Behav Ecol Sociobiol (1991) 28:61–76

Behavioral Ecology and Sociobiology

Self-organizing pattern formation on the combs of honey bee colonies

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Received April 18, 1990 / Accepted August 21, 1990

Summary. A characteristic pattern of brood, pollen, and honey develops on the combs of a honey bee colony, consisting of three distinct concentric regions – a central brood area, a surrounding rim of pollen, and a large peripheral region of honey. That the pattern is consistent and well-organized suggests its adaptive value for the colony, yet the mechanism of pattern formation has not been elucidated. Two hypotheses are presented. The blueprint (or template) hypothesis suggests that there are particular locations specified for the deposition of eggs, pollen and honey, i.e., the pattern develops as a consequence of the bees filling in the comb according to the orderly arrangement latent in the blueprint. An alternative is the "self-organization" hypothesis: pattern emerges spontaneously from dynamic interactions among the processes of depositing and removing brood, pollen and honey, without a plan specifying spatial relationships. Computer simulation of the self-organization hypothesis demonstrates how the colony-level pattern can emerge and how, using only local cues and simple behavioral rules, the bees can create an overall, global pattern of which they have no concept.

Introduction

1 The pattern

The typical feral honey bee colony comprises approximately 25000 adult worker bees and a single queen (Seeley 1985). In addition to the adult bees the colony includes brood (developing eggs, larvae and pupae) and a variable amount of accumulated food, namely honey and pollen. Brood and food are stored within the hive in a series of parallel wax combs subdivided into approximately 100000 cells. A characteristic well-organized pattern develops on the combs, consisting of three distinct concentric regions – a central brood area, a surrounding rim of pollen, and a large peripheral region of honey (Fig. 1). Inasmuch as the colony is a 3-dimensional structure, this pattern is most pronounced on the central combs which intersect a large portion of the roughly spherical volume of brood. The formation of this pattern of cell utilization is the subject of this report.

2 Presumed adaptive significance of the pattern

The well-organized pattern suggests its adaptiveness. A compact brood area may help to ensure a precisely regulated incubation temperature for the brood and may facilitate efficient egg laying by the queen. The location of pollen in the rim adjacent to the brood area, where it is readily accessible to the nurse bees, may promote efficient feeding of the nearby larvae.

The pattern is not only well-organized, but also consistent throughout the season. This feature may also be adaptive. Each day honey and pollen from tens of thousands of foraging grips are deposited into the cells, stores of honey and pollen cells are continually consumed, hundreds of eggs are laid, mature adult bees emerge from their cells, and diseased brood is removed. Despite the regular turnover in which cells are often refilled with something other than what they recently held, the stability of the pattern is maintained.

The presumed adaptive significance of the pattern raises the important question of what mechanisms account for its origin and maintenance. How does this colony-level pattern emerge from the activities of thousands of bees? I present two alternative hypotheses.

3 Alternative hypotheses of the pattern formation process

The conventional view, which I call the blueprint or template hypothesis, suggests that honey, pollen and eggs are deposited in particular locations on the comb. Many authors, in describing the characteristic pattern on the comb, seem to imply that this is the mechanism of pattern formation that operates in the honey bee col-

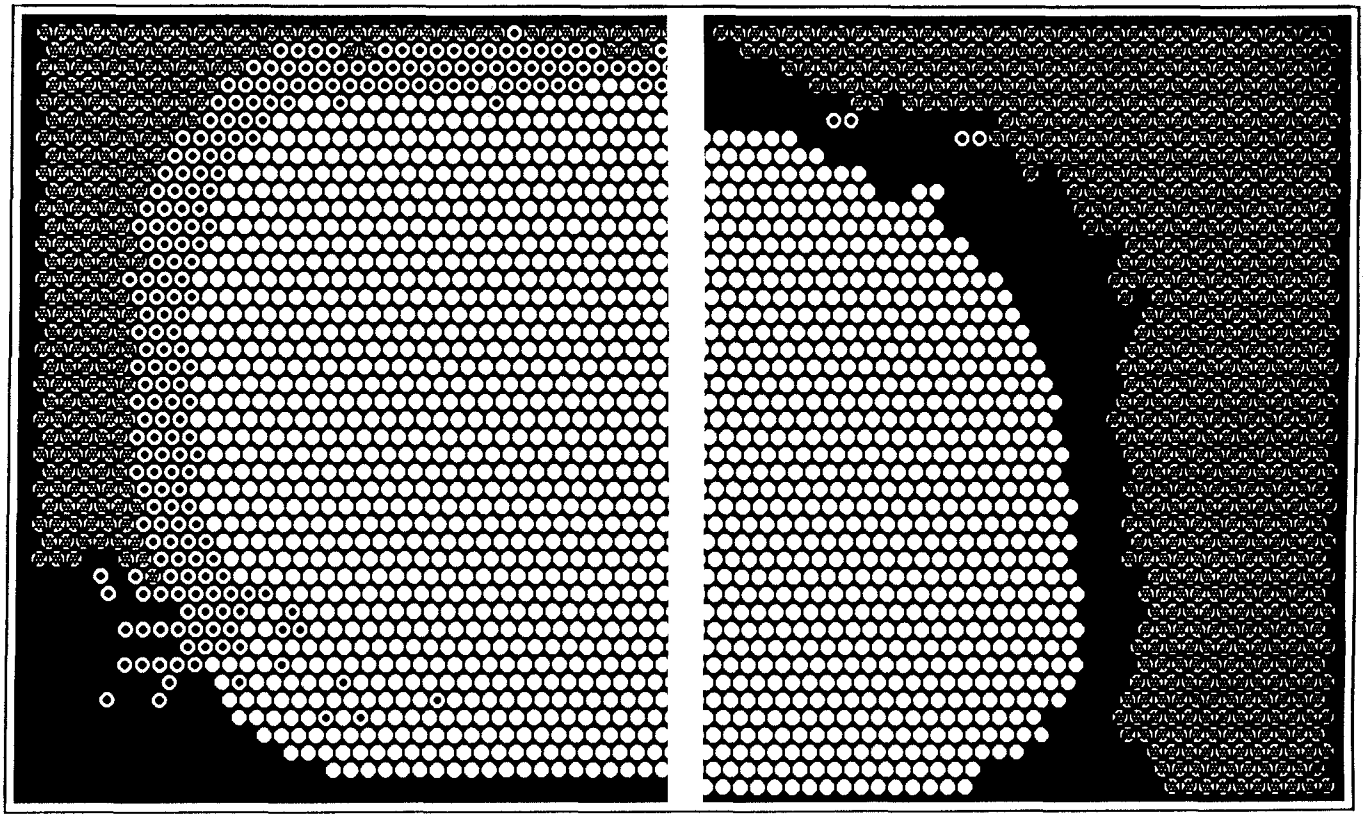


Fig. 1. Tracings made of frames taken from a standard 10-frame honey bee colony. *Left side:* the left half of a frame showing the characteristic pattern of centrally-located brood, a band of pollen and a large peripheral region of honey. *Right side*: the right half of a frame taken from a hive during early August when little pollen or honey was available. There is band of empty cells in the region previously containing pollen. Symbols are as follows: *White circles* = brood, *white circles with a black dot* = pollen, *grey areas* = honey, and *black* = empty cells

ony. For example, Winston (1987) states, "Pollen is usually placed in cells next to the brood nest, where it is easily accessible to nurse bees." Seeley and Morse (1976) note that "Honey and pollen are stored above and alongside the brood nest." In describing the feral colony, Seeley (1983) states, "Honey is stored in the upper region, pollen is packed in a narrow band directly below, and brood is reared in the lowermost portion of each comb."

As in a blueprint, the details of a plan specify the spatial relationships among the components. This is the way a crew of workmen build a house. An architect designs a building and draws up a plan. The workmen who actually construct the building merely arrange the component building blocks according to the static, predetermined blueprint. Can this type of mechanism explain the orderly pattern of brood, pollen and honey on the combs? Perhaps the bees have some means of knowing where on the comb to deposit eggs, pollen or honey. If so, the pattern could develop simply as a consequence of the bees filling in the comb according to the orderly arrangement latent in the blueprint. An excellent example of such a biological template is messenger RNA, which specifies a particular sequence of amino acids in a protein chain. We cannot understand how a particular protein forms by examining the constituent amino acids. We must refer to the RNA template.

In the case of pattern formation on the combs, this raises the question of the nature of the presumed blueprint. Conceivably, a temperature gradient from the center of the comb to the periphery could indicate where eggs, pollen or honey are to be placed. Alternatively the blueprint may be innate, with each bee knowing that the central portion of the comb is reserved for brood, and that pollen and honey are to be placed more peripherally.

Although, in principle, the blueprint hypothesis could account for the pattern formation, it is contradicted by certain observations which were the initial stimulus for this study. In particular, the blueprint hypothesis in inconsistent with the observation that pollen and honey are often deposited throughout the comb. As documented in the Results section, this becomes particularly obvious when adult bees eclose, vacating their pupal cells within the brood area, or when an empty comb is placed into a hive. In both situations, these empty cells are frequently filled with pollen or honey.

These observations suggested that a different mechanism must account for the process of pattern formation. In place of the blueprint hypothesis I propose a "self-organization" hypothesis in which the pattern emerges spontaneously from the interactions among the processes of depositing and removing materials from cells. If we understand the underlying constituents of the pattern formation process – egg laying, pollen and honey deposition, honey and pollen consumption, and the emergence of mature bees from their pupal cells – we can understand how the pattern arises. In this self-organizing system, there is no blueprint to specify locations for brood, pollen and honey; The dynamic relationships among the component processes of deposition and removal are sufficient to organize a pattern on the combs. The self-organization hypothesis is counterintuitive because a global, spatial pattern emerges automatically from processes based solely on local cues that lack long-range spatial information.

This report demonstrates how pattern formation on the comb of honey bee colonies emerges automatically through a self-organizing process. First, I present the behavioral rules of the bees and a set of parameter values which have been estimated from observations of the developing pattern, experimental manipulations, and previously published data. Then, through computer simulation, I combine these rules and parameter values into a model demonstrating the validity of the self-organization hypothesis. In the discussion I comment on the shortcomings of the blueprint hypothesis and speculate on certain adaptive features of the self-organization hypothesis. Finally, I discuss the role of self-organization in biological processes in general, and in colony-level processes of social insects in particular.

