Inspection and Feeding of Larvae by Worker Honey Bees (Hymenoptera: Apidae): Effect of Starvation and Food Quantity

Zhi-Yong Huang[1,2] and Gard W. Otis[1]

Honey bee larvae are frequently inspected and, sometimes, provided with food by adult workers, but the stimuli that elicit the important task of food provisioning have never been investigated. Larvae with their food experimentally deprived received more frequent inspection and feeding visits from nurse bees than normally fed larvae, suggesting that there could be a "hunger signal." Food-deprived larvae with artificially supplied larval food received the same rate of feeding visits from nurse bees as did normally fed larvae but still received more inspection visits. These results suggest that stimuli eliciting feeding are different from those for inspection. They also support the hypothesis that worker bees deposit food in a larval cell only when the quantity of food is below a certain minimum threshold that is perceived during larval inspections. A model is presented regarding the stimuli from larvae that result in worker feeding behavior.

INTRODUCTION

Social insects are characterized by a highly organized social structure and their well-coordinated colony behavior (Wilson, 1971). Coordination in an insect

[1] Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.
[2] Present address: University of Illinois, Department of Entomology, 320 Morrill Hall, 505 South Goodwin, Urbana, Illinois 61801.

society, as in any complex system, is dependent upon communication between the individual society members (Wiener, 1961; Seeley, 1989). In highly evolved social insects, colony members communicate about many aspects of social life, including caste status, hunger, danger, and food sources (Wilson, 1971).

Communication in an insect society takes place between the queen (and/ or king) and workers, among workers, and in hymenopteran insects, between workers and the brood. Studies on communication in insect colonies have been mostly focused on the first two categories. For example, chemicals signaling the presence of queen as well as effecting an array of other responses from workers, have been identified in both honey bees (Butler *et al.*, 1961, 1964; Slessor *et al.*, 1988) and ants (Edwards and Chambers, 1984; Glancey, 1986). Queen paper wasps *Polistes fuscatus* stimulate worker foraging via behavioral interactions (Gamboa *et al.*, 1990). Ants, bees, and termites all have elaborate chemical systems to communicate alarm and defense (reviewed by Winston, 1987; Hölldobler and Wilson, 1990). Recruitment to a food source has been studied in even more detail, with 18 chemicals identified as trail pheromone component(s) in ants (reviewed by Hölldobler and Wilson, 1990). Worker honey bees communicate the direction and distance of a food source via the well-known dance language (Frisch, 1967).

Communication between adult workers and the brood has not been studied as intensively as has communication among adult workers of insect colonies. Since the larval stage of many highly eusocial insects (ants, bees, wasps) depends completely on adult workers for progressive food provision, interactions should be common between workers and larvae in the process of larval feeding. Indeed, acoustical hunger signals from larvae to solicit food from adult wasps have been described in several species of *Vespa* (Ishay and Landau, 1972; Ishay, 1975). The phenomenon is considered widespread in the genus *Vespa*, and also occurs in *Dolichovespula* and *Vespula* (reviewed by Matsuura and Yamane, 1990). In some ants, such as *Formica sanguinea* and *Solenopsis invicta*, larvae solicit liquid food from workers by rocking their heads back and forth and flexing their mandibles (Hölldobler, 1968; O'Neal and Markin, 1973, cited by Hölldobler and Wilson, 1990).

Honey bee larvae are also fed progressively by adult worker bees. Bees engaging in brood care are 1 to 3 weeks old and are often termed "nurse bees." Nurse bees have well-developed hypopharyngeal and mandibular glands (King, 1933; Haydak, 1957; Simpson *et al.*, 1968), the glands which produce larval food. They spend much of their time in the brood area, inspecting cells and feeding larvae (Lineburg, 1924; Lindauer, 1953). Based on 272 h of observations, Lindauer (1953) calculated that, on average, a larva was inspected 1926 times for a total of 72 min but only fed during 143 visits. These observations suggest that worker bees, probably during inspection, assess whether a larva requires food or not in order to allocate food resources efficiently within the

colony. However, the stimuli that elicit feeding behavior in honey bees are presently unknown.

