demography and rate of behavioral development, we plotted for each colony the percentage of bees from the focal cohort that initiated foraging as a function of the percentage of old bees (the oldest cohort) in the colony (censused at the end of each trial of both experiments 1 and 2). For each trial, these two variables were significantly negatively correlated (trials 1 and 2, $r = 0.98$, $P < 0.05$; trial 3, $r = 0.99$; $P < 0.01$; $n = 4$ colonies per trial). Figure 2 depicts these results for all three trials together ($n = 12$ colonies). There was a significant negative regression with a slope of -1.03, indicating that a 1% decrease in the percentage of old bees caused a roughly 1% increase in the proportion of young bees initiating foraging during the experimental period.

Experiment 3: effects of removing all young bees on endocrine and exocrine correlates of behavioral development

Bees sampled from old-bee colonies had low JH titers, even in the absence of brood. In three out of three trials, reverted bees had significantly lower JH titers 1 day after the removal of young bees compared to foragers sampled just prior to young bee removal (Fig. 3, insets).

JH titers apparently started dropping soon after young bees were removed. In trial 1 there was already

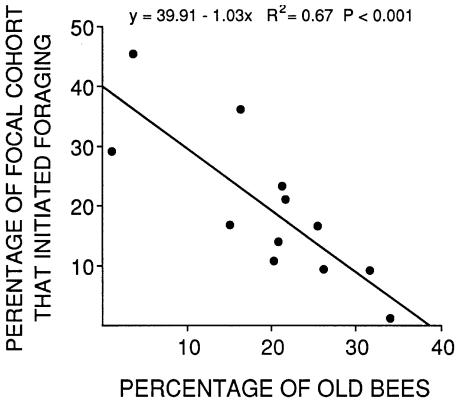

Fig. 2 Relationship between colony age demography and worker behavioral development. Behavioral development was quantified by calculating the percentage of the focal cohort that initiated foraging. Percentage of old bees was calculated from censuses taken at the end of each trial of experiment 1 and 2. Each *point* represents one of the 12 colonies used in these experiments

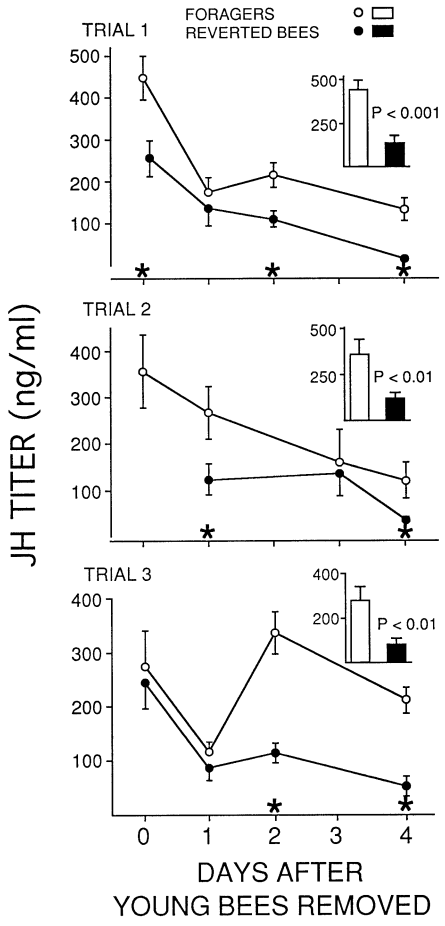

Fig. 3 Changes in mean (±SE) blood titer of JH for foragers and reverted bees in response to removal of young bees, in the absence of brood (n = 10 individual bees per point). Reverted bees on day 0 were collected 2.5 hour in trial 1, and 10 minutes in trial 3, after young bees were removed. A comparably early sample was not taken in trial 2. *Inset*: differences in JH titer between foragers on day 0, just before young bees were removed, and reverted bees on day 1. P values give the results of two-tailed t-tests (with unequal variance in trials 2 and 3). Significant differences ($P < 0.05$, t-tests) between foragers and reverted bees on a given day are indicated by an *asterisk*