Materials and methods

1 Study site and honey bee colonies. All observations were made in Ithaca, New York with colonies of Italian bees (*Apis mellifera ligustica*). Except as noted, the data presented were obtained from colonies of bees kept in observation hives. The hives were scaled-down versions of the hive described by Visscher and Seeley (1982). The internal dimensions of each hive were 78 × 46.5 × 4.5 cm, and each contained three full-depth Langstroth frames and about 8500 bees. The hives were housed inside a building, and covered with panels of styrofoam insulation. The colony was fitted with a plexiglass tunnel which provided the bees with access to the outdoors. Additional data were obtained from colonies of bees kept outdoors in one- or two-story full-depth supers.

2 Observation procedures and data collection. Observations of individual bees were made by marking bees with colored numbered plastic tags glued to the thorax (Opalithplättchen, Chr. Graze, Endersbach, FRG). The movements and activities of the bees were recorded through tracings made on a glass pane placed over the glass wall of the observation hive. Tracings also permitted changes in the contents of cells to be monitored without removing the frame from the hive. The hives were diffusely illuminated at all times. Additional illumination provided by a flashlight or high-intensity lamp permitted brief, more detailed examinations of cell contents or the behaviors of individual bees. At the end of an observation period, the overlying glass pane was removed and photographed against a white background to provide a permanent record. Further details of each experiment are presented along with the results.

3 Computer simulations. Computer simulations were done using the Pascal programming language on a Macintosh II computer. A hexagonal grid of 3300 cells (75 cells × 44 cells) was simulated. This represents the approximate number of cells on one side of a full-depth Langstroth frame.

4 Statistical analyses. Unless otherwise stated, measurements are given as the mean ± one standard deviation. Statistical tests are *t*-tests, either Student's or the test for equality of two proportions using arcsine transformations (Sokal and Rohlf 1969).

Results

1 Self-organization hypothesis: behavioral rules and parameter values

An hypothesis for pattern formation should explain not only the ultimate concentric pattern of brood, pollen and honey, but also the development of the pattern from its initial stages. In addition, a parsimonious explanation should be able to account for both the initial and mature patterns through the same process.

I describe how the cell use pattern emerges by considering each process that affects the deposition and removal of eggs, pollen and honey. For each kind of material, I describe how the behavioral rules of the individual bees contribute to its spatial pattern on the comb, and I estimate the parameter value describing its rate of deposition or removal.

Brood input: egg-laying by the queen. The maximum egg-laying rate of a queen honey bee has been reported to be between 1000 and 2000 eggs/day (0.7 to 1.4 eggs/min) (Brünnich 1923, Nolan 1925, Bodenheimer 1937). An additional estimate of the egg-laying rate in observation hives was obtained by following the queens of 2 hives for 8 periods of 30 to 90 min each for a total of 350 min. The average egg-laying rate per period was 0.71 ± 0.3 eggs/min (range 0.4 to 1.1 eggs/minute), essentially the same as that in full-sized colonies. Values at the lower end of the range corresponded to times when the hive had few available empty cells, while greater values were obtained when an empty frame was placed in the hive.

In addition to providing data on the rate of oviposition, the observations also reveal the pattern of egg laying. Figure 2 is a typical record made by tracing the path of the queen as she laid. It shows the locations of 43 consecutive ovipositions (indicated by numbered cells) made over a one-hour period, 9:00 AM to 10:00 AM. The empty frame was placed in the colony the previous evening. Prior to the morning observation period, 82 eggs were laid in the central portion of one side of the frame (indicated by white circles). As shown, these initial ovipositions were not placed contiguously but were somewhat scattered. Although the subsequent (numbered) ovipositions filled many of the gaps, the queen's pattern of egg laying was nonetheless quite unsystematic. The queen often zigzagged over the frame, doubling back to a spot where she just oviposited. Rather than exhaustively searching for all available cells in an area, the queen successively returned to an area to fill in empty cells.

This raises the question of how the queen selects cells. Each cell is carefully inspected prior to oviposition, but not every empty cell is accepted. The queen apparently takes into account the presence of nearby brood, since her ovipositions are restricted to cells relatively close to other cells which contain brood. In addition, she appears to take into account the boundaries of the comb upon which she is laying, since it is observed that, on an empty comb, eggs are initially laid in the central area of the frame.

Figure 3 (derived from the tracing of Fig. 2, and from similar data) demonstrates the marked tendency for eggs to be laid near one another. Of 143 ovipositions, the mean distance to the nearest egg (i.e., the number of intervening non-brood cells) was 0.7±1.0. For 95% of all ovipositions, there were less than 3 intervening nonbrood cells.

Thus, the queen's route while ovipositing can be characterized as an unsystematic zigzagging across the comb, during which each egg is laid, on average, within a few cell lengths of the nearest brood-containing cell. How a compact brood area develops from this pattern of movement will become more apparent after examining the dynamics of honey and pollen cells.

Honey and pollen input. Pollen foragers returning from the field select a cell in which to deposit their pollen loads. In contrast, the honey foragers regurgitate their nectar to the food storer bees which then select a cell in which to unload. Where do these bees place their nectar and pollen?

The original observation which prompted the formulation of the self-organization hypothesis was that pollen and honey was regularly, and unexpectedly, deposited within the brood area. This can be observed both in observation hives and in full-sized colonies. An example is shown in Fig. 4a, a tracing of a frame from an observation hive. Although much of the pollen is confined to a band at the edge of the brood area, and most of the honey is in the periphery, pollen and honey are also seen scattered within the brood area.

To document the pattern of honey and pollen deposition more precisely, two types of experiments were performed. In the first, an empty frame was placed in an observation hive in the morning (prior to foraging) and was removed at the end of the day. The location of each cell containing honey or pollen was recorded by photographing the frame. This was repeated five times in late August, 1989, at a time when both nectar and pollen were being collected. Figure 5 is a typical record. Pollen and honey are found throughout the frame. Cells near the edge of the frames received little nectar and pollen, and cells in the upper portion of some frames were used in preference to cells lower on the frame. Nonetheless, there is no indication that the bees select particular regions for pollen and honey, or that a portion of the frame is reserved for brood, as would be predicted by the blueprint hypothesis. (The predominance of nectar on the frame reflects the relatively greater rate at which the bees were bringing in nectar during this period.)

Although bees deposited pollen and honey in cells throughout an empty frame, perhaps the pattern of deposition would be different on frames containing brood. The central brood area could potentially provide bees with a cue enabling them to deposit nectar and pollen in specific locations. Therefore, a second set of experiments was performed.

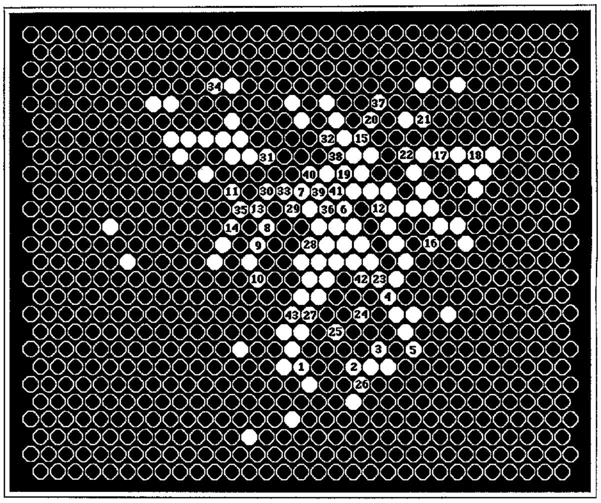


Fig. 2. The central portion of an initially empty frame showing cells in which the queen oviposited. An empty frame was placed in the colony on the evening of May 15, 1989. The queen was observed for 1 h (9:00 AM to 10:00 AM May 16). The numbers indicate the 43 consecutive ovipositions observed during this period. *White circles* indicate 82 ovipositions made prior to the period of observation

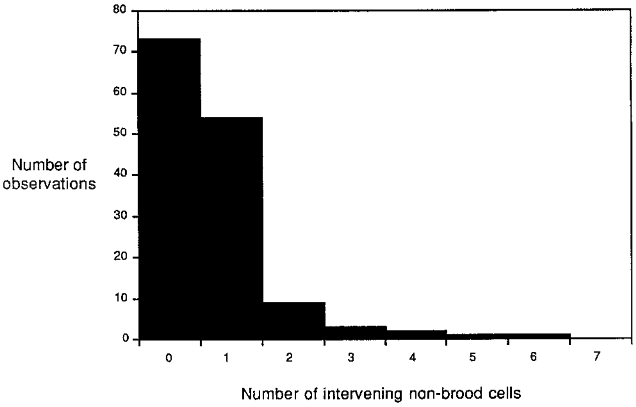


Fig. 3. Frequency histogram demonstrating the tendency of the queen to lay eggs near other brood cells. The *bars* represent the frequency with which eggs were deposited at specified distances to the nearest brood cell. Values indicate the number of intervening non-brood cells for each of 143 ovipositions

A frame with a large central brood area was chosen from a single-super outdoor colony. A block of approximately 100 cells (10 cells by 10 cells) was cut from the brood area in the center of the frame and another block of 100 cells was removed from the honey area in the periphery. Replacement sections were cut from a single dark empty frame that had previously been used for brood rearing. (This could be determined readily by the presence of cocoons lining the cells.) The sections were held in place with a small amount of melted beeswax. The frames were then returned to their original locations in the hive. Over next several days, the frames were removed briefly and the cells containing honey, pollen or