Lineburg (1924) suggested that nurse bees determine the quantity of food present in cells, since he observed that all larvae that were approximately the same age and position on the comb have about the same amount of food at all times. Similarly, Lindauer (1953) hypothesized that the quantity of food present in a cell might act as a cue for the feeding. In this study, we conducted experiments to determine whether nurse bees visit and/or feed food-deprived larvae at different rates than well-fed larvae and whether food quantity influences food provisioning by other nurse bees.

MATERIALS AND METHODS

Observation Hive

Experiments were conducted at the Apiculture Field Laboratory, University of Guelph, in the summer of 1987, using a colony kept in a four-frame observation hive. The bees were a genetical mixture of European bee subspecies (*Apis mellifera* L.) that is maintained at the University of Guelph. The colony was well established and had a population of about 12,000–15,000 worker bees during the experimental period. The temperature of the observation hive room was maintained at $31 \pm 1°C$ and kept dark except during the actual observation periods, when the room was illuminated with multiple fluorescent lights. The bees had free access to the outdoors via a glass-covered wooden tunnel.

Effect of Food Deprivation on Worker Feeding Behavior

This experiment was designed to determine whether starved larvae are treated differently than normally fed larvae. Larvae were "starved" according to the following procedure. One of the glass walls of the observation hive was replaced with a piece of plexiglass. Three movable doors (size, 8.5×9.5 cm) were made in the plexiglass wall, positioned over the third frame down from the top. During each trial, four larvae, all about 4 days old, of similar size, and in adjacent cells, were selected from a patch of comb located under one of the three doors. Access to larvae by worker bees was denied by pinning a piece of plexiglass (size, 2×2 cm) perforated with numerous 1.0-mm-diameter holes over the four cells.

These experimental larvae were prevented from being fed for either 0, 2, 4, or 6 h. The cover was then lifted from the cells and visits of bees to experimental larvae were immediately timed for 1 h. Some larvae that were starved for 4 or 6 h were removed by workers (8 and 24%, respectively); only data from larval groups in which all four larvae survived to the capping stage were analyzed.

A visit to a larva was classified as a "feeding visit" if a worker inserted its head and thorax into a larval cell for more than 10 s or an "inspection" if a worker inserted its head and thorax, or just its head, into a larval cell for less than 10 s. Ten seconds was used as a criterion because in the study by Brouwers et al. (1987, Fig. 3), over 90% (124 of 135) of feeding visits were observed to be more than 10 s. Three trials were conducted for each starvation period (2, 4, or 6 h), and six trials for the control (0 h), therefore a total of 60 larvae was observed. One-way ANOVA and regression analyses were performed with SAS (SAS Institute Inc., 1985).

Effect of Food Addition on Worker Feeding Behavior

This experiment was conducted to test whether the quantity of food around larvae or other stimuli from larvae are responsible for eliciting feeding behavior by worker bees. Larvae were artificially fed worker jelly collected as follows. Brood combs with 3- to 4-day-old larvae were taken from two source colonies unrelated to the observation colony. The larvae were carefully removed from cells with an aspirator and the worker jelly was collected and placed into a small glass vial. The worker jelly was either used immediately or stored at $-10°C$ for subsequent use. Only worker jelly stored for less than 48 h was used, to ensure that the experimental worker jelly was as similar as possible to the worker jelly newly deposited by nurse bees. Frozen worker jelly was thawed and brought to the temperature of the observation hive room before being added to larval cells.

Four adjacent cells, each with a 4-day-old larva, were covered for 4 h as in the first experiment. At the end of this starvation period, the larval cells were uncovered, and worker jelly was added experimentally to two cells randomly chosen among the four. Each of the two cells received approximately 15 μl worker jelly. Care was taken not to touch the larvae, as that resulted in a higher probability of their consumption by workers. After the addition of food, all worker visits to the four cells were timed and classified as in the previous experiment.

Data were pooled for the two starved and the two fed larvae for each set of four larvae, and paired t tests (Steel and Torrie, 1980) were performed across the six trials.

RESULTS

Effect of Food Deprivation on Worker Feeding Behavior

Normally fed larvae were fed at a rate of 1.5 $\pm$ 0.2/h, with an average duration of 70.7 $\pm$ 8.6 s/visit. These results are very consistent with those reported by Brouwers et al. (1987), which were 1.7 times/h and 77.63 $\pm$ 9.9

s/visit. Since the observations of Brouwers *et al.* (1987) were made on a colony maintained under red light, which bees cannot perceive (Frisch, 1967), these results indicate that the lighting condition in our study had no detectable effect on the feeding behavior of bees.

