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Decentralized control of drone comb construction in honey bee colonies

Abstract Honey bee colonies furnish their nests with two types of comb distinguished by cell size: large cells for rearing males (drone comb) and small cells for rearing workers (worker comb). The bees actively regulate the relative quantity of each type, a behavior likely to be important in setting a colony's sex ratio. Experimental analysis of the information pathways and control mechanisms responsible for this regulation found the following results. The amount of drone comb in a nest is governed by negative feedback from drone comb already constructed. This feedback depends on the workers having direct contact with the drone comb in their nest, but does not depend on the queen's contact with the comb. The comb itself, rather than the brood within it, is sufficient to provide the negative feedback, although the brood may also contribute to the effect. These findings show that drone comb regulation does not depend on the queen acting as a centralized information gatherer and behavioral controller. Instead, the evidence points to a decision-making process distributed across the population of worker bees, a control architecture typical of colony organization in honey bees and other large-colony insect societies.

Key words Honey bee · Comb · Drone · Decentralized control · *Apis mellifera*

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Introduction

A distinguishing feature of insect societies, and a major contributor to their great ecological success, is the elaborate and precise coordination of thousands of individuals in the pursuit of colony ends (Wilson 1971; Hölldobler and Wilson 1990). In large-colony species such as the honey bees and army ants, coordinated colony behavior depends largely on a system of decentralized control (Wilson and Hölldobler 1988; Bourke and Franks 1995; Seeley 1995; Gordon 1996; Bonabeau et al. 1997). In such systems, coordination is not imposed by leaders who receive and integrate information from throughout the colony and then issue appropriate commands to multitudes of effector subunits. Rather, coordination emerges from the separate behavioral choices of thousands of insects using simple rules of thumb and relying only on local information. With an appropriate set of decision rules, the parallel actions of thousands of workers can produce colony behaviors far exceeding the capacities of individual workers.

While such decentralized control may be the rule in large colonies, smaller societies rely on centralized direction by the queen for at least some aspects of social coordination. In the paper wasp *Polistes fuscatus*, for example, the queen occupies a critical position in a feedback loop regulating colony foraging effort (Reeve and Gamboa 1987). Similarly, queens of the halictid bee *Lasioglossum zephyrum* appear to coordinate worker behavior by leading returning foragers to brood cells ready for provisioning (Breed and Gamboa 1977). Theoretical considerations suggest that such a central regulatory role for queens may be limited to species with small colony sizes. Small size should facilitate the speed and thoroughness of the queen's assessment of colony needs, as well as her ability to regulate the behavior of her workers. Queens in large colonies may be unable to determine colony state rapidly enough to keep up with changing circumstances, or to effectively communicate colony needs to a large labor force. If, as is the case for

paper wasps, the queen's manipulations induce workers to oppose their individual fitness interests, she must be able to monitor their behavior and enforce obedience. Such policing is likely to be effective only in small colonies. Thus it is not surprising that central coordination by the queen has been reported only in species with colony populations numbering in the dozens.

Nonetheless, these considerations do not rule out effective queen control of some colony functions, even in large societies which coordinate most of their behavior through decentralized processes. The queen may effectively govern a particular task, as long as special circumstances facilitate her evaluation of the colony's need for that task. Furthermore, if her interests in the completion of this task coincide with those of the workers, she need not rely on coercion to summon an appropriate worker response to her evaluation of colony need.

A potential case of centralized queen control in a large-colony species may be found in the regulation of drone comb construction by honey bees. Cell diameters in natural honey comb have a bimodal distribution: the smaller size (approximately 5.2 mm wall to wall) is used to rear worker brood and the larger size (approximately 6.4 mm) is used to rear male (drone) brood (Taber and Owens 1970). Both sizes may also be used to store pollen or honey. (Colonies also build specialized cells for rearing queens, but these are constructed in much smaller numbers and typically only when a colony is preparing to swarm or to replace a dead or declining queen.) The relative quantity of each cell type appears to be a closely regulated feature of honey bee nests (Seeley and Morse 1976; Page et al. 1993). The percentage of drone comb by area in feral nests collected in upstate New York has been shown to cluster fairly tightly around 17% (standard deviation = 3%) (Seeley and Morse 1976). Experiments on hived colonies show that this proportion is actively maintained in response to perturbations of drone comb quantity: colonies provided with ample drone comb build significantly less new drone comb than colonies supplied exclusively with worker comb (Free 1967; Free and Williams 1975). From a functional standpoint, such regulation should be quite important to colony fitness, since the amount of drone comb in its nest sets an upper limit on the number of drones that a colony can rear, and thus plays a role in setting a colony's sex ratio (Allen 1963, 1965).