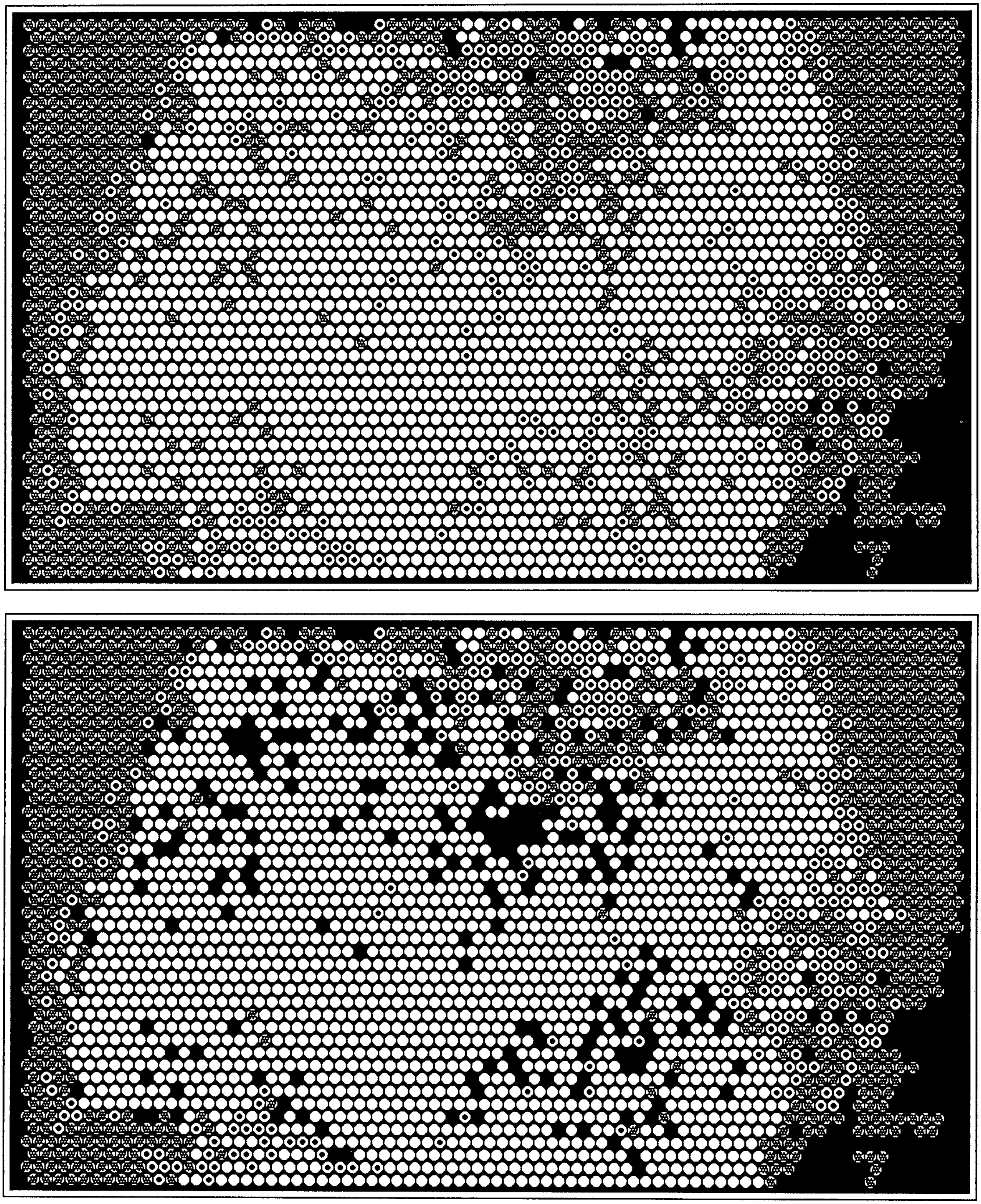


Fig. 4. 2 tracings of the same frame showing the preferential emptying of honey and pollen from cells nearby brood. The upper tracing was made on July 6, 1988 at 7:00 PM, at the end of the foraging day, and the lower tracing was made the following morning at 8:00 AM, prior to foraging. Note the preferential emptying of honey and pollen cells within the brood area or at the edge of the brood area. Symbols as in Fig. 1. See Table 2 for statistical analyses

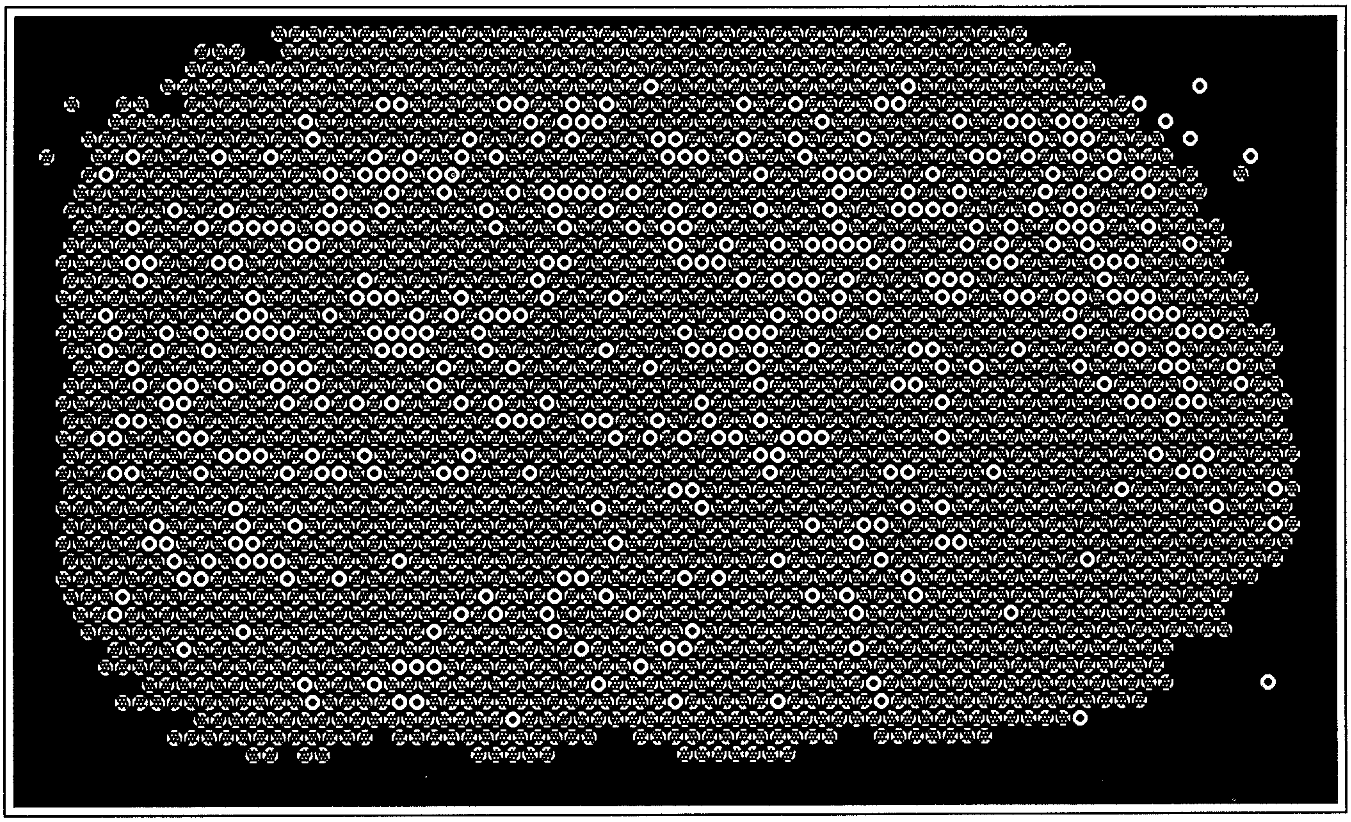


Fig. 5. Tracing of a frame showing the pattern of deposition of honey and pollen on an empty frame. An empty frame was placed in an observation hive at the beginning of the day (prior to the start of foraging), and removed at the end of the day. Symbols as in Fig. 1

eggs were counted in both the central and peripheral block. Counts were made on each of the first 3 days, and at less frequent intervals thereafter. (In 1 of the 4 replicates, counts were not taken on days 1 and 2 because of rain). Data were collected from both sides of each frame, and each side was scored separately.

The results (Table 1) show that, over the first 3 days, pollen and nectar were deposited with equal frequency both in the central block of cells and in the peripheral block of cells. There was no significant difference between the central and peripheral block with respect to the average number of cells containing honey, pollen or eggs on the first 3 days ($p<0.001$, t-test comparison between means). Clearly, neither the presence of brood nor a central brood area prompts the bees to deposit honey and pollen in particular locations.

However, on subsequent days, the contents of the implanted blocks changed considerably. Greater numbers of eggs were laid in the central cells than in the peripheral cells, reflecting the queen's reluctance to lay eggs outside the central brood area. In addition, the number of honey and pollen cells declined in the central area. Clearly, removal, rather than deposition alone, affects the pattern of materials in the central brood area. The important role of honey and pollen removal is addressed in the next section.

Honey and pollen removal. In striking contrast to the essentially random pattern of deposition, the removal of both honey and pollen occurs preferentially from cells nearby brood. The pattern of removal was demonstration in 2 types of experiments. In the first experiment I document the overall dynamics across the comb. In the second experiment I focus attention on individual cells and the behavior of the bees which visit them.