Starvation caused a significant increase in the rates of both inspections (ANOVA, $P < 0.001$; Fig. 1A) and feeding visits ($P < 0.001$; Fig. 1B). Both inspection and feeding responses increased with increasing starvation time, up to 4 h. However, after 2 h of starvation, the rate of feeding visits had already increased significantly (Tukey's studentized range test, $P < 0.05$), while the rate of inspection was the same ($P > 0.1$) as for the control larvae.

Starved larvae received more feeding visits than unstarved larvae, but did they receive more food? If starved larvae received feeding visits shorter than normal, then the total feeding time (a product of the number and the duration of visits) could remain the same for all treatments despite the increase in visits. However, we found no difference in the durations of individual feeding visits (ANOVA, $P > 0.2$) among the four treatments. In fact, there was a positive linear relationship between the length of time larvae were starved and the total time workers spent feeding them (Fig. 2). The total feeding time per hour for unstarved larvae was 119.2 ± 15.7 (mean $\pm$ SE) s (0 h in Fig. 2), which agrees well with the 2-min total feeding time per h reported by Lindauer (1953) for similarly aged larvae.

Effect of Food Addition on Worker Feeding Behavior

Starved larvae that were experimentally provided with worker jelly received a mean rate of inspection 12.4 ± 2.6/h, while starved larvae with no food added received 10.0 ± 2.5/h; this difference is not significant (paired t test, $P > 0.4$; Fig. 3A). However, food addition to starved larvae significantly reduced feeding visits, from a rate of 3.3 ± 0.5 to 1.8 ± 0.3/h (paired t test, $P < 0.05$; Fig. 3B).