How do the bees regulate the quantity of drone comb? The builders' behavior must somehow be informed by the current relative amount of each kind of comb in the nest. Evaluating such a global feature of nest design seems a daunting challenge for a single bee, given the large difference in scale between a worker and the entire nest. The queen, however, is uniquely well-placed to evaluate precisely this feature. She spends most of her time walking on the comb and measuring cell diameters in order to decide which kind of egg to lay (fertilized eggs for worker cells and unfertilized eggs for drone cells). If she keeps a running tally of how many of each cell type she encounters, she could perceive any imbalance in the relative amounts of drone and worker comb. If she can communicate the presence of such an imbalance to the workers, they could correct it by adjusting the kind of new comb they build.

Moreover, the queen would not have to impose an evaluation of drone comb need different from that dictated by the workers' own genetic interests. This is despite their haplodiploid mechanism of sex determination, which introduces potential conflict between singly-mated queens and their daughters over sex allocation ratios (Trivers and Hare 1976). Workers, more closely related to their sisters than to their brothers, should prefer a more female-biased sex ratio than does the queen, who is equally related to both her sons and daughters. In honey bees, a high degree of polyandry ameliorates this conflict by lowering the relatedness of workers to their sisters. Honey bee queens mate with 7–17 males (Taber and Wendel 1958; Adams et al. 1977) and at any given time use the sperm of at least three males, and probably many more (Page and Metcalf 1982; Page et al. 1984; Page 1986). These levels of polyandry should drive the workers' preferred sex ratio very near the 1:1 value favored by queens (Charnov 1978). Hence, queens and workers should experience little disagreement over the desired amount of drone comb in the nest, and its attendant effects on sex allocation.

Nothing is known of the queen's role in regulating drone comb construction, nor indeed anything else about the behavioral mechanisms underlying this phenomenon. Hence I explored the information pathways used by bees to assay the relative amount of drone comb already in the nest and adjust their new construction accordingly. I sought cues which might inform the bees of the state of their comb, paying particular attention to the queen's participation in evaluating these cues.

Methods and materials

Study sites, bees and hives

The study consisted of five experiments, described in detail in the Results section. Experiments 1–4 were carried out at a Cornell University bee yard near Ithaca, New York State (42°26′N, 76°30′W), from June to September 1994. Experiment 5 was performed at the MacArthur Agro-Ecology Research Center near Lake Placid, Florida (27°9′N, 81°12′W), from March to May 1995. Queenright colonies of 15000–25000 bees were housed in hives consisting of two ten-frame full-depth Langstroth hive bodies (37.5 × 46.5 × 24.5 cm). The number and type of combs in the hives depended upon the experimental treatment, as described below. Colony populations were near the mean for feral nests in the Ithaca area (23,400: Seeley and Morse 1976). The hive volumes (84 l) were within the range of feral nest volumes but larger than the feral median of 45 l in order to discourage swarming and to allow room for feeder jars (Seeley and Morse 1976). The bees were commercial hybrids, principally *Apis mellifera ligustica*.

Measurement of comb areas

The basic experimental design compared the relative area of drone comb built by colonies under different treatments. Two methods were used to measure these areas. For the first method, used in all

five experiments, I traced the combs onto a sheet of glass, marking both the comb outlines and the borders between comb regions composed of drone cells and regions composed of worker cells. Because the bees typically built large homogeneous expanses of one comb type or the other, and because the two cell sizes are readily distinguishable by eye, these borders could be traced fairly precisely. The glass tracing was copied onto a sheet of paper which served as a permanent record of the construction. These images were then digitized, using an Agfa Arcus Plus scanner and a Macintosh Centris 650 computer. A 10-cm scale line was added to these digitized images in Adobe PhotoShop, and NIH Image (public domain software developed by the United States National Institutes of Health and available on the Internet by anonymous FTP from zippy.nimh.nih.gov) was then used to measure the absolute area of the drone and worker sections. The relative amount of drone comb was calculated as the ratio of drone comb area to total comb area.