Experiment 1. First, I marked the contents (brood, pollen, or honey) of all cells on one side of a frame by tracing on a pane of glass laid against the glass wall of the observation hive. Then, after a specified time interval, I made note of which cells had been emptied in the intervening period, and which still contained honey and pollen. Because observations were made during a period of little or no foraging (overnight in two cases and on a cloudy day with little foraging in a third case), the changes on the frame largely reflected the removal of honey and pollen, rather than a combination of deposition and removal which would complicate the analysis. Results of the three trials are given in Figs. 4 and 6, and Table 2. Figure 4a and 4b are the tracings from the first trial (July 6, 7:00 PM to July 7, 8:00 AM, 1988) showing the overnight emptying of honey and pollen cells. Here, all of the emptied pollen and honey cells were from cells located within 2 cells or less from a cell containing brood; no pollen or honey cells further from brood were emptied. Similar, but less striking results, were found in the other two trials. Figure 6, based on the data from all three trials, illustrates how brood prox-

Table 1. Results of the experiment in which blocks of empty cells were implanted in the central brood area and the periphery of the frame. Data are mean numbers of cells with honey, pollen or brood (± st. dev.) [*N*]. Significance values are *t*-test comparisons between means

Day	Central block			Peripheral block			Significance
Honey							
1	25.8	(±20.6)	[6]	16.2	(± 9.2)	[6]	NS
2	40.7	(±32.2)	[6]	37.8	(±20.0)	[6]	NS
3	39.5	(±32.3)	[8]	51.9	(±24.5)	[8]	NS
> =4	23.1	(±17.0)	[14]	74.1	(±14.2)	[14]	<0.001
Pollen							
1	0.8	(± 2.0)	[6]	0.7	(± 1.6)	[6]	NS
2	1.2	(± 2.5)	[6]	1	(± 1.7)	[6]	NS
3	1.5	(± 2.5)	[8]	1.4	(± 1.8)	[8]	NS
> =4	17.1	(±14.2)	[14]	4.4	(± 1.6)	[14]	0.03
Brood							
1	0	(± 0.0)	[6]	0	(± 0.0)	[6]	NS
2	0	(± 0.0)	[6]	0	(± 0.0)	[6]	NS
3	0	(± 0.0)	[8]	0	(± 0.0)	[8]	NS
> =4	16.5	(±28.1)	[14]	0	(± 0.0)	[14]	0.04

imity affects the probability that a honey or pollen cell will be emptied. For each of the three trials I divided the honey and pollen cells on the frame into 11 classes indicating the distance of the cell to the nearest brood cell (The 0 category represented a honey or pollen cell adjacent to brood, and category 10 included all cells 10 cell widths or more from the nearest brood cell.) I then calculated the percentage of honey or pollen cells in each category that were emptied in the intervening period. The graphs indicate that a cell containing honey or pollen is more likely to be emptied if there is brood nearby.

In Table 2 I analyze the data in a different manner. For the 3 trials, the distance to the nearest brood cell was measured for each pollen and honey cell. The mean distance was calculated for honey and pollen cells that were not emptied and compared to the mean distance for cells that were emptied. Both honey and pollen cells that were emptied were significantly closer to brood in all trials ($p < 0.001$).

Presumably, the emptying of cells is the result of consumption of honey and pollen by the bees, rather than a reshuffling from cell to cell. For pollen this is almost certainly true since bees have no known way to move pollen from one cell to another. It is possible, though unlikely, that the observed loss of honey from nearby the brood results from bees moving honey out of the brood area.

Experiment 2. If honey and pollen are removed at a greater rate from cells nearby brood, then one would expect the bees to visit these cells more frequently or spend more time within these cells compared with the more peripheral cells, or both. This was demonstrated by comparing the visitation rate of pollen cells located in or adjacent to the brood area versus cells located in the periphery. For each of 7 trials, 10 pollen cells were randomly chosen from within or adjacent to the brood area and another 10 cells randomly chosen more peripherally, at least 3 cells from the nearest brood cell. At 8 min intervals over a 2-h period, observations of each cell were made, and the presence or absence of a bee in the cell was recorded. In addition, throughout the observation period all the cells were watched for bees depositing pollen in any of the cells. No bees were observed to make pollen deposits; therefore the occupancies are assumed to reflect visits in which bees were removing (presumably consuming) pollen from the cell, rather than packing pollen shortly after a deposit. The results are presented in Table 3. For each trial the occupancy (the proportion of the observations in which a cell was occupied by a bee) for the cells near brood was greater than the occupancy of peripheral cells. For all 7 trials, the mean occupancy was significantly greater for the central versus peripheral cells ($p = 0.001$). For 5 of the 7 trials, the cells were examined the next day to determine whether the increased occupation actually reflected the removal of pollen. All 50 of the peripheral cells still contained pollen, but only 40% of the 50 central cells had pollen. The most likely explanation of these results is that most of the pollen consumption occurs in the brood area. Probably it is the younger bees that eat most of the pollen feed to the larvae. These nurse bees restrict most of their activity to the brood area (Seeley 1982), and may therefore account for the preferential removal. Alternatively, all of the workers may be consuming pollen at a similar rate, but they might preferentially feed from cells near brood. Whatever the explanation, these experiments further demonstrate an increased removal of pollen in those cells surrounded by, or nearby, the brood.

Although it would have been desirable to perform the equivalent experiment comparing the occupancy of honey cells nearby brood with those in the periphery,

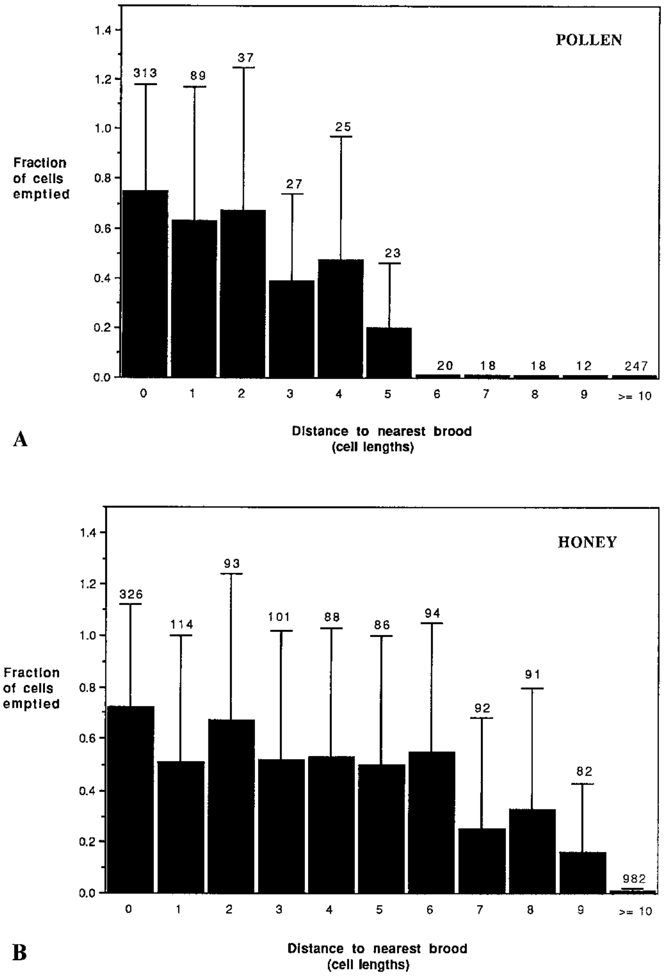


Fig. 6. **A** The proportion of pollen cells emptied as a function of proximity to brood. **B** The proportion of honey cells emptied as a function of proximity to brood. Each of the 11 categories (0 to > = 10) is a measure of the distance of a cell to its nearest brood neighbor (0 indicates a cell adjacent to brood, > = 10 indicates a cell with 10 or more intervening non-brood cells). For each category the fraction of cells emptied during the period is indicated. The plots show the averages of the 3 trials of Table 2. *Error bars* are one standard deviation. *Numbers* above each bar are the total number of cells in each category for all 3 trials. (In **A**, bars in categories 6 to > = 10 represent values of 0.) Further details of each trial are given in Table 2

this is not feasible since it is not possible to know whether a worker in a honey cell is visiting the cell to deposit nectar or to remove honey.

From experiment 2 I have estimated the relative rate at which pollen is removed from a central versus a peripheral cell, a parameter which I call the preferential removal factor. This was calculated from the data of Table 3. For each trial I took the ratio of the occupancy of central versus peripheral cells. The mean of these ratios (9.98) is the preferential removal factor for pollen used in the simulations below. Lacking an experimental estimate for the preferential removal of honey, I have used the value obtained for pollen cells. This is justified on the basis of Fig. 6, which shows that the probability distributions for cell emptying as a function of distance to the nearest brood cell are similar for both honey and pollen cells. Although, for the honey cells, there is less of a difference between the central and peripheral cells, there is clearly preferential removal from nearby brood.

Honey and pollen parameter values and additional assumptions of the model. Several other biological parameters enter into the model of pattern formation. The brood development time is taken as 21 days, the average time a worker bee requires to complete its development from egg to adult stage (Winston 1987). The model disregards drone brood and the loss of brood from developmental errors (diploid males) or disease.

The other important parameters concern honey and pollen deposition and removal rates. During the foraging season a typical colony collects 60 kg honey (Seeley 1985). A reasonable estimate for the amount of honey consumed during that period is 35 kg, or about 60% of the input. A colony collects about 20 kg of pollen through the season, but consumes nearly all of that, overwintering with perhaps 1 kg. Since pollen input and consumption are nearly equal, pollen generally does not accumulate in the colony to a significant extent. However, wide fluctuations in pollen foraging regularly occur as pollen-producing plants come into bloom, and result in periods in which net pollen input is relatively large and available cells rapidly fill with pollen. In 1 study the ratio of incoming pollen foragers to nectar foragers,

Table 2. The relationship between the emptying of a pollen or honey cell and the distance of that cell to the nearest brood cell. Data are distances (in cell lengths) to the nearest brood cell (mean ± s.d. [*N*], where *N* = number of cells examined). A distance of zero corresponds to cells that are adjacent. All differences between the mean distances for cells emptied and cells not emptied are significant, $p < 0.001$

Colony number date time interval	Pollen cells		Honey cells	
	Emptied	Not emptied	Emptied	Not emptied
# 1 July 6-July 7 1900 h–0800 h	0.01 ± 0.1 [71]	0.7 ± 1.3 [313]	0.03 ± 0.2 [143]	3.1 ± 3.0 [706]
# 2 July 26-July 27 1500 h–0900 h	1.6 ± 1.5 [71]	13.9 ± 6.3 [338]	3.4 ± 1.6 [14]	22.0 ± 6.9 [277]
# 2 July 29-July 30 0700 h–0800 h	1.0 ± 1.3 [29]	7.9 ± 5.6 [7]	6.5 ± 2.5 [274]	14.1 ± 3.5 [735]

Table 3. The proportion of observations in which centrally-located versus peripherally-located pollen cells were occupied by a worker bee. Each trial consisted of 10 pollen cells randomly chosen from within the center of the brood area and 10 cells randomly chosen from the periphery, outside the brood area. Each cell was observed 15 times at 8 min intervals over 2 h. Data are the proportion of the observations in which the cell was occupied by a bee. Differences between the means for the central versus the peripheral pollen cells (based upon arcsine transformations of the proportions) are significant, $p=0.001$

Trial	Colony	Date	Location of pollen cell			Ratio (center/periphery)
			Center	Periphery		
1	2	June 5	0.13	0.07		1.73
2	2	June 6	0.22	0.01		16.50
3	1	June 8	0.10	0.00		–
4	1	June 9	0.35	0.02		17.33
5	1	June 12	0.11	0.02		5.67
6	2	June 13	0.43	0.03		16.25
7	1	June 23	0.16	0.07		2.40
					Mean	9.98
					St. dev.	7.48

averaged across the day for many days, was 0.26 ± 0.19 (mean ± st. dev.) with a range of 0.06 to 0.83. (calculated from Visscher and Seeley 1982). In the simulations presented below I vary the pollen input within this range to reflect the fluctuations in pollen availability.

