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Nonrandom Visitation of Brood Cells by Worker Honey Bees (Hymenoptera: Apidae)

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The visitation pattern by worker honey bees to cells in the brood nest was monitored on an artificially created brood pattern consisting of about one-fourth brood cells evenly distributed among empty cells. The majority (63%) of the observed workers selectively entered larval cells. In contrast, some workers avoided egg cells when presented a choice of egg vs empty cells. The results suggest that larvae produce a general signal indicating their presence to worker bees. Eggs also seem to produce a signal, which is perceived to be different from the one from larvae.

KEY WORDS: honey bee; *Apis mellifera;* brood signal; communication.

INTRODUCTION

Communication among members is important in insect societies, as it facilitates colony coordination and integration. Recognition of castes is an important part of communication, as it is a precondition for many other interactions. For example, in a honey bee colony, a queen first has to be recognized as a queen before other activities such as retinue formation and feeding with royal jelly (Snodgrass, 1956) can proceed. Similarly, the ability of worker bees to recognize brood, its stage of development, sex, and caste, is a prerequisite for appropriate brood care and larval feeding (Free, 1987).

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Workers distinguish pupae of different ages (Free and Winder, 1983). They apparently can distinguish the larvae of all three castes, since drone, worker, and queen larvae receive food with different compositions (Gontarski, 1954; Shuel and Dixon, 1959; Jung-Hoffmann, 1966). In addition, nurse bees can also recognize the age of larvae, as the feeding behavior and composition of larval food vary with larval age (Shuel and Dixon, 1959; Jung-Hoffmann, 1966; Thrasyvoulou *et al.*, 1983; Brouwers, 1984; Beetsma, 1985; Brouwers *et al.*, 1987). Workers also seem to determine the food requirement of larvae during the inspections (Huang, 1988).

The recognition of certain brood characteristics and other conditions could occur at different levels. A worker could determine all those characteristics in one step without actually entering each cell. Alternatively, it could perceive a series of signals in steps: first differentiating brood cells from other cells, perhaps without actually going into each cell; then determining whether the brood is in the egg or larval stage; and finally, after receiving more elaborate stimuli, recognizing the actual age of either egg or larva and responding accordingly.

To understand further brood–worker behavioral interactions, we tested whether worker bees would visit brood cells preferentially among a patch of mixed cells. A positive finding would suggest that brood has a relatively long-range (over the cell depth) signal or cue to communicate its presence to worker bees, resulting in an increased nursing efficiency.

MATERIALS AND METHODS

The experiment was conducted at the field laboratory of the University of Guelph, Ontario. The honey bees were a mixture of European bee races (*Apis mellifera* L.). A four-frame observation hive was established in late May 1987. The colony population was estimated to have 12,000–15,000 worker bees (estimated by counting bees in samples of quadrate on each frame) throughout the summer. The room temperature was controlled at 31 ± 1°C and kept dark except during the actual observation periods, when the room was illuminated with multiple fluorescent lights. Previous study (Huang and Otis, 1990) under the same lighting conditions reported highly consistent data on feeding behavior compared to those conducted under red light (Brouwers *et al.*, 1987).

A cohort of 100 worker bees less than 18 h old was color-marked with Testor's paint and introduced to the observation hive. About a week later, a queen was confined on an empty comb for 24 h in a source hive to obtain large numbers of eggs less than 24 h old. A brood pattern as shown in Fig. 1 was created by destroying every other egg both vertically and horizontally. This comb and another one (with larvae 1 to 3 days old and some capped brood) from the same source colony replaced two of the original combs in the observation hive at the start of the experiment. The location of each cell with an egg