a significant difference ($t = 2.83$, $P < 0.01$) between foragers sampled just before old bees were removed and reverted bees sampled 2.5 h later (day 0 comparison, Fig. 3). An alternative interpretation of this result is that our sample was drawn from a group of foragers that for some reason already had lower JH titers. This possibility, however, is not consistent with two other results. First, in trial 3, we collected bees just 10 min after colony formation, and these bees had high JH titers, typical of foragers. There was no difference in JH titers between these bees and those sampled just prior to colony formation ($t = 0.25$, $P > 0.5$, day 0 comparison, Fig. 3). Second, in all three trials reverted bees continued to show a drop in JH over time, suggesting an ongoing effect. Continued effects of young bee removal were clearly seen in all three trials, because JH titers were significantly lower on day 4 than on day 0 (trials 1 and 3), or day 1 (trial 2) ($t = 3.13$, 2.56, 5.82, $P < 0.01$, 0.05, 0.001, for trials 1, 2, and 3, respectively). These results suggest that low JH in reverted bees was a consequence of changes in colony age demography. Results from trial 3 also argue against the possibility that the drop in JH was due to an exposure to foreign brood, because the brood used for the 5-min presentation was from the old-bee colony's parent colony.

Bees that continued foraging in the old-bee colonies also showed a decrease in JH titers in all three trials (Fig. 3). These foragers had significantly lower titers on day 1 compared with the starting levels on day 0 in trials 1 and 3 ($t = 4.3$ and 2.31, $P < 0.001$ and 0.05, respectively). This difference is not significant for trial 2 ($t = 0.92$, $P > 0.3$). Despite the drop in JH in foragers, foragers and reverted bees generally showed different endocrine profiles. Foragers had significantly higher JH titers than reverted bees on days 1–4, except for day 1 for trials 1 and 3, and day 3 for trial 2. JH titers were more variable for foragers than for reverted bees. For example, in trial 3 the JH titers of foragers increased significantly ($t = 5.01$, $P < 0.001$) on day 2 to a level similar to day 0, which was just before young bees were removed. This rebound was not detected in

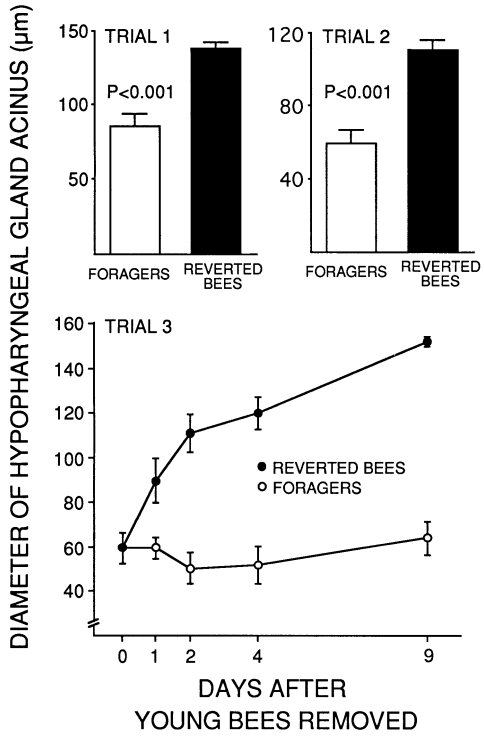

Fig. 4 Changes in mean (± SE) hypopharyngeal gland size for foragers and reverted bees in response to removal of young bees in the absence of brood (n = 10 individual bees per point). Bees were sampled on day 22 in trial 1 and on day 7 in trial 2. For trial 3, the same bees were also used for JH analyses in Fig. 3, trial 3. A quadratic ($Y = 65.21 + 21.9\ X - 1.6\ X^2$, $F = 24$, $P < 0.0001$) relationship best describes the change of size in nurses, while a straight line with slope 0 (slope = 1.1 ± 1.1, $t = 1$, $P > 0.3$) best describes the forager data, indicating no change with time

the other two trials. Inter-colony variation in the JH titers of foragers has been observed previously in more typical colonies (Robinson et al. 1989, Z.-Y. Huang and G.E. Robinson, unpublished work).