Because this method relied on my visual discrimination of cell types, rather than direct measurement of cell diameters, I checked its results by employing a more painstaking approach on some of the data of experiment 5. The combs built in this experiment were photographed using color slide film and the slides projected onto a vertical screen consisting of a sheet of plate glass (65 × 50 cm) covered on one side with white paper. This screen design allowed me to measure the comb image from the back of the screen without obstructing the projector. The size of the image was adjusted so that all combs were projected at the same magnification. Because the combs were built in identical Langstroth frames, the width of the frame's topbar was used as a scale. A zoom lens attachment on the projector was adjusted until the width of the topbar image was exactly 22.7 mm, as measured with a pair of calipers. This scale, somewhat larger than actual size, gave a crisp image in which the cells were large enough to be easily measured.

Cells were sampled with a 19-mm square grid of fine lines marked on the screen. Every cell which fell onto an intersection of the grid was measured with calipers to the nearest 0.1 mm. A histogram of cell width frequencies was then plotted for each colony, using all the data from all treatments for that colony. In all cases the distribution for each colony was clearly bimodal, but with considerable overlap. For each colony the cell width at the trough between the two peaks was taken as the boundary value between worker and drone cells. All of a colony's sampled cells which were larger than its boundary value were taken to be drone cells and all cells smaller than it were taken to be worker cells. A small number of cell measurements fell at exactly the boundary value (2.0 ± 1.3% of sampled cells in each colony/treatment combination). Half of these were designated worker cells and half drone cells. The relative amount of drone comb built by each colony in each treatment was calculated as the ratio of the number of drone cells to the total number of cells. Because the likelihood of sampling a cell depended on its area, this ratio was expected to equal the ratio of areas calculated with the first method, described above.

Statistical analysis

Drone comb proportions were subjected to arcsine square root transformation in order to improve the normality of the data (Sokal and Rohlf 1981). For those experiments in which the transformed values fitted a normal distribution (as determined by Kolmogorov-Smirnov tests for goodness of fit), the treatment means were compared either by a one-tailed paired t-test (experiments 1–4) or by a repeated measures analysis of variance followed by multiple F-tests (experiment 5) (Kiess 1989). A one-tailed test was used because the null hypothesis (no regulation of drone comb quantity) is only rejected if the amount of drone comb constructed is less in treatments with ample drone comb than in treatments with no drone comb. Because the data in one treatment of experiment 1 were not normally distributed, the values were instead compared by Wilcoxon's signed-ranks test. All analyses were carried out on Apple Macintosh computers using Microsoft Excel 5 or StatSoft Statistica. Measurements are reported as mean ± SD.
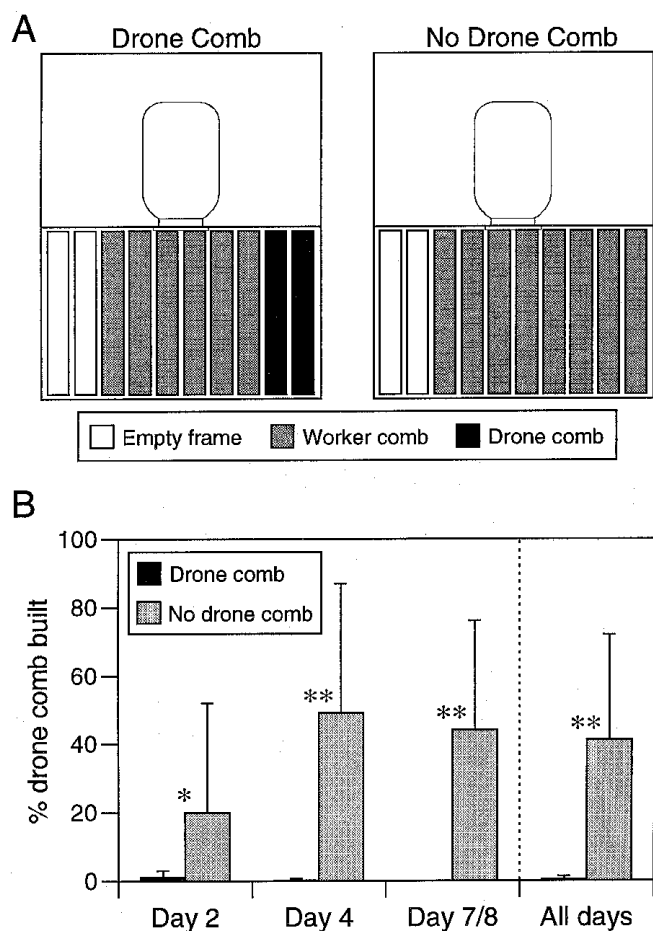
Results

Each experiment compared the proportion of drone comb built by colonies across treatments differing in the amount or kind of drone comb already in the hive and the degree of contact the workers or the queen had with the comb. In each experiment, each colony received each treatment exactly once.

