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Honeybee colony integration: Worker–worker interactions mediate hormonally regulated plasticity in division of labor

(social organization/juvenile hormone/*Apis mellifera*/ontogeny/information acquisition)

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ABSTRACT **Adult workers in honeybee (*Apis mellifera*) colonies exhibit plasticity in hormonally regulated, age-based division of labor by altering their pattern of behavioral development in response to changes in colony conditions. One form of this plasticity is precocious development: levels of juvenile hormone increase prematurely and bees begin foraging as much as 2 weeks earlier than average. We used two experimental paradigms inspired by developmental biology to study how bees obtain information on changing colony needs that results in precocious foraging. An analog of "cell culture," with bees reared outside of colonies in different sized groups, revealed that worker–worker interactions exert quantitative effects on endocrine and behavioral development. "Transplants" of older bees to colonies otherwise lacking foragers demonstrated that worker–worker interactions also affect behavioral development in whole colonies. These results provide insights to a long-standing problem in the biology of social insects and further highlight similarities in the integration of activity that exist between individuals in insect colonies and cells in metazoans.**

A central question in insect sociobiology is how the activities of individual workers are integrated to enable colonies to develop and produce reproductives despite changing internal and external conditions. The regulation of age-based division of labor among workers demands a high level of colony integration. Honeybees (*Apis mellifera*) generally work in the nest for the first 3 weeks of adult life and then spend their final 1–3 weeks foraging, but they can accelerate, retard, or reverse their behavioral development in response to changes in colony or environmental conditions, or both (1). It is important for colony survival and reproduction that bees respond accurately to the need for a particular worker activity because the shift from nest duties to foraging requires complex physiological changes (2).

We studied how workers obtain information that influences one form of plasticity in behavioral development: precocious development, in which bees begin foraging as much as 2 weeks earlier than average (2). Precocious foraging may occur naturally in colonies deficient in foragers due to a seasonal surge in birth rates or because of the loss of foragers to predators; it can also be induced experimentally in colonies that lack older bees (1).

It is unlikely that an individual worker has the capacity to acquire and integrate information on the global state of its colony, in which a dozen different activities, performed by tens of thousands of individuals, may be proceeding simultaneously. The regulation of plasticity in worker behavioral development thus may be similar conceptually to the regulation of plasticity in cell development: workers, like cells, develop in response to local stimuli in ways that are appropriate at the global level. Cell development is mediated by interactions with the extracellular matrix (3, 4) and with other cells (5–7). Similarly, worker development may be mediated by interactions with components of the nest and with other workers. Lindauer (8) proposed that workers perceive colony needs via stimuli emanating from the nest during "patrolling" behavior. Ribbands (9) and Free (10) suggested that information on colony needs is somehow conveyed during trophallaxis (social feeding); both of these mechanisms could underlie the acquisition of information that results in plasticity in behavioral development. Based on two experimental approaches rooted in developmental biology, we report that worker–worker interactions play an important role in mediating behavioral development in honeybees.

MATERIALS AND METHODS

To separate the effects of worker–worker interactions from worker–nest interactions on development, individuals were reared outside their colonies, like cells in culture. Newly emerged bees from the same colony were reared for 1 week in either isolation, small groups, or a colony and then assayed. Colony-reared bees could have had worker–worker or worker–nest interactions, group-reared bees only worker–worker interactions, and isolated bees neither.

One-day-old bees were obtained by placing combs of sealed brood from colonies in a 33°C incubator. They were reared in the laboratory in plastic Petri dishes (60 × 15 mm) and given a 40% (wt/vol) sucrose solution in an inverted Eppendorf tube with holes in the lid. Most dishes were maintained at 34°C in darkness except when food was replenished.