Although these larvae received worker jelly in their cells, it is unlikely that they were in a different "hunger state" than similarly starved larvae that did not receive food. This is because the larvae were starved for 4 h before food was added, and observations were started immediately after food addition. In spite of this fact, the feeding rate to them dropped compared to those with no food added. Did the rate drop to the same level as that of unstarved larvae? We found that the rate of feeding visits received by starved larvae with food added (1.8 ± 0.3, labeled WJ in Fig. 3B) was not significantly different (t test, $P > 0.4$) from the rate of feeding visits received by the unstarved control in the previous experiment (1.5 ± 0.2/h, labeled 0 h in Fig. 1B).
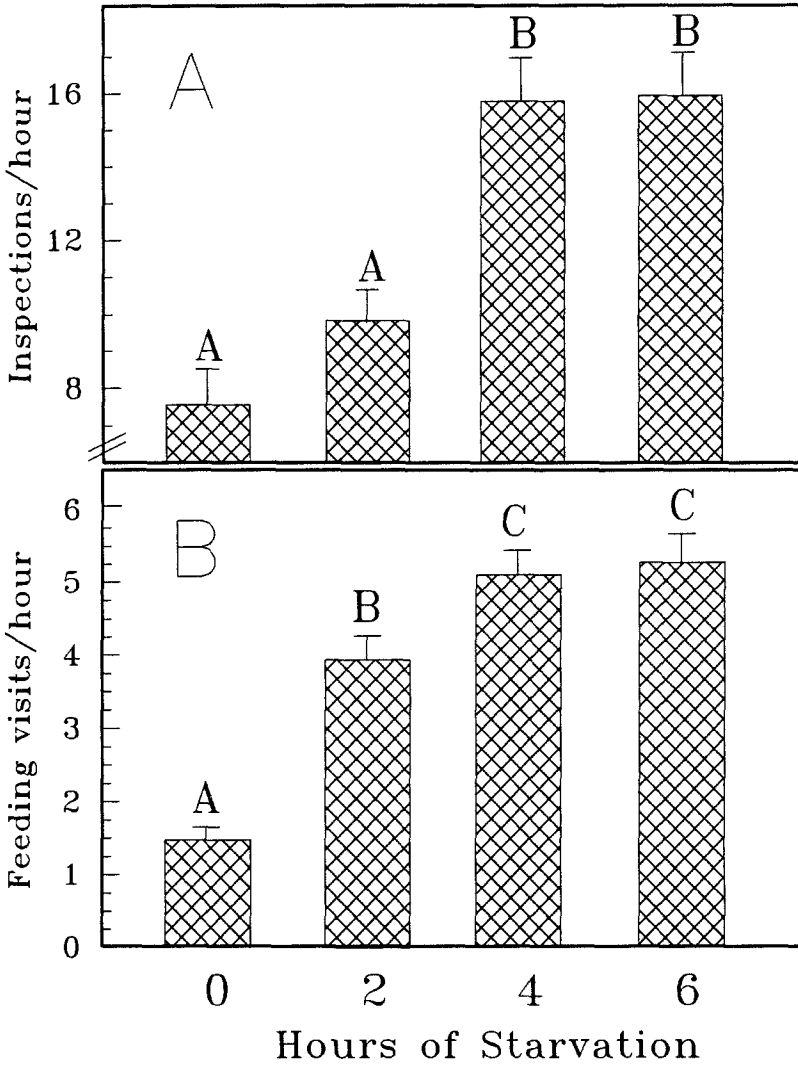