In three out of three trials, there was a significant increase in hypopharyngeal gland size in reverted bees, even in the absence of brood (Fig. 4). In trial 3, the only trial in which this variable was analyzed over time, two-way ANOVA revealed significant differences in hypopharyngeal gland size between foragers and reverted bees ($F = 120.96$, $P < 0.001$) and among sampling times ($F = 13.13$, $P < 0.001$). Hypopharyngeal gland size increased significantly over time in reverted bees; a quadratic relationship best described these data (see Fig. 4; $F = 24.3$, $P < 0.01$). Hypopharyngeal gland size did not increase over time in foragers ($F = 0.98$, $P = 0.33$).

Discussion

The results of this study demonstrate that plasticity in age-related division of labor in honey bee colonies is socially regulated. Behavioral development can be accelerated, delayed, or reversed, solely by manipulating colony age structure.

In experiment 1, behavioral development was accelerated in response to forager depletion. We hypothesize that this was because young workers were exposed to fewer older bees. Because forager depletion probably also caused a decrease in the amount of fresh nectar and pollen brought into the nest, the observed accelerated development could also have been due to changes in the nest environment. However, this alternative explanation is not consistent with the results of experiments 2 and 3. In experiment 2, there was no fresh nectar and pollen entering the colony because foragers were confined for several days. Yet delayed, rather than accelerated, behavioral development was observed in this experiment. Huang and Robinson (1992) also found that confined foragers can inhibit the behavioral development of younger bees. Confining foragers to colonies by simulated rain resulted in delayed behavioral development in two of three trials in experiment 2. In these two trials, the confinement lasted for 4 and 9 days, respectively, and there was active foraging in the control colonies. In trial 1, there was no significant difference in the proportion of bees from the focal cohort that initiated foraging in the forager-confined colony relative to the control colony. Compared to trials 2 and 3, the confinement period in trial 1 was shorter (only 3 days). In addition, foraging activity during the time the old bees were confined was severely curtailed in the control colonies, due to cool, rainy weather (Fig. 1 depicts number of foragers during the post-confinement period only). This in effect eliminated any differences between control and confined colonies, because foragers in control colonies were also confined by inclement weather. We suspect that these two differences decreased the effect of the treatment and thus explain the lack of a significant effect in trial 1.

Results of experiments 1 and 2 also showed a strong negative relationship between the percentage of old bees present in a colony and the proportion of the focal cohort that initiated foraging. The slope was close to unity, suggesting a 1:1 ratio between forager "loss" and forager "production". These results suggest that the number of foragers in a colony is precisely regulated, apparently via a negative feedback system.

Results of experiment 3 demonstrate that physiological changes associated with behavioral reversion can occur in response to changes in colony age structure, even in the absence of brood. Although some methodological details and results varied from trial to trial in experiment 3, the main results were consistent in all three trials: lower JH titers and more developed hypopharyngeal glands after the removal of young bees. Decreased JH titers were detected in both reverted bees and foragers, while redevelopment of hypopharyngeal glands was detected only in reverted bees. Since the first foragers sampled as "reverted bees" (just 10 min after young bees were removed) had high levels of JH (and probably degenerated hypopharyngeal glands), it is doubtful that they were responding to the brief exposure to brood in the same way that reverted bees were on subsequent days. Those sampled on subsequent days had low JH and regenerated hypopharyngeal glands and likely were functioning as real (reverted) nurse bees. Studies conducted with glass-wall observation hives have revealed that foragers do occasionally enter brood cells (Darrell Moore, personal communication), but since they have degenerated hypopharyngeal glands (Winston 1987), they probably engage in little, if any brood care behavior. These considerations weaken the conclusions that can be drawn from "reverted bees" sampled immediately after young bees were removed. However, other lines of evidence support the notion that the low JH titers detected in reverted bees on subsequent days represent a drop in JH that is associated with young bee removal. JH titers in reverted bees continued to decline over time, and we were careful to sample only bees with clear evidence of foraging experience (worn wings); numerous studies conducted to date indicate that foragers in the summer have high JH titers (e.g., Robinson et al. 1989; Huang et al. 1994; Huang and Robinson 1995).