2 Computer simulations

To appreciate the consequences of the complex dynamics of deposition and removal for brood, pollen and honey, and the effects of various parameter values describing their rates, the biological details described above have been incorporated into a computer model which simulates the time course of filling of one side of a standard Langstroth frame (approximately 3300 cells) located in the central portion of the hive. The results of the simulations allow us to determine whether the behavioral rules and parameter values described in the preceding sections are sufficient to generate the observed pattern, and to determine which components of the model are necessary for the pattern formation.

The following summarizes the key behavioral components of the simulation:

1) Starting from the center of the frame, the queen moves randomly over the comb and lays eggs in any empty cell that is less than 4 cells to the next nearest brood cell. The maximum egg-laying rate is 1 egg/min. After 21 days the brood cell is vacated.

2) Honey and pollen are deposited in randomly-selected cells, either empty or partially filled with the same substance.

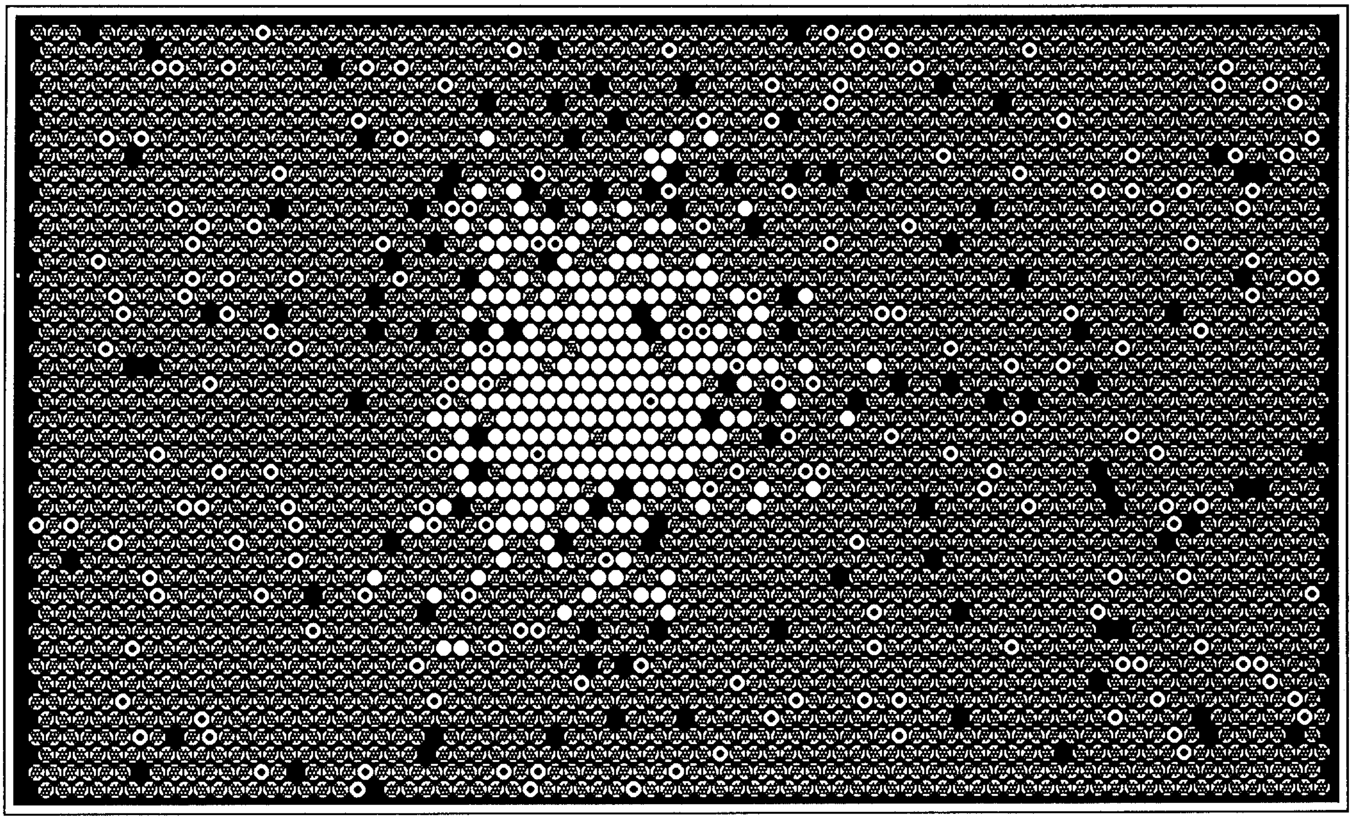
3) Honey and pollen are removed from randomly selected cells. However, the amount removed from each cell is linearly proportional to the number of immediate brood neighbors surrounding the cell. Cells completely surrounded by 6 brood cells are emptied at 10 times the rate of cells without adjacent brood. (The value of 10 is the estimated preferential removal factor).

The parameter values specifying the egg-laying rate and rates of honey and pollen deposition and removal were incorporated into the simulations as follows. During each min of the simulation a maximum deposition and removal rate was specified. However, only during the initial stages of the simulation are these maximal rates achieved, since the frame begins to fill and it becomes more difficult (at times impossible) to find cells in which to deposit honey and pollen or in which to oviposit. Parameter values were chosen to agree with the values given in the previous section (*Honey and pollen parameter values and additional assumptions of the model*). Thus, in the first simulation (Fig. 7) the ratio of honey removal to honey input was 0.59, the ratio of pollen removal to pollen input was 0.99, and the average ratio of pollen input to honey input was 0.21 (range = 0.03 to 0.56).

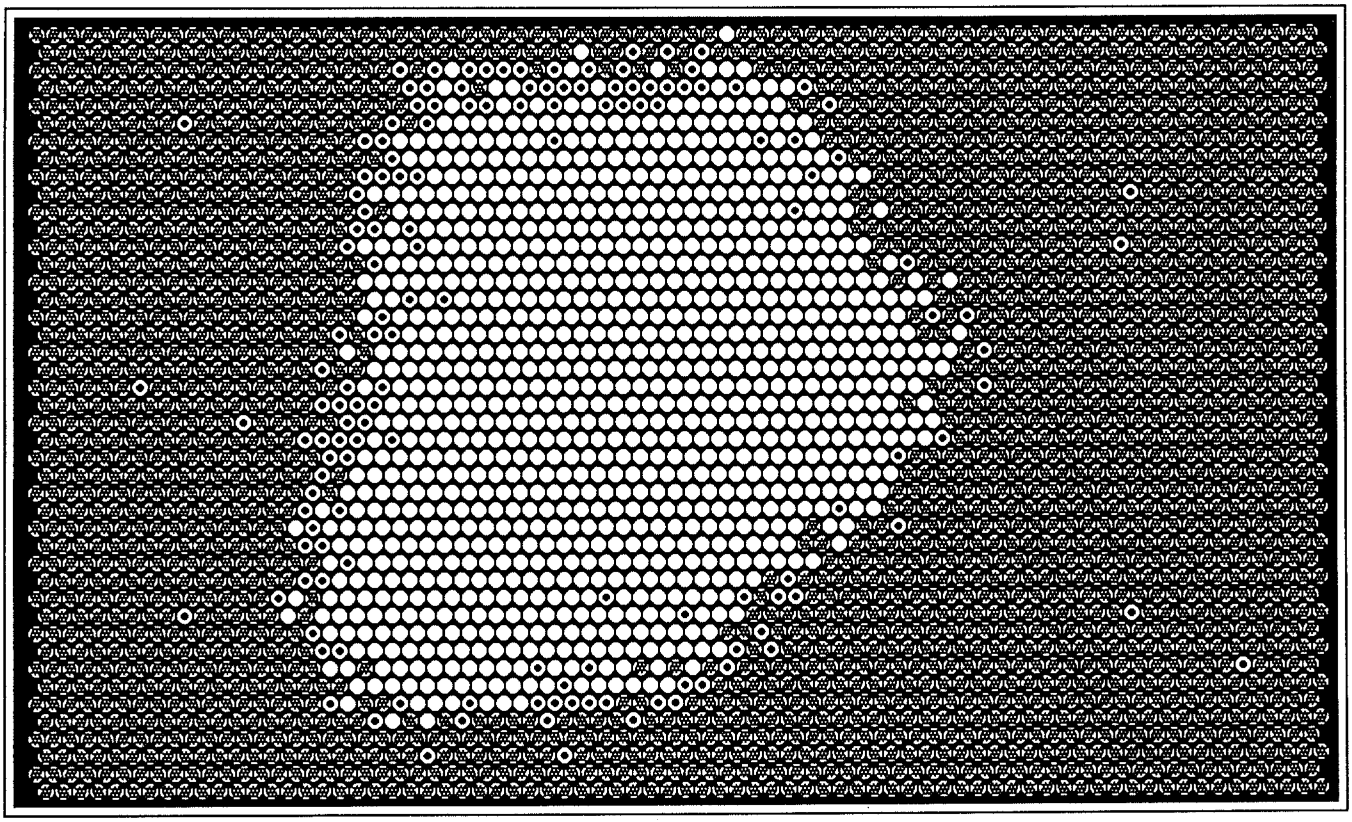
The simulation, depicted in Fig. 7, shows how the characteristic cell utilization pattern develops. Initially (Fig. 7a), pollen and honey accumulate throughout the frame as bees deposit their loads randomly on the empty frame, and the queen wanders over the frame from her central starting point. Similar to what is seen in Fig. 2 (an actual tracing of the queen's oviposition sequence), the center of the frame begins to fill with eggs, but a compact brood area has not yet developed. In fact, many of the cells interspersed among the eggs contain honey and pollen. I call this the early "disorganized" stage.