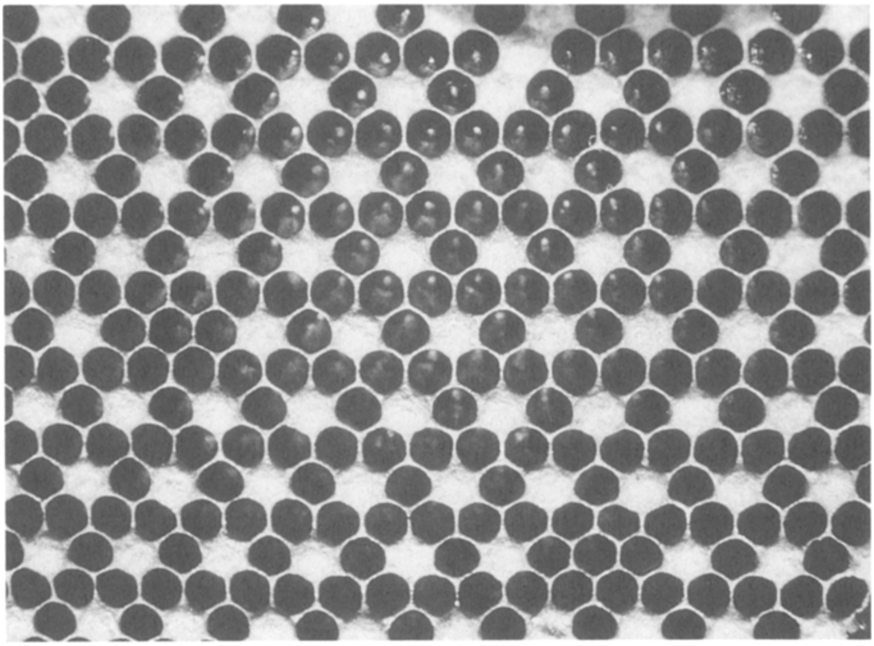

Fig. 1. Special brood pattern created to study the visitation of worker bees to brood vs empty cells. The pattern was created by destroying every other egg both horizontally and vertically. Approximately one of four cells contained an egg (or a larva after it hatches). This photo was taken after the experiment was over, when all cells contained capped brood.

and the perimeter of the area with eggs were marked on the glass surface of the observation hive. The queen in the observation hive remained confined within a 12 × 12-cm iron-mesh screen cage during the experiment, through which she could be fed by workers and laid some eggs within. No signs of queen rearing were observed.

Observations started the next day and terminated 6 or 7 days later, when some cells were beginning to be capped by worker bees. During observations, a marked bee was randomly chosen in the brood area, and its visitation sequence to cells was recorded. When a bee inserted its head or head and thorax into a cell, the content of the cell (e.g., none, egg, larva) was recorded. Observation of a particular bee was terminated when it left the delineated brood area. Observations (50 ± 10 min) were made once a day between 0800 and 1800, and five to eight marked bees were followed during each time period. Two trials were performed for this experiment in the same observation hive 20 days apart. Observations were made of 7- to 13-day-old bees in trial 1 and 10- to 15-day-old bees in trial 2.

Two things can happen if a bee can discriminate brood cells from empty ones: it either enters brood cells preferentially or does so to empty cells. A bee could also not discriminate brood cells from empty cells and thus visit both types of cells "randomly"; i.e., the visitation rates to either cells depend on their relative abundance. The serial visitations to both cell types by a worker bee during an observation bout can be described by a binomial distribution. We used the following formula to calculate the probability of a bee either (1) preferentially entering brood cells—this hypothesis is tested when observed number of visits to brood cells are larger than expected—or (2) preferentially entering empty cells—this is tested when observed number of visits to brood cells are smaller than expected:

$$\sum P(>Y) = \frac{N!}{T!X!} p^T (1 - p)^X, \qquad T = Y, Y + 1 \cdots N \tag{1}$$

$$\sum P(<Y) = \frac{N!}{T!X!} p^T (1 - p)^X, \qquad T = Y, Y - 1 \cdots 0 \tag{2}$$

where p is the probability that a randomly-selected cell contains brood. The value p is the ratio of brood cells to total cells (brood + empty), which was close to 0.25 in both trials. X is the number of observed visits to empty cells, Y to brood cells, and N is the total of X and Y. A value of $\Sigma P((>Y)$ or $\Sigma P(<Y)$ smaller than 0.05 indicates that it is unlikely (i.e., $P < 0.05$) that such an event would occur if the bee is visiting indiscriminately, and the null hypothesis can thus be rejected for that particular bee. Experimental-wise significance was tested by χ^2 test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results reported in Tables I and II are itemized by each "bee bout"—the numbers of visits to both empty and brood cells by a bee during one observation period. Since bees were not individually identified, the same bee could potentially be recorded in several bee bouts here. Nevertheless, due to the relatively large number of bees compared to the bee bouts (200 vs 86), this should not be a serious bias.

With a few exceptions, workers bees biased their visits to larval cells, and the majority (59 and 69% for trials 1 and 2, respectively) of them tested significant at 5% level or higher (Tables I and II). χ^2 test indicated that both these proportions were too high to be attributable to the associated type I error rate ($P < 0.001$). At the other extreme, two bees (Nos. 24 and 25, Table II) bias their visits strongly toward empty cells, but only one (No. 24) was significant; the other one was marginal. For cells with eggs, the picture appears to be quite different. Among a total of 24 bees observed, only 2 bees (No. 1 in Table I and No. 1 in Table II) significantly preferred egg cells, while more bees (5 of 12 in