While it has been previously shown that reverted nurses can actually regain the ability to feed brood (Rösch 1930; Milojévic 1940), this is the first report of hypopharyngeal gland regeneration in broodless colonies by former foragers. Reverted bees actually were exposed to brood for 5 min before sampled, but it is unlikely that such a brief exposure could provoke the observed changes in JH and hypopharyngeal gland size, especially for the bees sampled on day 1, after only

a single exposure. JH titers can change rapidly (Trumbo et al. 1995), but there are no accounts of endocrine changes within a 5-min time frame, much less one accompanied by a change in gland structure. Our results do not rule out the possibility that factors from the brood can also influence reversion. But brood is not a necessary stimulus for JH titers to decline and for the regeneration of hypopharyngeal glands.

Results from reverted bees in experiment 3 also are consistent with previous studies on the relationship between JH and hypopharyngeal gland development. Low titers are typically associated with well-developed hypopharyngeal glands, while JH treatment induces premature hypopharyngeal gland degeneration (Rutz et al. 1974; Beetsma and Houten 1974; Jaycox et al. 1974; Jaycox 1976). Hypopharyngeal glands regenerated in reverted bees, but not in foragers, even though foragers also experienced a drop of JH titer. Perhaps hypopharyngeal glands did not also regenerate in foragers, despite a drop in JH, because for the most part the drop was not as severe or did not last as long as in reverted bees. Alternatively, perhaps hypopharyngeal gland development is not regulated by JH under all conditions. For example, Rutz et al. (1977) reported that removal of the corpora allata (the glands that produce JH) had no effect on hypopharyngeal gland development in young bees, but this finding is difficult to interpret in the absence of hormone titer data to evaluate the effects of the allatectomy. Huang et al. (1994) found that soldiers have larger hypopharyngeal glands than do foragers, despite their similar age and JH titers. These results, coupled with our findings, suggest that the relationship between hypopharyngeal gland development and JH is more complex than previously suspected.

Bees that continued foraging in the old-bee colonies also showed a decrease in JH titers in all three trial of experiment 3, though titers were usually higher than for reverted bees. Why did they continue to forage? One possibility is that levels of JH in these bees never dropped below the minimum levels that are associated with foraging (*c*. 100 ng/ml; see Huang et al. 1994). Another possibility is that high JH titers are not required for continued foraging. This suggestion is consistent with findings of foraging in early spring and late fall by bees with low JH titers (Huang and Robinson 1995).

Colony age demography and the regulation of age-related division of labor

Previous studies (Winston and Punnett 1982; Winston and Fergusson 1985, 1986; Fergusson and Winston 1988; Kolmes and Winston 1988; Naumann and Winston 1990a, b) have given the impression that several colony and environmental factors are involved in the regulation of age-related division of labor in honey bee colonies, and that they sometimes act in a variable manner. While the regulation of division of labor undoubtedly is complex, we are encouraged in our attempts to understand this process by initially focusing on a single factor, colony age demography. We suggest that the results of most studies aimed at identifying environmental and colony factors that regulate division of labor, even those with conflicting results, can be consistently explained on the basis of the effects of this factor.

There are contradictory results on the relationship between the size of a colony's adult worker population and individual behavioral development. Winston and Punnett (1982) found a positive correlation between population size and the age at which bees began foraging. Similarly, Winston and Fergusson (1985) reported that decreases in colony population, due to depleting workers of unknown ages, lowered the age at first foraging. However, Naumann and Winston (1990a) reported no such effects of similar non-specific worker depletion. Results of our experiments suggest one possible explanation for this discrepancy: perhaps a greater proportion of older workers were depleted in Winston and Fergusson (1985) relative to Naumann and Winston (1990a). The findings of Giray and Robinson (1994) also demonstrate that colony population does not have an independent effect on age at first foraging – typical behavioral ontogeny was observed in small colonies, but varying the proportion of young bees to old bees affected the age of first foraging significantly.