The rate of juvenile hormone (JH) biosynthesis was used as a physiological measure of behavioral development. JH has been shown to regulate behavioral development in honeybees (1); young bees working in the nest have low blood titers and low rates of biosynthesis of JH, whereas foragers have high titers (11, 12) and high rates (13). Rates of biosynthesis for the corpora allata were determined *in vitro* with a radiochemical assay (14–16). Huang *et al.* (13) recently adapted this assay for adult worker honeybees and, in addition, demonstrated that rates of JH biosynthesis are highly correlated with JH blood titers in worker bees. The corpora allata–corpora cardiaca complex (CA) from an individual bee was removed and incubated for 3 hr in 50 μl of medium (17) containing 100 μM L-[^{3}H-*methyl*]methionine (specific activity, 200 mCi/mmol; 1 Ci = 37 GBq; New England Nuclear). JH was extracted with 250 μl of isooctane (Fisher, HPLC grade) and quantified by liquid scintillation spectrometry (Biosafe II cocktail; Research Products International). The values obtained were corrected by a blank incubation for each assay.

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Abbreviations: JH, juvenile hormone; CA, corpora allata–cardiaca complex.

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Gland pairs from each individual bee were cultured and assayed individually (13).

To test effects of isolation on behavioral development, 1-day-old bees (n = 970) from two source colonies were mixed randomly and each was marked individually with a colored, numbered plastic tag (Oplallithpläthen, Endersbach, F.R.G.). Half of the bees were reared in isolation, as described above. The other half were introduced into an unrelated colony with a population of ≈50,000 bees ("nursery colony"). An additional 2000 one-day-old bees from the same two source colonies were each marked with a paint spot and introduced to the nursery colony. Rates of JH biosynthesis were determined for 10 isolated bees and 10 colony-reared bees when they were 6 days old. The following day, 295 tagged, isolated bees, 283 tagged, colony-reared bees, and 1400 paint-marked bees were used to create a "single-cohort colony" (18). The colony was given one comb of honey and pollen, one empty comb, and a queen unrelated to the workers. Observations of foraging were made when bees were 11–16 days old. Foragers were classified as bees that returned with either pollen loads or a distended abdomen (signifying nectar or water foraging) or that made round trips longer than 4 min (19). A census taken on day 17 indicated that at least 157 tagged, isolated and 257 tagged, colony-reared bees were present during the observation period.

To test effects of worker–worker interactions on behavioral development in whole colonies, we used an approach analogous to transplant experiments in developmental biology. Transplant experiments assess the effects of one cell type on another by transplanting a specific group of cells from one individual to another (20). For each experiment, a pair of single-cohort colonies was established, each with 1500 one-day-old bees from the same source colonies, one empty comb, one comb approximately one-fourth filled with nectar and one-eighth filled with pollen, and a queen unrelated to the workers obtained from the same commercial source. Three to 6 days later, 200 older bees were collected from a different colony (located >9 km away), chilled, marked with paint, and introduced into the "treatment" single-cohort colony. The older bees were either pollen foragers or soldiers. Soldiers were bees that emerged from the hive in response to an experimentally induced disturbance; soldiers and foragers are the oldest bees in a colony (21). "Control" single-cohort colonies in experiments A1, A2, and B1a received 200 one-day-old bees from the same colony that was used to supply bees for the treatment single-cohort colony. The control colony in experiment B2 received pollen foragers. Observations of foraging were made when resident bees were 8–12 days old, depending on the experiment (see Table 1). The number of foragers was determined by marking bees that returned with either pollen loads or a distended abdomen during 1 hr of observations. Foraging observations were made during times when no conspicuous orientation flights occurred. The same two queens were used in experiments A1, B1a, and B2; they were alternated in the treatment and control colonies in experiments A1 and B1a to minimize any possible queen effects.

RESULTS

Colony-reared bees showed low rates of JH biosynthesis, typical for their age (13), whereas isolated bees showed high rates precociously (Fig. 1). Rates of JH biosynthesis for 1-week-old isolated bees were similar to those of 24-day-old foragers (t = 0.8, $P > 0.3$) and significantly higher than 1-week-old colony-reared bees (t = 8.6, $P < 0.001$).