Several simulation days later (Fig. 7b) the characteristic well-organized pattern has formed. The central brood area is now compact. Honey and pollen in the periphery have segregated into a peripheral region, almost entirely honey, and a band of pollen adjacent to the brood area. How has this transformation occurred?

Observing the progression of the simulation reveals the interacting processes that contribute to the pattern formation. First, the central compact brood area results, in part, from the queen's tendency to lay eggs near one another. Even though pollen and honey are deposited within the brood area at the same time, their preferential consumption nearby the brood does not permit their

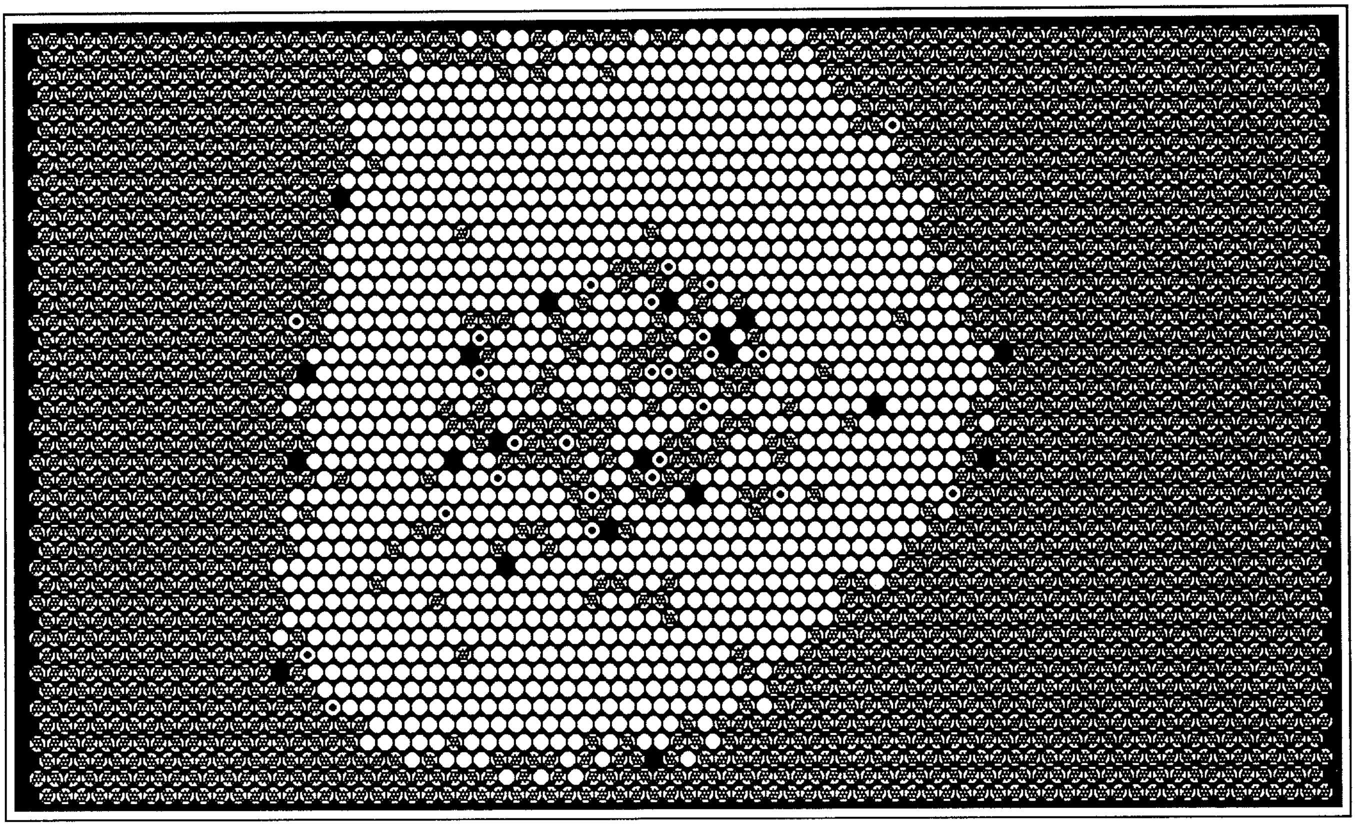


A



B

Fig. 7A–C. Computer simulation of pattern formation on the comb. **A** Day 1; **B** Day 7; **C** Day 22. Symbols as in Fig. 1. Parameter values as follows: ratio of honey removal to honey input = 0.59, ratio of pollen removal to pollen input = 0.99, average ratio of pollen input to honey input = 0.21 (range = 0.03 to 0.56), preferential removal factor = 10. See text for further details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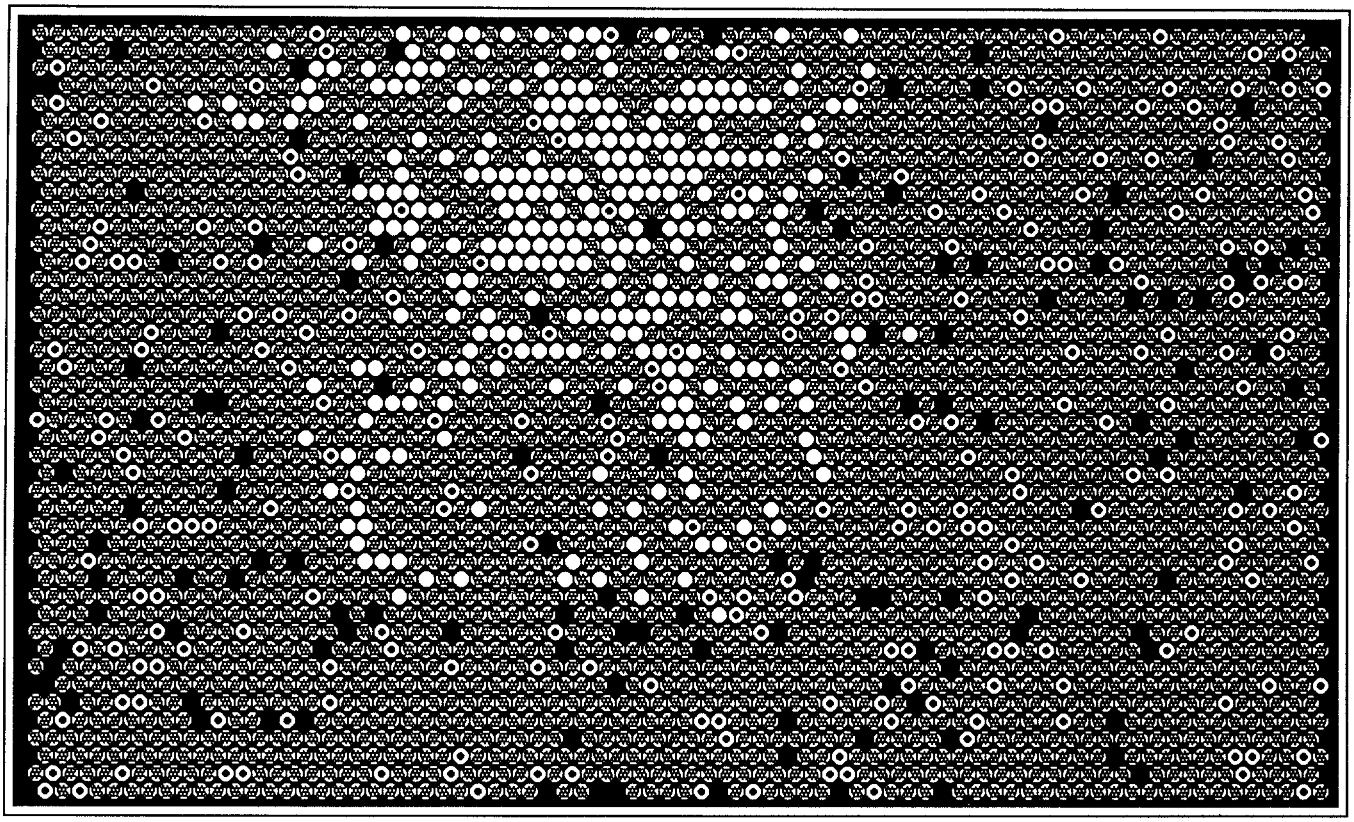
accumulation within the brood area. Thus, the brood area is continually freed of honey and pollen and filled with eggs, enhancing its compact structure. Even though the queen appears to be rather inefficient by continually retracing her steps, we see that it is necessary for her to repeatedly return to an area in order to discover new empty cells which are gradually cleared of pollen and honey. It would only be efficient to search for oviposition sites exhaustively and thoroughly in one area if that area were reserved for eggs. Since many of the cells nearby brood are often filled with honey and pollen, a better search strategy may be to quickly and cursorily pass through an area, and later return when more cells are emptied.

A second process explains the segregation of honey and pollen in the periphery. Since both are deposited randomly, initially pollen as well as honey appear in the periphery. However, recall that in a typical colony, on average, 95% of the collected pollen is consumed. With the normal fluctuations in pollen availability, much of the time there is a daily net loss of pollen. Thus, pollen is consumed in the periphery at nearly the same rate as it is deposited. Since honey is brought into the colony at a much greater rate than pollen, cells in the periphery that have been emptied of their pollen are therefore more likely to be replaced with honey. Gradually any pollen deposited in the periphery is removed, leaving this region almost entirely honey. Where, then, is pollen stored?

Eventually the only place a available for storage of pollen is in the band of cells adjacent to the brood. Once a cell in the brood area is occupied by an egg, it remains an occupied brood cell for the next 21 days as the bee develops. But in the interface zone between the brood and the peripheral stores of honey, the preferential removal of honey and pollen continually provides a region where cells are being emptied at a relatively high rate. These cells with a high turnover rate are available for pollen. The only other region where empty cells regularly become available are those cells in the brood area from which fully developed bees emerge. As shown in Fig. 4, bees readily put both honey and pollen in this area, but it is rapidly removed. This is also seen on day 22 of the simulation (Fig. 7c) in the region where the first group of "bees" emerges after their 21 days of development, and honey and pollen appear in the center of the comb.