Using larger, more typical, colonies than in our study, Kolmes and Winston (1988) found no differences in the age at first foraging in response to specific depletions of either young or old bees. These results are not consistent with those of our first experiment. Perhaps this is because Kolmes and Winston (1988) monitored the behavioral development of bees that were 1 day old at the time the depletion was made, while we monitored 14- to 26-day-old bees. It is possible that younger bees would not show changes in behavioral development in Kolmes and Winston (1988) because middle-aged individuals were able to respond first to the demographic changes caused by the depletions, restoring a more typical balance between preforagers and foragers. Alternatively, perhaps the effects of colony age demography on division of labor vary with population size. An important focus of future work will be to study the control of division of labor with more natural colonies than used here or elsewhere (Huang and Robinson 1992; Giray and Robinson 1994).

Changes in resource availability also have been suggested to play a role in the regulation of age-related division of labor in honey bee colonies. There is anecdotal evidence that workers initiate foraging at younger ages when nectar and pollen is plentiful (Nikeil and Armbruster 1937, cited in Sekiguchi and Sakagami

1966; Nowogrodzki 1983; Kolmes 1985; Seeley 1985). These observations suggest that factors associated with abundant food resources, such as increased dance communication or food processing, may accelerate behavioral development. Increased activity levels have been observed in both foragers and younger bees after exposure to tactile stimulation ("dorsoventral abdominal vibrational dances"; Schneider et al. 1986a; Schneider 1987). Similarly, workers apparently can be recruited to become nectar-storers after exposure to another form of tactile stimulation ("tremble dance"; Seeley 1992). In both cases, the behavioral change is relatively fast and it has not been shown that behavioral development also is accelerated. Alternatively, or in addition to those possible factors, increases in resource availability could lead to accelerated behavioral development in younger bees because of possible reduced exposure to foragers. Increased food availability causes increased foraging activity by existing foragers (von Frisch 1967; Seeley 1992), which leads to their spending less time in the hive and suffering increased activity-induced mortality. This suggestion may also explain why accelerated behavioral development occurred in colonies experimentally deprived of beeswax (Fergusson and Winston 1988). Since honey is the energy source for wax production, perhaps extreme wax deprivation leads first to a short-term response, increased foraging intensity by existing foragers, and then to an earlier onset of foraging by younger bees.

The effects of a colony's food stores on age-related division of labor also are not clear. Fukuda (1960) suggested that a shortage of pollen stores in the hive can lead to accelerated behavioral development, but interpretation of his results is difficult because the genetic and age structure of the two colonies (control and pollen-reduced) could be different. A second paper (Free 1967) has been cited as providing evidence that changes in pollen stores lead to changes in rates of behavioral development; this study demonstrates short-term changes in the proportion of foragers collecting pollen or nectar, but does not document more long-term changes in behavioral ontogeny.

There also are contradictory results on the relationship between the amount of brood in a colony and individual behavioral development. Winston and Punnett (1982) found no correlation between the amount of sealed (pupal) brood and the age at first foraging. Winston and Fergusson (1985) reported that the age at first foraging was negatively correlated with the area of eggs and larvae (unsealed brood), but not with area of pupal brood. However, in a subsequent study (Winston and Fergusson 1986), the age at first foraging did not vary in colonies with either low, medium, or high amounts of eggs and larvae. These inconsistent results suggest that amount of brood does not have an independent effect on worker behavioral development in honey bee colonies.

Results of our experiments 2 and 3 and this review of the literature suggest that colony age demography can shape the structure of the labor pool by causing workers to be "prepared" to perform certain tasks before the actual need arises. While under natural conditions it might be assumed that specific demographic changes will be reliably associated with specific increases in task need, this study demonstrates that these two factors can be experimentally uncoupled. Moreover, under our experimental conditions, behavioral development was more strongly influenced by colony age demography than task need. In experiment 2 the need for foraging was presumably *increased* because foragers were confined, but behavioral development of younger workers was delayed, rather than accelerated. In experiment 3, the need for brood care was *decreased* because no brood was present, but endocrine and exocrine changes associated with behavioral reversion still occurred. Perhaps the need for nurse bees still existed to care for the queen but the widespread reversion we observed suggests that the queen was not the main cause for reversion. Analyses of the relative influences of colony age demography and specific task needs on behavioral development in other insect societies would perhaps reveal the social and ecological correlates of systems that rely on one factor more strongly than the other.