A high rate of JH biosynthesis in young, isolated bees is linked to precocious behavioral development. Sixty-seven of 157 bees isolated for 1 week and then introduced into a single-cohort colony started foraging when they were only

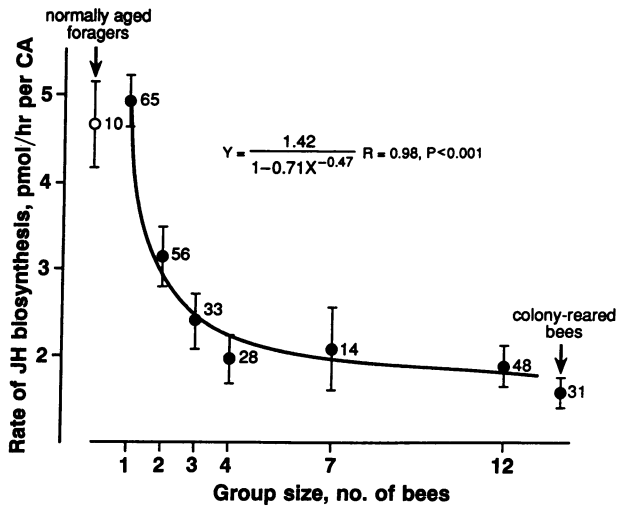

FIG. 1. Effect of group size on adult worker honeybee development, measured by determining individual rates of JH biosynthesis. Newly emerged bees from the same colony were reared for 1 week either in isolation, small groups, or a colony and then assayed. The 24-day-old pollen foragers were sampled from a different colony (13). Rates of JH biosynthesis (mean ± SE) decreased with increasing group size; this relationship is best described as a reverse sigmoid function (R^2 = 0.98, $P < 0.001$). Rates for individuals reared in groups ≥4 bees were not significantly different than for colony-reared bees (Tukey's test, $P > 0.05$). The number of bees assayed is indicated (n = 28 groups of 2, 11 groups of 3, 7 groups of 4, 2 groups of 7, and 5 groups of 12 bees).

13–16 days old, compared with 3 of 257 one-week-old sister bees that were colony-reared ($P < 0.001$, G = 129.3, 1 df). One-week-old isolated bees from this experiment also had significantly higher rates of JH biosynthesis than 1-week-old colony-reared bees (6.14 ± 0.59 SE vs. 1.05 ± 0.30 pmol/hr per CA, respectively, n = 10 for each group, t = 7.7, $P <$ 0.001). Isolated bees foraged precociously but otherwise exhibited normal foraging behavior: most (85%) took multiple foraging trips, demonstrating an ability to orient to their hive, and some (10%) returned with conspicuous loads of pollen—i.e., with tangible evidence of foraging success. Bees reared in isolation (n = 20) did not have developed ovaries, which can occur in small groups of worker bees maintained without a queen for longer periods of time (22).

JH biosynthesis varied inversely with group size (Fig. 1). Mean rates for 1-week-old individuals in groups of ≥4 bees were not different than colony-reared bees despite the lack of a nest environment, suggesting that isolated bees and those in groups of 2 and 3 developed prematurely due to a lack of other workers rather than a lack of nest-related stimuli. Despite the low mean rates, however, there were some individuals in groups of ≥4 bees with "forager-like" rates of JH biosynthesis. Using as a criterion the mean rate of JH biosynthesis for foragers minus 1 SD (2.84 pmol/hr per CA), the percentages of bees with "forager-like" rates of JH biosynthesis were 77%, 45%, 27%, 25%, 14%, and 18% for group sizes of 1, 2, 3, 4, 7, and 12, respectively.

Transplanting 200 older bees (foragers and soldiers) from a typical colony to a single-cohort colony when resident bees were 4 days old inhibited precocious foraging of resident bees but transplanting young bees from the same colony did not (Table 1, experiment A1). Inhibition of precocious foraging also occurred when the transplant was made when resident bees were 7 days old (Table 1, experiment A2), but the inhibitory effect was significantly ($P < 0.001$) diminished relative to the effect observed in experiment A1.