This model is robust. Simulations under different parameter conditions demonstrate that the pattern formation is not dependent upon a particular narrow range of parameter values. The characteristic concentric pattern forms as long as the following conditions hold: (1) Eggs are deposited near previously laid eggs; (2) The preferential removal factor is greater than one; (3) The ratios of pollen and honey deposition and consumption correspond to rates typically found in nature. (These rates are as follows: a value close to 1 for the ratio of pollen removal to deposition, a value less than 1 for the ratio of honey removal to deposition, and a value less than 1 for the ratio of pollen foraging to honey foraging.) In contrast, the pattern does not form if these three conditions are not met.

For example, Fig. 8 demonstrates the importance of preferential removal of honey and pollen nearby brood.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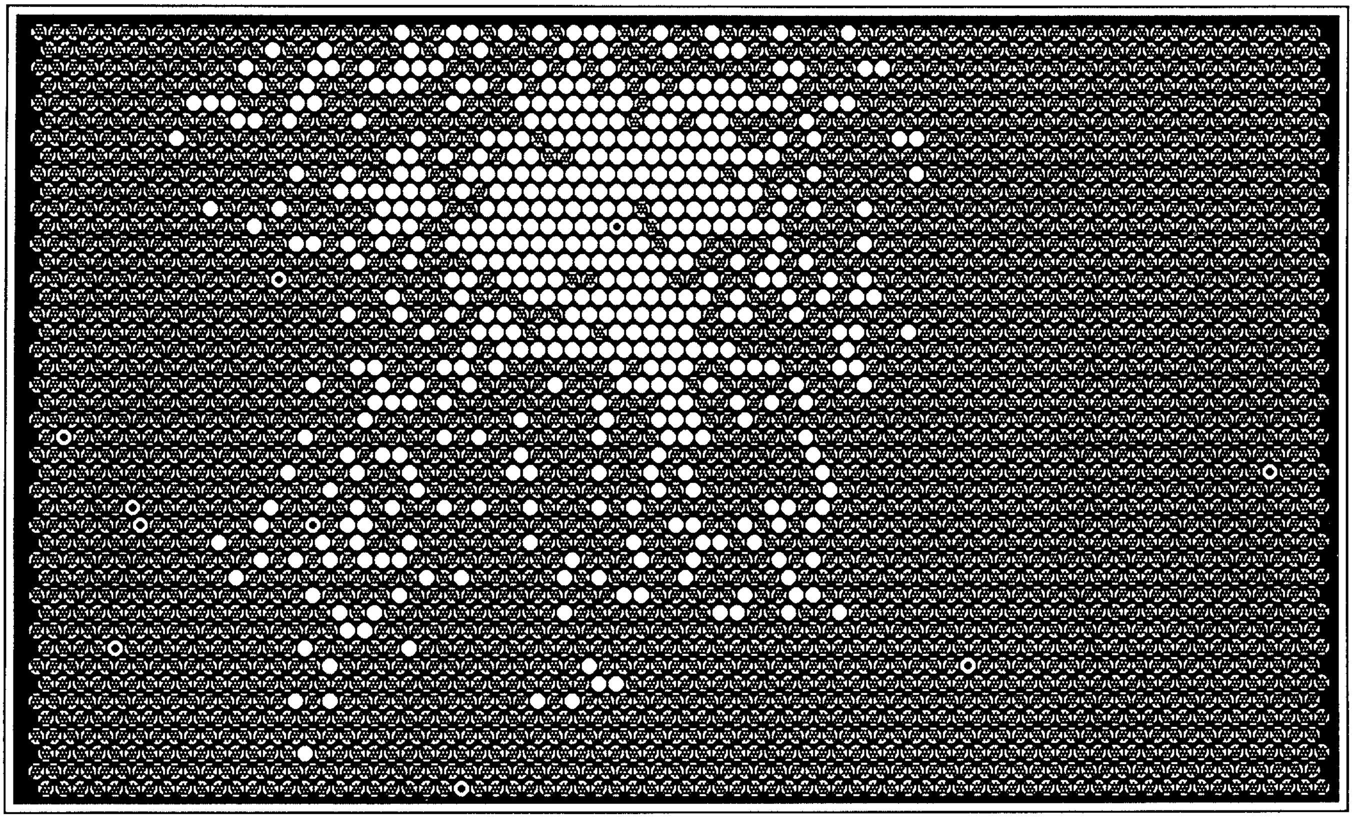
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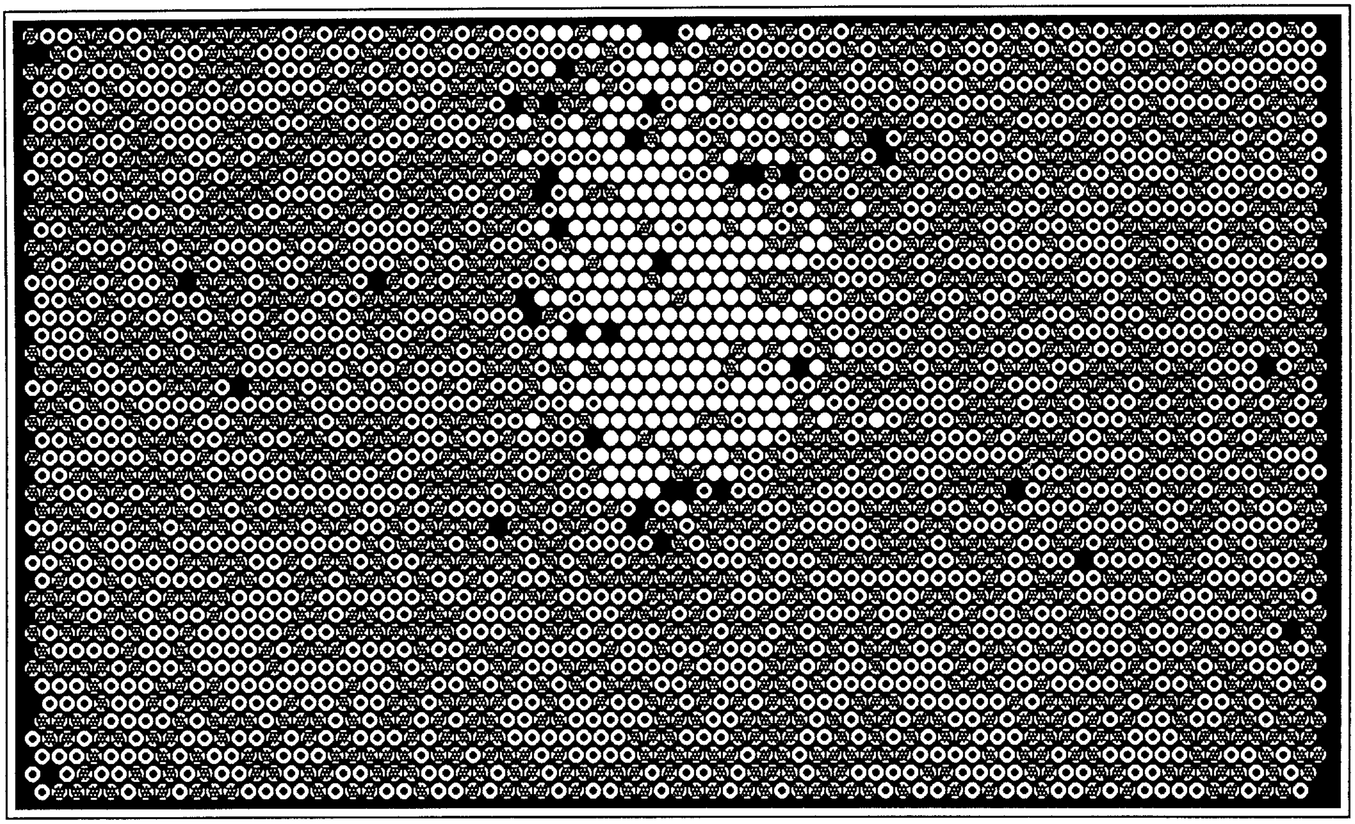
Fig. 8 A, B. Computer simulation with identical parameter values as in Fig. 7, except for the elimination of the preferential removal of honey and pollen nearby brood (preferential removal factor = 1). Symbols as in Fig. 1. The characteristic pattern with a compact central brood area does not form. **A** Day 1; **B** Day 7