Table I. Results of Trial 1

Brood stage	Bee age (days)	Bee bout	No. visits to brood[a]	No. visits to empty[a]	$\Sigma P(>Y)^b$	$\Sigma P(<Y)^c$
Eggs	7	1	6 (2.3)	3 (6.7)	0.01**	
		2	5 (3.3)	8 (9.7)	0.21	
		3	1 (2.3)	8 (6.7)		0.30
		4	6 (5.5)	16 (16.5)	0.49	
		5	4 (3.7)	11 (11.3)	0.54	
		6	4 (3.7)	11 (11.3)	0.54	
		7	4 (3.3)	9 (9.7)	0.42	
		8	4 (1.8)	3 (5.2)	0.07	
Eggs	8	9	7 (6.3)	18 (18.7)	0.44	
		10	3 (2.3)	6 (6.7)	0.40	
		11	6 (5.5)	16 (16.5)	0.48	
		12	3 (2.8)	8 (8.2)	0.58	
Larvae	9	13	6 (3.3)	7 (9.7)	0.08	
		14	7 (3.8)	8 (11.2)	0.06	
		15	8 (2.5)	2 (7.5)	0.00***	
		16	11 (3.8)	4 (11.2)	0.00***	
		17	5 (1.8)	2 (5.2)	0.01**	
		18	11 (3.5)	3 (10.5)	0.00***	
		19	7 (2.8)	4 (8.2)	0.01**	
Larvae	10	20	4 (1.3)	1 (3.7)	0.02*	
		21	8 (2.8)	3 (8.2)	0.00**	
		22	7 (2.0)	1 (6.0)	0.00***	
		23	3 (2.3)	6 (6.7)	0.40	
		24	4 (4.0)	12 (12.0)	0.60	
		25	4 (2.5)	6 (7.5)	0.23	
Larvae	11	26	8 (3.0)	4 (9.0)	0.00**	
		27	6 (5.8)	17 (17.2)	0.53	
		28	8 (3.3)	5 (9.7)	0.01**	
		29	5 (3.3)	8 (9.7)	0.21	
		30	1 (2.3)	8 (6.7)		0.30
		31	3 (2.3)	6 (6.7)	0.40	
		32	8 (2.0)	0 (6.0)	0.00***	
		33	9 (3.0)	3 (9.0)	0.00***	
Larvae	12	34	11 (2.8)	0 (8.2)	0.00***	
		35	9 (2.8)	2 (8.2)	0.00***	
		36	9 (2.5)	1 (7.5)	0.00***	
		37	4 (4.3)	13 (12.7)		0.57
		38	4 (3.3)	9 (9.7)	0.42	
		39	10 (4.3)	7 (12.7)	0.00**	
Larvae	13	40	6 (5.5)	16 (16.5)	0.49	
		41	5 (3.0)	7 (9.0)	0.16	
		42	8 (2.3)	1 (6.7)	0.00***	
		43	7 (3.3)	6 (9.7)	0.02*	
		44	6 (2.0)	2 (6.0)	0.00**	
		45	4 (3.0)	8 (9.0)	0.35	
		46	8 (3.8)	7 (11.2)	0.02*	

[a] "No. visits to brood" and "No. visits to empty" represents the number of observed visits to brood (egg/larval) cells and empty cells, respectively. The ratio of brood cells to total cells in this trial is 240/957. The numbers in the parentheses are the expected number of visits to brood cells and empty cells, if random, respectively. They were calculated as, respectively, 0.251 × total visits (No. visits to brood + No. visits to empty) and (1 − 0.251) × total visits.

[b] "$\Sigma P(>Y)$" is the calculated accumulative binomial probability for a bee to visit the observed number of brood cells and more, when assuming the bees are visiting the brood cells randomly. This test was done on bee bouts which biased toward brood cells.

[c] "$\Sigma P(<Y)$" is the calculated accumulative binomial probability for a bee to visit the observed number of brood cells and less, when assuming the bees are visiting the brood cells randomly. This test was done on bee bouts which biased against brood cells.

Table II. Results of Trial 2.