In three experiments transplants of old bees (foragers or soldiers) also inhibited precocious foraging when hive en-

Table 1. Effects of worker–worker interactions on precocious behavioral development in adult worker honeybees, as evidenced by transplanting 200 older bees to colonies otherwise lacking older bees

Exp.	Description	Resident bees that foraged precociously, no.	Transplanted bees that foraged, no.	Significance, *P*
A	Inhibition of precocious foraging by transplant of old bees			
1	Transplant on day 4, foraging observations on day 11			
	Treatment: pollen foragers (20%) and soldiers (80%) added, entrance open	3	48	<0.001
	Control: 1-day-old bees added, entrance open	46	1	
2	Transplant on day 7, foraging observations on day 11			
	Treatment: pollen foragers added, entrance open	25	18	<0.001
	Control: 1-day-old bees added, entrance open	44	1	
B	Mechanism of inhibition: worker–worker interactions (colony entrance open or closed) or worker–nest interactions (only when colony entrance open)			
1a	Transplant on day 3, foraging observations on day 7			
	Treatment: soldiers added, entrance closed from day 3 to day 7	2	31	<0.001
	Control: 1-day-old bees added, entrance closed from day 3 to day 7	41	5	
1b	Transplant on day 4, foraging observations on day 7			
	Treatment: foragers added, entrance closed from day 4 to day 6	0	24	<0.001
	Control: 1-day-old bees added, entrance closed from day 4 to day 6	23	0	
2	Transplant on day 4, foraging observations on days 9 and 10			
	Treatment: pollen foragers added, entrance closed from day 4 to day 8	1	36	>0.7
	Control: pollen foragers added, entrance open	2	18	

Significance values are based on *G* tests for heterogeneity (23). The inhibitory effect in experiment A2 is significantly less ($P < 0.01$) than in experiments A1, B1a, B1b, or B2, presumably because the transplant occurred when resident bees were older. Experiment B2 tested the effects of transplanting older bees when the colony entrance was either open or closed; precocious foraging was inhibited in both cases (hence the nonsignificant *P* value), which is consistent with the results of experiments B1a and B1b.

trances were closed for 3 or 4 days, starting immediately after the transplant was made (Table 1, experiments B1a, B1b, and B2). A transplant of soldiers significantly inhibited precocious foraging relative to a transplant of 1-day-old bees when both hive entrances were closed in experiment B1a. This experiment was replicated (experiment B1b) using foragers instead of soldiers and the result was identical. Transplants of foragers to both colonies in experiment B2 inhibited precocious foraging whether or not the hive entrance was open.

DISCUSSION

The finding that social interactions mediate plasticity in age-based division of labor provides insight into the question of how the activities of individual workers are integrated into a smoothly functioning colony. Worker–worker interactions play other important roles in the integration of colony division of labor (1), including the regulation of relatively short-term changes in foraging behavior. Differential treatment by nest workers during food transfer influences the intensity of nectar foraging behavior in honeybees (24) and the likelihood of continuing to collect the same type of resource in honeybees (25) and *Solenopsis invicta* ants (26). The results presented here demonstrate that social interactions also play a role in the regulation of more long-term changes in behavior.

Social isolation induced precocious endocrine and behavioral development. These results are consistent with findings that precocious foragers develop in colonies lacking older bees (1) and precocious foragers have high JH titers (18) and high rates of JH biosynthesis (unpublished results). In addition, young bees given JH treatments become precocious foragers (27–31). Thus, although isolation experiments did not expose bees to conditions encountered in nature, at least some processes underlying honeybee behavioral development occurred under these atypical conditions. Moreover, isolation induced the development of precocious foragers that functioned normally in a colony. Isolation also has been reported to affect behavioral development in ants (32, 33) but it is not known whether these effects are due to a lack of worker–worker or worker–nest interactions, or both.

Mean rates of JH biosynthesis varied inversely with group size, indicating quantitative effects of worker interactions on rates of development. However, our results also suggest that precocious development by some individuals also occurred in groups of all size. This is consistent with the observation that a small proportion of bees develops precociously in colonies that lack older bees (34).