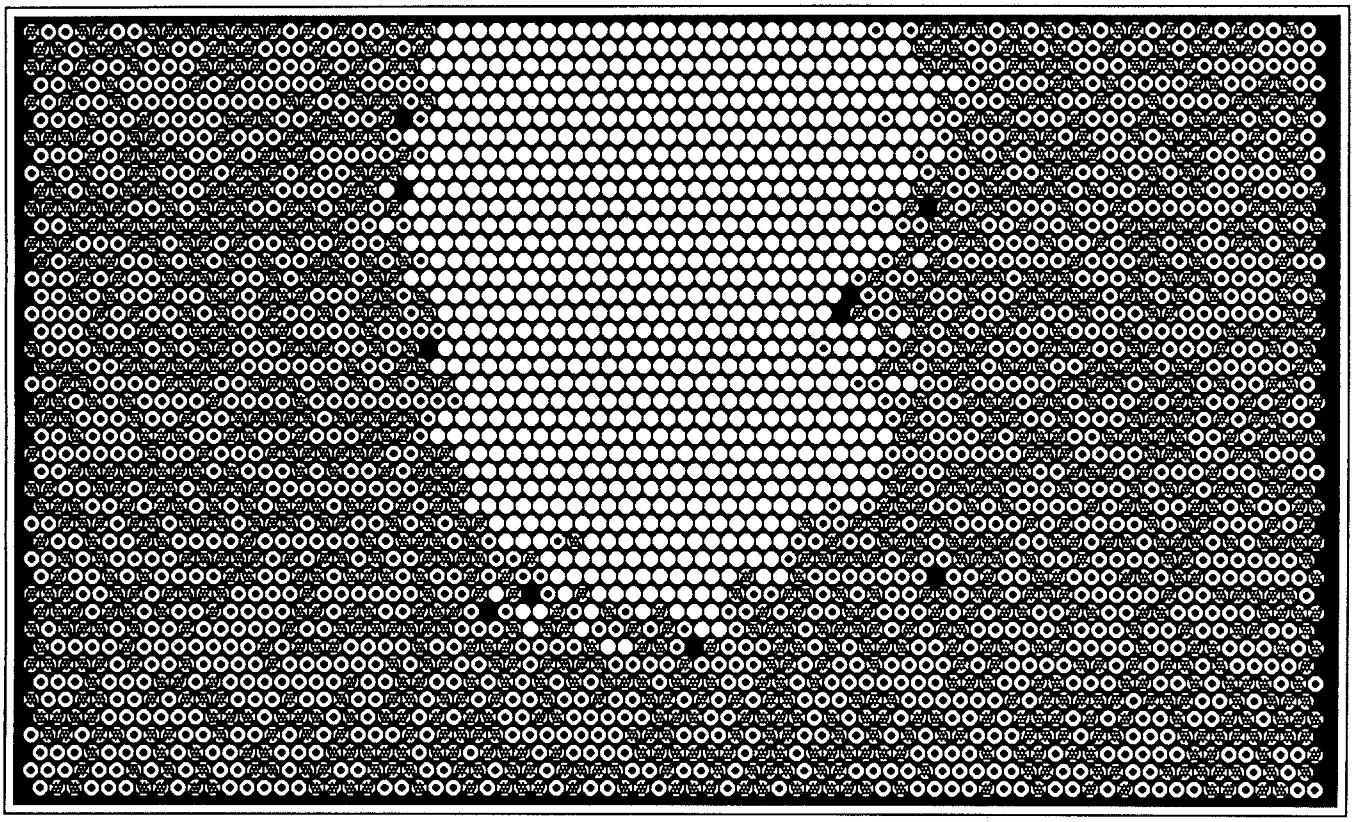


Fig. 9 A, B. Computer simulation with parameter values as in Fig. 7, except that the values for pollen input and removal were equal to those for honey. Symbols as in Fig. 1. The characteristic segregation of honey and pollen does not occur. **A** Day 1. **B** Day 7

In this simulation, its value is 1 instead of a 10, thus eliminating preferential removal. The other parameters values have not been changed, but are the same as those used in Fig. 7. In this situation honey and pollen deposited near the brood are removed at the same rate as in the periphery, so that a compact brood area cannot form. Although pollen is gradually removed from the periphery for the reasons previously discussed, an interface zone for pollen deposition cannot develop. Without preferential removal of honey and pollen nearby brood, the characteristic concentric pattern cannot develop.

Figure 9 demonstrates the effect of altering only the normal ratio of honey to pollen deposition. With the foraging and removal rates of honey and pollen equal, there is no segregation of pollen and honey into separate regions. The periphery becomes a random mixture of honey and pollen. As expected, the compact brood area will develop since preferential removal of honey and pollen occurs nearby brood.

Discussion

1 Comparison of the hypotheses, and comments on the self-organization hypothesis

Despite its attractive simplicity, the blueprint hypothesis does not adequately describe the pattern formation process. If there exist particular regions for the deposition of brood, nectar and pollen, it is difficult to explain the deposition of honey and pollen throughout an empty frame (Fig. 5) and in the center of the brood nest (Fig. 4 and Table 1). The only way to rationalize these depositions is to view them as aberrations on the part of a few bees, or as errors resulting when a high rate of honey or pollen input reduces the available number of storage cells. Another major shortcoming of this hypothesis is that it disregards the processes of honey and pollen removal.

The self-organization hypothesis directly addresses these issues. It includes the observed random pollen and nectar deposits throughout the frame as an integral component of the pattern formation process, and shows how the early disorganized stage is gradually transformed into the final mature form. The preferential removal of honey and pollen nearby brood plays a crucial organizing role.

Several other features of the self-organization hypothesis are particularly attractive. It is tempting to speculate that the bees, depositing pollen and honey randomly in any available cell, may be able to quickly and efficiently unload without the necessity of a more time-consuming search for the "proper" storage site. Simultaneously, the preferential emptying of cells in the brood area automatically provides additional cells in which the queen can lay, thus contributing to a compact brood area. Preferential food removal also results in an interface zone between the honey and brood where cells are continually being emptied. When forage is available, this region becomes the pollen zone. An elegant feature of this self-organizing process is that this potential pollen storage area does not arise through the workers reserving an area for pollen and refraining from placing honey there. Rather, the interface zone persists simply and automatically because of the high rate of turnover in the contents of these cells.

Another interesting, and perhaps important, feature of the self-organization process is that perturbations of the normal pattern are quickly and automatically "repaired" without any special repair processes. For example, the continual emergence of adult bees results in irregular graps in the compact brood nest, as does disease (e.g., chalkbrood, a fungal infection that kills larvae, which are then removed by adult bees). Similarly the sporadic blossoming of forage plants results in large influxes of pollen and nectar which are often stored within the brood area in cells vacated by bees which have completed metamorphosis. The pattern is "self-repairing" after these perturbations, quickly returning to its previously ordered state. This feature of the pattern formation process may contribute to efficient colony functioning.

Another, more speculative, implication of the self-organization hypothesis is that rate of egg laying and pollen collection may be self-regulated. The preferential removal of pollen and honey as a function of nearness to brood suggests a positive feedback situation. As the amount of brood on the comb increases, the rate of nearby honey and pollen removal would also increase, providing more empty cells in which the queen can lay. With enough positive feedback, a band of excess empty cells might develop surrounding the brood, thus allowing the queen to lay eggs at her physiological maximum rate. If the width of this empty zone is indeed a function of the amount of nearby brood, then the size of the pollen storage area may be automatically regulated in proportion to pollen requirements. As the amount of brood increases, consumption of honey and pollen nearby the brood would probably increase as well, thus increasing available pollen storage space in parallel with the nutritional needs of the developing larvae.

2 Mechanisms of self-organization in biology and in insect societies

Understanding how structures develop is one of the major mysteries in biology. In the field of morphogenesis and developmental biology, the classic example is the question of how complex embryonic structures arise from the apparently homogeneous tissue of the fertilized egg. The early embryologists put forth their rival hypotheses of preformation and epigenesis (Maynard Smith 1986; Gould 1977). The preformationists believed that the structure and spatial relationships of the adult were latent in a miniature homunculus inhabiting the egg, and that development proceeded from the inherent template or blueprint merely by an increase in size. In contrast, the epigeneticists maintained that the complexity of adult form developed gradually within the undifferentiated embryonic tissues through intrinsic processes.

An important theoretical breakthrough was the work of the English mathematician Alan Turing who showed how a system of chemicals (which he called morphogens) reacting together and diffusing through a tissue could generate regular patterns even if they are initially distributed randomly throughout the medium (Turing 1952). Turing's reaction-diffusion model demonstrates self-organization, and has become a classic paradigm for cellular differentiation and morphogenesis (Murray 1989 and references therein).

More recently, a variety of mechanisms of pattern formation have been presented, some derived from Turing's work and others more distantly related. These models of self-organization may explain skin pigmentation patterns of animals (Murray 1981), patterns of stripes in the visual cortex (Swindale 1980), shell patterns of mollusks (Ermentrout et al. 1986) and even visual patterns experienced during drug-induced hallucination (Ermentrout and Cowan 1979). Although the descriptive term self-organization does not refer to a unique morphogenetic mechanism, all these systems have in common that each can be analyzed as an assemblage of constituent subunits, such as individual cells, which are organized through the local interactions.

In the context of pattern formation on the combs, the subunits are the cells of brood, pollen and honey that make up the pattern. Order on the comb emerges solely through the dynamics of filling and emptying of cells, without any foreman outside the system directing these processes, nor by the bees following a blueprint showing which cells to fill or empty.

Earlier studies of social insects focussed on various crucial aspects and ingredients of self-organization, but none was able to incorporate all the components into a complete functioning process. Wilson (1971), speaking of "mass action" and the building of complexity in insect societies, states that the "remarkable qualities of social life are mass phenomena that emerge from the meshing of ... simple individual patterns." Wilson and Holldöbler (1988) emphasize that "The individual ant need operate only with 'rules of thumb', elementary decisions based upon local stimuli that contain relatively small amounts of information". Seeley's analyses of social foraging in honey bees (1989a, b; also Seeley and Levien 1987) stress that the coordinated activities of the honey bee colony arise through decentralized decision making, and the ability of individual bees to independently gather information about the colony's needs and adjust their behaviors accordingly. Highly coordinated colony processes emerge in the absence of a control hierarchy and with the autonomous functioning of the individual.

Part of the problem of understanding colony-level phenomena is the difficulty of gaining an intuitive grasp of how self-organization works. Complex manmade structures are not self-organizing, but instead have their order imposed from the outside, by means such as a blueprint. Lacking familiarity with self-organizing processes, it is therefore difficult for us to predict how complex colony-level patterns of social insects emerge spontaneously from the simple interactions of thousands of subunits. To overcome this difficulty, a recent fruitful approach has been to analyze the phenomena with mathematical models and computer simulations which readily incorporate the inter-related activities of thousands of individuals. Examples include studies of the foraging patterns of ants (Pasteels et al. 1987; Deneubourg et al. 1989; Goss and Deneubourg 1989; Goss et al. 1989; Deneubourg et al. 1990), nest construction by termites (Deneubourg 1977) and honey bees (Belić et al. 1986), food selection in honey bees (Seeley et al. 1990), and thermoregulation in honey bees (Heinrich 1981; Omholt 1987). The process of pattern formation on the combs of honey bee colonies is yet another example of how complex behaviors and orderly structures emerge through mechanisms of self-organization.

Wilson (1971), in his discussion of the building of complexity in societies, stated that, "the reconstruction of mass behavior from a knowledge of the behavior of single colony members is the central problem of insect sociology." These models of self-organization bring us a step further towards the goal of understanding the remarkable colony-level phenomena of social insects.

Acknowledgements. I gratefully acknowledge the support of Sigma Xi, a National Science Foundation Graduate Fellowship, on Olin Fellowship, and the U.S. Department of Agriculture (grant NY(C)-191407). I thank Tom Seeley and Kirk Visscher for thought-provoking discussions, and Richard Nowogrodski, Tom Seeley, and Paul Sherman for meticulous reading of the manuscript. Special thanks to Mi Won Camazine for assistance with many experiments.

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