Brood stage	Bee age (days)	Bee bout	No. visits to brood[a]	No. visits to empty[a]	$\Sigma P(>Y)$	$\Sigma P(<Y)$
Eggs	10	1	6 (1.6)	1 (5.4)	0.00***	
		2	4 (2.3)	6 (7.7)	0.18	
		3	1 (5.7)	24 (19.3)		0.01**
		4	3 (4.6)	17 (15.4)		0.30
		5	1 (3.7)	15 (12.3)		0.09
Eggs	11	6	0 (2.7)	12 (9.3)		0.04*
		7	2 (4.1)	16 (13.9)		0.18
		8	0 (3.0)	13 (10.0)		0.03*
		9	0 (3.4)	15 (11.6)		0.02*
		10	1 (3.9)	16 (13.1)		0.07
		11	1 (4.4)	18 (14.6)		0.05*
		12	1 (4.1)	17 (13.9)		0.06
Larvae	12	13	14 (4.4)	5 (14.6)	0.00***	
		14	7 (2.7)	5 (9.3)	0.01**	
		15	9 (3.7)	7 (12.3)	0.00**	
		16	6 (3.2)	8 (10.8)	0.08	
		17	6 (2.7)	6 (9.3)	0.04*	
		18	4 (2.3)	6 (7.7)	0.18	
Larvae	13	19	7 (2.3)	3 (7.7)	0.00**	
		20	6 (2.7)	6 (9.3)	0.04*	
		21	8 (3.4)	7 (11.6)	0.01**	
		22	11 (3.2)	3 (10.8)	0.00***	
		23	12 (4.8)	9 (16.2)	0.00***	
Larvae	14	24	0 (2.7)	12 (9.3)		0.04*
		25	0 (2.5)	11 (8.5)		0.06
		26	10 (3.0)	3 (10.0)	0.00***	
		27	4 (1.6)	3 (5.4)	0.05*	
		28	13 (3.4)	2 (11.6)	0.00***	
		29	9 (3.7)	7 (12.3)	0.00**	
		30	4 (1.6)	3 (5.4)	0.05*	
Larvae	15	31	5 (3.0)	8 (10.0)	0.15	
		32	4 (3.2)	10 (10.8)	0.40	
		33	12 (4.1)	6 (13.9)	0.00***	
		34	9 (3.9)	8 (13.1)	0.01**	
		35	11 (4.1)	7 (13.9)	0.00***	
		36	7 (3.9)	10 (13.1)	0.07	
		37	13 (4.6)	7 (15.4)	0.00***	
		38	4 (2.1)	5 (6.9)	0.13	

[a] The ratio of brood cells to total cells is 280/1223 in this trial. The numbers in the parentheses are the expected number of visits to brood cells and empty cells, if random, respectively. They are calculated as, respectively, 0.229 × total visits (No. visits to brood + No. visits to empty), and (1 − 0.229) × total visits. Other column headings the same as in Table I.

trial 2) preferentially visited empty cells over egg cells (χ^2 test, $P < 0.01$). Preferential visitation to larval cells was observed so frequently (62% of bees observed with both trials pooled, $P < 0.001$) that it probably represents a real biological phenomenon. In addition, the data suggest that cells with eggs are at times avoided. The different behavior of bees to egg and larval cells seems to indicate the brood signal is stage specific; i.e., the egg cells are recognized as different from larval cells without entering the cells first.

Thirty-seven percent of the worker bees failed to make the distinction between empty and larval cells. Those bees may have been less experienced or less specialized on brood rearing, or they may have been doing brood nest-related tasks other than brood tending. It has been well established that the temporal schedule of bees is not a rigid one. Individuals of the same age can behave quite differently in the same colony, with a rather high degree of plasticity (Lindauer, 1953; Kolmes, 1985).

Since a majority of bees preferentially selected cells with larvae over empty ones, it appears that the larvae in the cells emitted a signal to indicate their presence, as proposed by Huang (1988). The apparent ability of larvae to indicate their presence by a general signal is not too surprising, because nurse bees would thus be able to locate larvae without entering every cell and, as a result, increase their efficiency of nursing. Eggs may also have a signal, although the signal seems repellent since some bees avoided egg cells. Lineburg (1924) observed apparent "pausing" of nurse bees over cells without entering, frequently when the cell contains an egg or a young larva. In addition, he suggested that the queen may sense eggs, larvae, or even debris in cells by hesitating over cells without entering, and workers may also avoid egg cells without entering them. We observed similar behaviors, with the bees pausing and lowering the antennae toward the cell before entering or rejecting a cell. It is possible the bees were sensing the cell content during these pauses, and then entering or ignoring the particular cell as dictated by their physiological and/or behavioral conditions at that moment.

Our observations, together with those of Lineburg (1924), suggest that the signal(s)/cue(s) indicating the existence of larvae and eggs is (are) chemical in nature. A few studies have shown that volatile chemicals from larvae could be used in various ways of communication. Free and Winder (1983) suggested that a relatively involatile pheromone on the body surface of brood is responsible for the recognition of worker and drone larvae and pupae. Koeniger and Veith (1984) chemically identified a brood pheromone which induces warming behavior by workers. More recently, Le Conte *et al.* (1989) reported that simple aliphatic esters emitted from drone and worker larvae are used by a parasitic mite *Varroa* as cues to find its suitable hosts. Further studies are needed to see if the above-studied chemicals could be used by worker bees for larval recognition prior to entering cells.

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