Fig. 1. Effect of larval starvation on the rate of inspection (A) and feeding (B) by worker bees. Each bar represents the mean from 12 larvae except that of "0" h, which was from 24 larvae. Error bars denote standard errors of the mean. Bars with different letters indicate that they are significantly different at the 5% level by Tukey's HSD test.
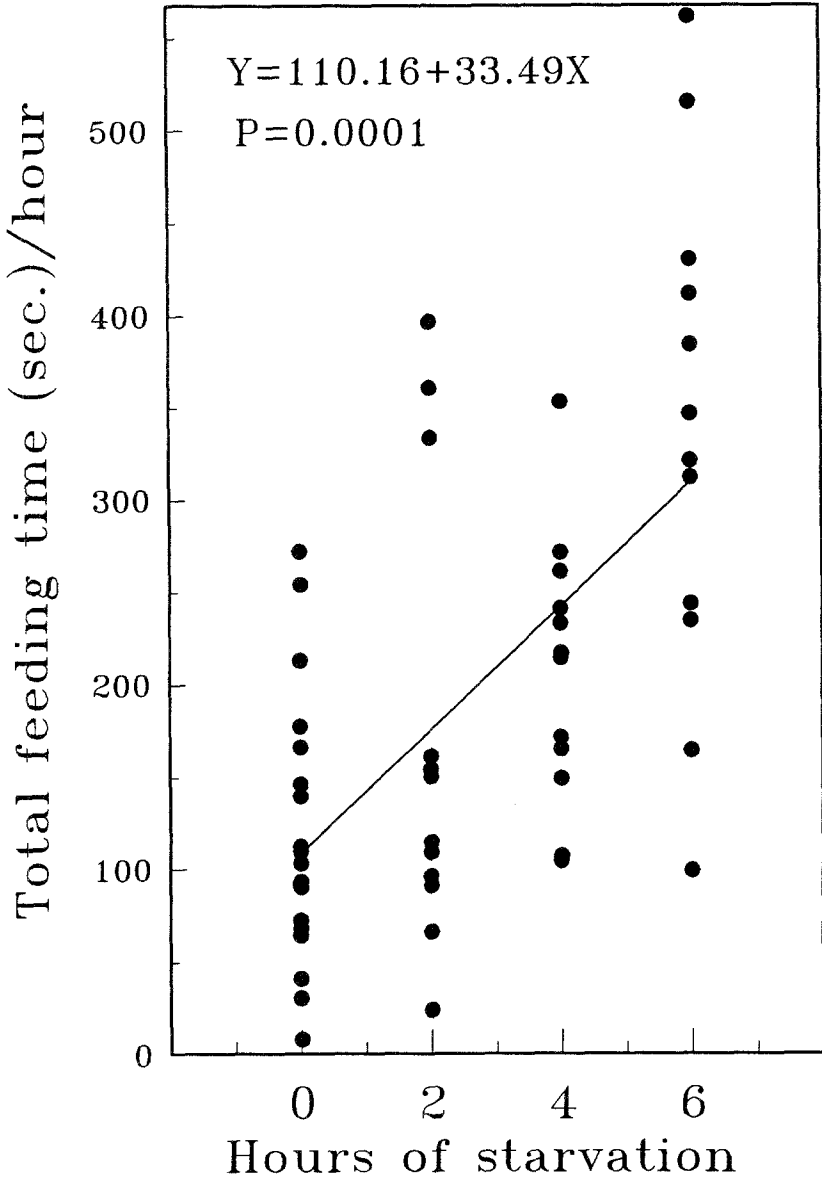
Fig. 2. Relationship between starvation period and time spent feeding by workers. Each data point represents the total feeding time received by a single larvae during the 1-h observation.
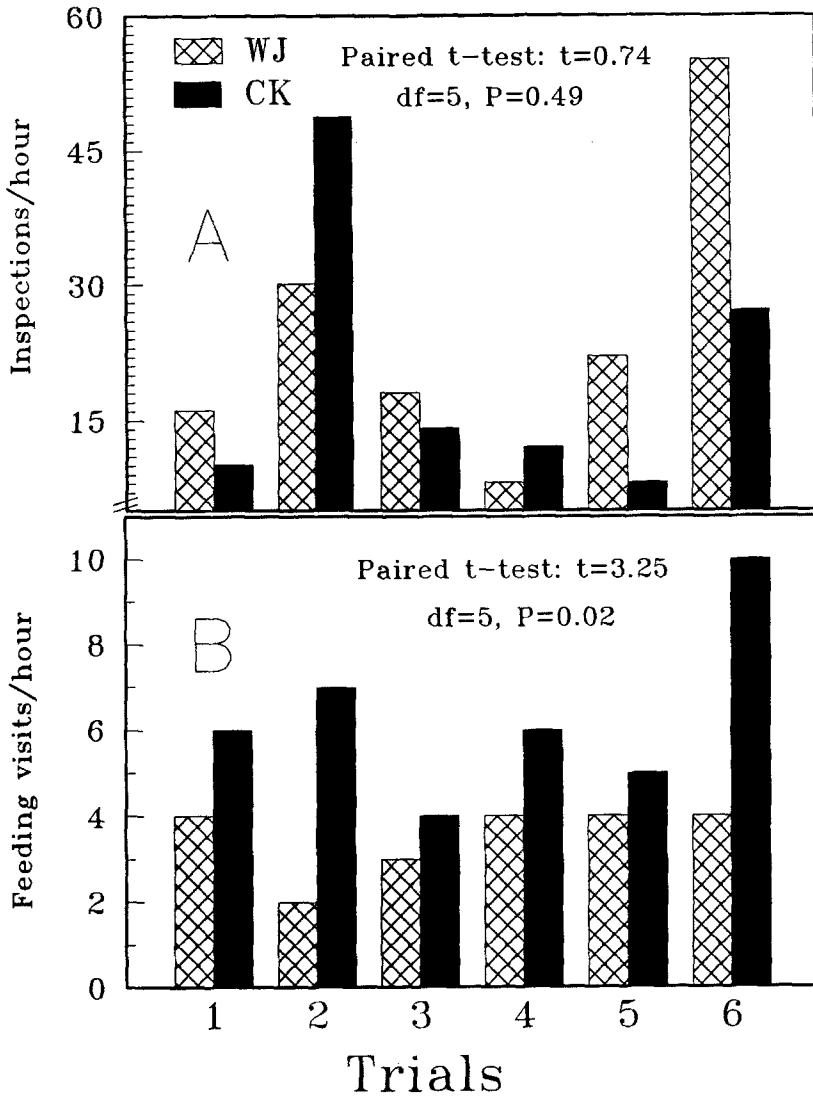
Fig. 3. Effect of food addition on the rate of inspection (A) and feeding (B) by worker bees. First, larvae were starved for 4 h, then two of the four larvae were randomly selected to receive worker jelly experimentally. Each bar represents the total number of feedings received by two larvae in 1 h, either by starved larvae with worker jelly added (WJ; hatched bar) or by starved larvae with no WJ added (CK; filled bar).