Transplant experiments demonstrated that worker–worker interactions affect behavioral development in whole colonies; young bees were inhibited from foraging precociously in the presence of foragers. An alternative explanation is that young bees were not inhibited from developing precociously but rather were only inhibited from foraging due to the presence of older, more experienced foragers. However, we observed weaker inhibition of precocious foraging with a transplant made when resident bees were 7, rather than 4, days old. This result suggests that more bees developed precociously in the colony that received the transplant later. In contrast, the timing of the transplant would be a less important variable according to the alternative explanation outlined above. We therefore favor the conclusion that very few precocious foragers were observed in transplant experiments because young resident bees were actually inhibited from developing precociously.

The transplanted bees inhibited precocious foraging even though they themselves were prevented from collecting fresh nectar or pollen, the most obvious way that they could have changed the nest environment. Furthermore, because in experiment B1a we transplanted only soldiers, rather than foragers, the inhibitory effect cannot be attributed to small amounts of nectar or pollen brought in by transplanted bees at the time of the transplant. These results suggest that in all four transplant experiments resident bees were inhibited from foraging precociously because they responded to the presence of the transplanted older bees directly rather than to changes in the condition of their nest.

Advanced insect colonies have long been likened to "superorganisms" (35, 36) and there are many parallels between

"sociogenesis" (37)—i.e., insect colony development—and the processes of morphogenesis that occur within a multicellular organism (37). Previous studies have shown that the regulation of developmental plasticity in a metazoan organism and an insect colony has common features (1). Our findings further highlight similarities between sociogenesis and morphogenesis by demonstrating that stimuli that mediate developmental plasticity in adult workers, as in cells, emanate from social interactions.

Worker interactions may affect honeybee behavioral development similarly to the ways that cell interactions affect cell development in some organisms (38). We hypothesize that there is an interplay between JH, which we designate as an intrinsic "activator" that promotes behavioral development, and an "inhibitor," an as-yet unidentified factor(s) transferred among workers that retards development. Precocious development by some workers in a colony deficient in older bees thus would be a consequence of young workers interacting relatively less frequently with older workers, receiving less inhibitor, and exhibiting an accelerated rate of JH increase. Normal rates of behavioral development in a colony with a mixed age structure may be a result of young workers receiving high levels of inhibitor from older workers relative to their intrinsic rate of JH increase. This model is more plausible if the production of JH and the inhibitor is coupled, with older bees producing or transferring higher levels of inhibitor than younger bees. Previously observed interindividual differences in honeybee behavioral development, due, in part, to worker genotypic variability (18, 34), may be based on variation in the production of, or sensitivity to, the proposed activator or inhibitor, or both. The effects of worker genotype thus would be analogous to the effects of cell lineage (5).

Social insect workers, like cells (39), are maintained in various differentiated states by flexible mechanisms. Our results have implicated worker–worker interactions, which may involve behaviors, volatile pheromones, surface molecules such as cuticular hydrocarbons, or chemicals exchanged during trophallaxis. The experimental approaches described here will be useful in identifying the precise "colony-level morphogens" that integrate age-based division of labor in insect colonies.