Implications for the activator-inhibitor model of honey bee behavioral development

Huang and Robinson (1992) proposed the activator-inhibitor model to explain how colony age demography can influence honey bee behavioral development via worker-worker interactions. The model draws upon knowledge of how cell-cell interactions affect the developmental fate of cells within organisms. The essence of this model is regulation via an interplay between an activator and an inhibitor. Regulation via activator-inhibitor mechanism is common in many other systems (e.g., regulation of: physical caste ratios in ants and termites, Wheeler and Nijhout 1984; Okot-Kotber et al. 1993; age of puberty onset in mammals, Price and Vandenbergh 1992; cell differentiation, Bode and Bode 1984; gene expression, Heitzler and Simpson 1991).

The results presented here are consistent with the activator-inhibitor model. According to this model, precocious development by some workers in a colony deficient in older bees is a consequence of young workers interacting relatively less frequently with older workers, being exposed to less inhibitor, and exhibiting an accelerated rate of JH increase. Delayed or reversed development in a colony with predominantly old bees may be a result of workers exposed to unusually high levels of inhibitor.

In experiment 1, behavioral development was accelerated in response to forager depletion. This may have

been due to young bees being exposed to fewer bees with high inhibitor levels during (as yet) unspecified worker-worker interactions. In experiment 2 all bees were confined to their colonies and delayed foraging occurred. This may have been due to young bees being exposed to more inhibitor because of increased contact with the confined foragers. Increased contact is expected because the foragers will be inside the hive all the time rather than spending a large proportion of time foraging; they will probably also live longer as a consequence of being prevented from foraging. That the confinement period in trial 1 was shorter, and no significant effect observed, suggests that effect of the inhibitor is time-dependent .

In experiment 3 all young bees were depleted from colonies and reversion occurred. The drop in JH titer detected in this experiment may have been due to drastically increased exposure of bees to other colony members with high inhibitor levels. We would have predicted a drop in JH in some, but not all, foragers on the basis of the hypothesis that the activator (JH) and inhibitor are coupled; a bee with a decreased JH titer should therefore be less inhibitory to other individuals. The results suggest that there is mutual, more widespread, inhibition among all foragers. This inhibition was only temporary in trial 3, but apparently not so for foragers in trials 1 and 2, at least for the brief duration of our experiment. Perhaps this difference is related to the fact that the old-bee colony in trial 3 was twice as large as those in trials 1 and 2.

Conclusions

Results of this study demonstrate that honey bee colony age demography can directly influence temporal division of labor. One important implication of these results is that labor schedules in a honey bee colony can be maintained independent of task needs. Workers apparently can develop physiological competency for a particular task before the need for the task actually arises. This is consistent with the idea that a colony of social insects has a "preferred state" (Schmid-Hempel et al. 1993), and the colony is "plastic" in the sense that it returns to that state after a disturbance.

Despite the prominent role played by colony age-demography, it is likely that some forms of worker-nest interactions related to task needs also play a role in regulating temporal polyethism. Further experiments are needed in which colony age demography is held constant and task need is varied.

Effects of colony age demography may act via the hypothesized activator-inhibitor mechanism, or other similar mechanisms. The activator-inhibitor model will undboubtedly be modified in the future, but at present it provides a heuristic tool to understand the roles of physiology, colony age demography, and labor needs in the regulation of division of labor.

Acknowledgements We thank J.C. Kuehn for expert help in maintaining the bees; D.W. Borst for graciously providing JH antiserum; and S.N. Beshers, L.M. Heuser, C. Wagener-Hulme, M.L. Winston, one anonymous reviewer, and especially T.D. Seeley for comments that greatly improved the manuscript. Supported by grants from NIH (MH42274-01) and USDA (92-37302-785 and 94-37302-0568) to G.E.R.

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Communicated by R.F.A. Moritz