DISCUSSION

Longer periods of starvation resulted in increased rates of both inspection and feeding visits. The feeding response seems to be more sensitive to starvation than the inspection response, because the rate of feeding visits was significantly elevated for larvae starved for 2 h, while the rate of inspections increased significantly only for larvae starved longer than 4 h. Moreover, rates of inspections were not affected by adding food to starved larvae, whereas that of feeding visits declined to the same level as for unstarved larvae. Differences in frequencies of inspection and feeding visits over time in the first experiment, as well as the uncoupling of the two responses in the second experiment, suggest that the two behavioral responses are based on different stimuli. One stimulus may elicit inspections of larvae, while another stimulus, related to the quantity of food in a larval cell, may be perceived during the inspection and modulates food provisioning behavior.

Similar to larvae in *Vespa* wasps and *Formica* ants, therefore, honey bee larvae also have a system to elicit food from workers. Based on the results presented here and those of Huang (1988), we propose a model explaining the interactions between larvae and workers during the feeding process. We propose that three different stimuli are involved in the regulation of larval feeding by adult workers. First, a larva has to be identified by workers as a larva. Huang and Otis (1991) have provided experimental proof for the presence of this stimulus. Worker bees seem able to tell whether a cell contains an egg, contains a larva, or is empty, without entering each cell, since their visitations to egg cells or larval cells are not random. The first stimulus probably not only directs different subsets of workers to their respective "goals" (egg, worker larva, or queen larva, etc.), but also is responsible for the base rate of inspection to those different cell occupants.

The second stimulus communicates the "hunger status" of the larva. This stimulus increases in intensity as the interval between feeding increases and an increase in stimulus strength elicits a higher rate of inspection. This stimulus is intrinsic to the larva, because adding food to larvae starved for 4 h did not change the rate of inspection visits compared to that for starved larvae with no food added. The stimulus, perhaps a chemical, is probably produced by larvae as a result of starvation and does not decrease in magnitude immediately after food is added to a cell. The second stimulus, therefore, is associated only with the elevated rates of inspection. A lack of this stimulus does not mean complete cessation of inspections, because the first stimulus, which establishes the basic rates of inspection, is always present.

The third stimulus is perceived by workers during larval inspection and induces food deposition. We suggest that this stimulus is not intrinsic to the larva, but related to the quantity of food present in the cell. This is deduced

from the fact that when larvae (deprived of food for 4 h with food later added) are still "starved," as indicated by the higher rates of inspection to them, the feeding rates to them are, nonetheless, not increased. The only difference between these larvae and those with no food added is, of course, the quantity of food. Thus when workers are presented with conflicting stimuli, i.e., larvae with "hunger signals" yet with abundant food in their cells, workers inspected them more often but feed them at a normal rate. Under natural conditions, an increased inspection rate would probably always result in increased feedings, since the hunger signal (second stimulus) will invariably be correlated with the amount of food in the cell (third stimulus); the uncoupling of the two stimuli in our experimental setup normally would not occur. According to this model, a worker feeds a larva as long as the food in a cell is below a certain threshold level, regardless of the hunger status of the larva; on the other hand, a hungry larva, by its hungriness via the hunger signal, can increase its chance of being inspected and therefore being fed more often than others. The reason for this "redundancy" of stimuli is not clear; perhaps by using another stimulus to determine the need for food deposition, workers can avoid feeding a larva that produces a false signal (i.e., cheating).

The threshold for food deposition may be modulated by a variety of factors. It is known that worker feeding behavior varies with different colony conditions. For example, if a colony is made broodless for a period of time, the first batch of larvae when brood rearing resumes receives significantly more food than do larvae in colonies that are continuously rearing brood (Brouwers, 1984; Brouwers et al., 1987). Eggs normally do not receive brood food, but in some colonies over 90% of eggs that were introduced to broodless colonies received food, while only a few (less than 5%) in other similarly treated colonies did so (Z.-Y. Huang, unpublished observation). Colony differences were consistent during the entire summer and suggests that there is genetic variation for the "food deposition thresholds."

Our model is consistent with the results of Lineburg (1924) and Lindauer (1953). Lineburg (1924) observed that, when a larva for some unknown reason was given extra food, it received less food subsequently, even though it was still inspected as often. Lindauer (1953) suggested that "quantity of food still present" might act as a releaser for food deposition, and the apparently numerous inspections may serve to estimate the amount of food in larval cells. The mechanism by which workers estimate the food quantity is not clear. Food of queen larvae is known to contain several volatile chemicals (Boch et al., 1979); it is conceivable that worker jelly also does, and workers may correlate the concentration of volatiles with the amount of food.