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1. Robinson, G. E. (1992) *Annu. Rev. Entomol.* **37**, 637–665.
2. Winston, M. L. (1987) *The Biology of the Honey Bee* (Harvard Univ. Press, Cambridge, MA), p. 281.
3. Grant, D. S., Tashiro, K.-I., Bartolome, S.-R., Yamada, Y., Martin, G. R. & Kleiman, H. K. (1989) *Cell* **58**, 933–943.
4. Wessel, G. M., Tomlinson, C. R., Lennarz, W. J. & Klein, W. H. (1989) *Development* **106**, 355–365.
5. Doe, C. Q. & Goodman, C. S. (1985) *Dev. Biol.* **111**, 206–219.
6. Corwin, J. T. & Warchol, M. E. (1991) *Annu. Rev. Neurosci.* **14**, 301–333.
7. Heitzler, P. & Simpson, P. (1991) *Cell* **64**, 1083–1092.
8. Lindauer, M. (1952) *Z. Vgl. Physiol.* **34**, 299–345.
9. Ribbands, C. R. (1952) *Proc. R. Soc. London Ser. B* **140**, 32–42.
10. Free, J. B. (1965) *Symp. Zool. Soc. London* **14**, 39–59.
11. Fluri, P., Lüscher, M., Wille, H. & Gerig, L. (1982) *J. Insect Physiol.* **23**, 61–68.
12. Robinson, G. E., Strambi, A., Strambi, C., Paulino-Simões, Z. L. & Barbosa, J. M. N. (1987) *Gen. Comp. Endocrinol.* **66**, 457–459.
13. Huang, Z.-Y., Robinson, G. E., Tobe, S. S., Yagi, K. J., Strambi, C., Strambi, A. & Stay, B. (1991) *J. Insect Physiol.* **37**, 733–741.
14. Pratt, G. E. & Tobe, S. S. (1974) *Life Sci.* **14**, 575–586.
15. Tobe, S. S. & Pratt, G. E. (1974) *Biochem. J.* **144**, 107–133.
16. Feyereisen, R. & Tobe, S. S. (1981) *Anal. Biochem.* **111**, 372–375.
17. Kaatz, H.-H., Hagedorn, H. H. & Engels, W. (1985) *In Vitro* **21**, 347–352.
18. Robinson, G. E., Page, R. E., Strambi, C. & Strambi, A. (1989) *Science* **246**, 109–112.
19. Winston, M. L. & Katz, S. J. (1981) *Can. J. Zool.* **59**, 1571–1575.
20. Spemann, H. (1918) *Wilhelm Roux Arch. Entwicklungsmech. Org.* **43**, 448–555.
21. Breed, M. D., Robinson, G. E. & Page, R. E. (1991) *Behav. Ecol. Sociobiol.* **27**, 395–401.
22. Velthuis, H. H. W. (1970) *Entomol. Exp. Appl.* **13**, 377–394.
23. Sokal, R. R. & Rohlf, F. J. (1981) *Biometry* (Freeman, San Francisco), 2nd Ed., p. 859.
24. Seeley, T. D. (1989) *Behav. Ecol. Sociobiol.* **24**, 181–199.
25. Lindauer, M. (1954) *Z. Vgl. Physiol.* **36**, 391–432.
26. Sorensen, A., Busch, T. M. & Vinson, S. B. (1985) *Behav. Ecol. Sociobiol.* **17**, 191–198.
27. Jaycox, E. R., Skowronek, W. & Gwynn, G. (1974) *Ann. Entomol. Soc. Am.* **67**, 529–534.
28. Jaycox, E. R. (1976) *J. Kans. Entomol. Soc.* **49**, 165–170.
29. Robinson, G. E. (1985) *J. Insect Physiol.* **31**, 277–282.
30. Robinson, G. E. (1987) *J. Comp. Physiol. A* **160**, 613–619.
31. Sasagawa, H., Sasaki, M. & Okada, I. (1989) *Appl. Entomol. Zool.* **24**, 66–77.
32. McDonald, P. & Topoff, H. (1985) *J. Comp. Psychol.* **99**, 3–14.
33. Lenoir, A. (1987) *Experientia* **54**, 219–241.
34. Page, R. E., Robinson, G. E., Britton, D. S. & Fondrk, M. K. (1992) *Behav. Ecol.* **3**, 173–180.
35. Wheeler, W. M. (1923) *Social Life Among the Insects* (Harcourt Brace, New York), pp. 3–6.
36. Ratnieks, F. L. W. & Reeves, H. K. (1992) *J. Theor. Biol.* **58**, 33–65.
37. Wilson, E. O. (1985) *Science* **228**, 1489–1495.
38. Bode, P. M. & Bode, H. R. (1984) *Pattern Formation* (Macmillan, New York), pp. 213–241.
39. Blau, H. M. & Baltimore, D. (1991) *J. Cell Biol.* **112**, 781–782.