Our model, though it involves three different stimuli, may still be too simple. For example, feeding behavior and composition of worker jelly vary with larval age (Jung-Hoffmann, 1966; Brouwers, 1984; Brouwers et al., 1987).

Thus workers seem able to recognize not only sex and caste, but also age of immature stages. It is not clear whether the "age indicator" is contained in the first stimuli in our model or whether an additional stimulus supplies this information just before food is added.

Honey bee brood is known to have many effects in the colony (see Free, 1987), such as stimulating pollen collection (Free, 1967), inhibiting the ovarian development of workers (Jay, 1970, 1972; Kubisova and Haslbachova, 1978), stimulating warming behavior (Koeniger and Veith, 1983), affecting rate of protein synthesis of hypopharyngeal gland (Brouwers, 1983; Huang and Otis, 1989; Huang et al., 1989), and inducing the capping of mature larvae by workers (Le Conte et al., 1990). However, few studies were pursued further in a communication perspective, and the precise nature of the stimuli that mediate these processes is known only for warming behavior (Koeniger and Veith, 1983) and cell capping (Le Conte et al., 1990). Our results suggest that a seemingly simple behavior such as larval feeding could involve a complex interplay of various stimuli from larvae, and workers need to make numerous decisions in the process.

ACKNOWLEDGMENTS

REFERENCES

Boch, R., Shearer, D. A., and Shuel, R. W. (1979). Octanoic and other volatile acids in the mandibular glands of the honeybee and in royal jelly. *J. Apic. Res.* **18:** 250–252.

Brouwers, E. V. M. (1983). Activation of the hypopharyngeal glands of honeybees in winter. *J. Apic. Res.* **22:** 137–141.

Brouwers, E. V. M. (1984). Glucose/fructose ratio in the food of honeybee larvae during caste differentiation. *J. Apic. Res.* **23:** 94–101.

Brouwers, E. V. M., Ebert, R., and Beetsma, J. (1987). Behavioural and physiological aspects of nurse bees in relation to the composition of larval food during caste differentiation in the honeybee. *J. Apic. Res.* **26:** 11–23.

Butler, C. G., Callow, R. K., and Johnston, N. C. (1961). The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic acid, a honeybee pheromone. *Proc. Roy. Soc. Lond. B* **155:** 417–432.

Butler, C. G., Callow, R. K., and Chapman, J. R. (1964). 9-Hydroxydec-trans-2-enoic acid, a pheromone stabilizing honey bee swarms. *Nature* **201:** 733.

Edwards, J. P., and Chambers, J. (1984). Identification and source of a queen-specific chemical in the Pharaoh's ant, *Monomorium pharaonis* (L.). *J. Chem. Ecol.* **10:** 1731–1747.

Free, J. B. (1967). Factors determining the collection of pollen by honeybee foragers. *Anim. Behav.* **15:** 134–144.

Free, J. B. (1987). *Pheromones of Social Bees*, Comstock, Ithaca, N.Y.

Frisch, K. von (1967). *The Dance Language and Orientation of Bees*, Harvard University Press, Cambridge, Mass.

Gamboa, G. J., Wacker, T. L., Scope, J. A., Cornell, T. J., and Shellman-Reeve, J. (1990). The mechanism of queen regulation of foraging by workers in paper wasps (*Polistes fuscatus*, Hymenoptera: Vespidae). *Ethology* **85:** 335–343.

Glancey, B. M. (1986). The queen recognition pheromone of *Solenopsis invicta*. In Lofgren C. S., and Vander Meer, R. K. (eds.), *Fire Ants and Leaf-Cutting Ants: Biology and Management*, Westview Press, Boulder, Colo., pp. 223–230.

Haydak, M. H. (1957). Changes with age in the appearance of some internal organs of the honeybee. *Bee World* **38:** 197–207.

Hölldobler, B. (1968). Verhaltensphysiologische Untersuchungen zur Myrmecophilie einiger Staphylinidenlarven. In Herre, W. (ed.), *Verhandlungen der Deutschen Zoologischen Gesellschaft* (Heidelburg, 1967) (Zoologischer Anzeiger Supplement, Vol. 31), Akademische Verlagsgesellschaft, Geest & Portig K.-G., Leipzig, pp. 428–434.

Hölldobler, B., and Wilson, E. O. (1990). *The Ants*, Harvard University Press, Cambridge, Mass.

Huang, Z.-Y. (1988). *Mechanism of Hypopharyngeal Gland Activation by Brood of Honey Bees (Apis mellifera L.)*, Ph.D. dissertation, University of Guelph, Ontario, Canada.

Huang, Z.-Y., and Otis, G. W. (1989). Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). *Insects Soc.* **36:** 264–276.

Huang, Z.-Y., and Otis, G. W. (1991). Non-random visitation of brood cells by worker honey bees (Hymenoptera: Apidae). *J. Insect Behav.* **4:** 177–184.

Huang, Z.-Y., and Otis, G. W., and Teal, P. E. A. (1989). Nature of brood signal activating the protein synthesis of hypopharyngeal gland in honey bees, *Apis mellifera* (Apidae: Hymenoptera). *Apidologie* **20:** 455–464.

Ishay, J. (1975). Frequencies of the sounds produced by the Oriental hornet, *Vespa orientalis*. *J. Insect Physiol.* **21:** 1737–1740.

Ishay, J., and Landau, E. M. (1972). *Vespa* larvae send out rhythmic hunger signals. *Nature* **237:** 286–287.

Jay, S. C. (1970). The effect of various combinations of immature queen and worker bees on the ovary development of worker honeybees in colonies with and without queens. *Can. J. Zool.* **50:** 661–664.

Jay, S. C. (1972). Ovary development of worker honey bees when separated from worker brood by various methods. *Can. J. Zool.* **48:** 169–173.

Jung-Hoffmann, I. (1966). Die Determination von Königin und Arbeiterin der Honigbiene. *Z. Bieneforsch* **8:** 296–322.

King, G. E. (1933). *The Larger Glands in the Worker Honey-Bee: A Correlation of Activity with Age and with Physiological Functioning*, Ph.D. dissertation, University of Illinois, Urbana.

Koeniger, N., and Veith H. J. (1983). Glyceryl-1,2-dioleate-3-palmitate, a brood pheromone of the honeybee (*Apis mellifera* L.). *Experientia* **39:** 1051–1052.

Kubisova, S., and Haslbachova, H. (1978). Effects of larval extracts on the development of ovaries in caged worker honey bees. *Acta Entomol. Bohemoslovaca.* **75:** 9–14.

Le Conte, Y., Arnold, G., Trouiller, J., Masson, C., and Chappe, B. (1990). Identification of a brood pheromone in honeybees. *Naturwissenschaften* **77:** 334–336.

Lindauer, M. (1953). Division of labour in the honeybee colony. *Bee World* **34:** 63–73, 85–91.

Lineburg, B. (1924). The feeding of honeybee larvae. *Bull. U.S. Dept. Agr.* **1222:** 25–37.

Matsuura, M., and Yamane, S. (1990). *Biology of the Vespine Wasps*, Springer-Verlag, Berlin.

O'Neal, J., and Markin, J. P. (1973). Brood nutrition and parental relationships of the imported fire ant *Solenopsis invicta*. *J. Ga. Entomol. Soc.* **8:** 294–303.

SAS Institute Inc. (1985). *SAS User's Guide: Statistics*, Version 5, Cary, SAS Institute Inc., N.C.

Seeley, T. D. (1989). The honey bee colony as a superorganism. *Am. Sci.* **77:** 546–553.

Simpson, J., Riedel, I. B. M., and Wilding, N. (1968). Invertase in the hypopharyngeal glands of the honeybee. *J. Apic. Res.* **71:** 29–36.

Slessor, K. N., Kaminski, L.-A., King, G. G. S., Borden, J. H., and Winston, M. L. (1988). Semiochemical basis of the retinue response to queen honey bees. *Nature* **332:** 354–356.

Steel, R. G. D., and Torrie, J. H. (1980). *Principles and Procedures of Statistics, a Biometrical Approach*, McGraw–Hill, New York.

Wiener, N. (1961). *Cybernetics: Or Control and Communication in the Animal and the Machine*, MIT Press, Cambridge, Mass.

Wilson, E. O. (1971). *The Insect Societies*, Harvard University Press, Cambridge, Mass.

Winston, M. L. (1987). *The Biology of the Honey Bee*, Harvard University Press, Cambridge, Mass.