Experiment 1: do colonies adjust their building to regulate the amount of drone comb in the nest?

This experiment was designed to replicate the previously reported regulation phenomenon, namely the tendency of colonies to build proportionately more drone comb when their nest has little drone comb. For the first phase of the experiment, six colonies were assigned to one of two treatments: those in treatment No Drone Comb received eight worker combs and those in treatment Drone Comb received six worker combs and two drone combs. The combs were largely filled with brood, pollen and honey. All colonies were also provided with two empty test frames in which to build new comb (Fig. 1A). The combination of filled combs and ample natural nectar sources available at that time of year (June) provided an excellent stimulus for comb building (Pratt 1997). However, in order to safeguard against unexpected nectar shortfalls, two feeder jars containing roughly 2 l of 1.75 M sucrose solution were placed in the otherwise empty upper section of each hive.

The colonies were left undisturbed for 1 week except for daily refillings of the feeder jars, as necessary, and three data collection checks. In these checks, any test frames on which comb had been built were removed and replaced with empty test frames. The areas of freshly built drone and worker comb were measured as described above. Some colonies occasionally attached new comb to the walls of the upper hive body, or to the top cover of the hive. This comb was discarded because it could not be reliably removed without damaging it and so rendering impossible the identification of comb type. Combs were collected 2, 4 and 7 days after setting up the experiment. Immediately after the final collection, the experiment was begun again with the treatments reversed: the three colonies previously in treatment No Drone Comb were assigned to treatment Drone Comb and vice versa. The schedule of feeding and checking was the same as in the first phase, except that bad weather delayed the final check until the eighth day.

In treatment No Drone Comb the colonies had far less, and in treatment Drone Comb somewhat more, than the 17.0 ± 3.0 areal percentage of drone comb found in feral colonies (Seeley and Morse 1976). Therefore, if the bees can adjust their building to regulate the amount of drone comb in their nest, I expected each colony to build relatively more drone comb when in treatment No Drone Comb than when in treatment

A

B

Fig. 1A,B Design and results of experiment 1 comparing the amount of new drone comb built when colonies began with no drone comb and when they already had ample drone comb. **A** Each hive consisted of two hive bodies, the lower one filled with frames and the upper one empty except for a feeder jar containing sucrose solution. **B** Relative amounts of new drone comb constructed are shown for different days following the start of the experiment; cumulative data for the entire experiment are shown to the right of the dotted line. Treatments were compared either by Wilcoxon's signed-ranks test (day 7/8) or by one-tailed paired t-tests (all other analyses) (*NS*: $P > 0.05$, *$P \leq 0.05$, **$P \leq 0.01$). *Bar heights* show the mean percentage drone comb across colonies; *error bars* show the standard deviations

Drone Comb. If they cannot regulate their drone comb supply, I expected colonies to show no difference in the relative amount of drone comb built in the two treatments.

All six colonies built comb throughout the experiment, although there was considerable intercolonial variation in both the total amount of comb built and in the proportion of drone comb built, even within a single treatment (Table 1). However, at every collection period the mean proportion of drone comb was significantly higher in treatment No Drone Comb than in treatment Drone Comb (Fig. 1B). I obtained the same result when all the data were combined in one test. Thus, the colonies clearly adjusted their new construction in order to regulate their drone comb supply.

Experiment 2: do bees need to be in direct tactile contact with the drone comb in order to properly regulate its quantity?

Given that the bees can adjust their building according to the relative proportion of each comb type in the nest, this experiment was designed to determine what kind of cue is used by the bees to inform their building decisions. That is, how do they know that they already have a sufficient quantity of drone comb and thus do not need to build much more? I tested whether detection of drone comb depends on the bees' having direct tactile contact with it. Alternatively, the bees might detect it at a distance, perhaps by means of a chemical cue or signal released by the comb or its contents.

The experiment employed the same six colonies used in experiment 1 and an identical design, except that the two drone combs in treatment Drone Comb and two of the worker combs in treatment No Drone Comb were separated from the rest of the colony by a single sheet of wire screen (mesh size: 5 mm) (Fig. 2A). This prevented any bees from walking on or antennating the drone combs, while allowing volatile chemicals to pass through. The screen in treatment No Drone Comb controlled for any ancillary influence of the screen on building behavior. Feeding and comb collection were the same as in experiment 1, except that comb was collected on the second and sixth days of each replicate.

If tactile contact is necessary for the assay of comb type, I expected the bees in treatment Drone Comb to behave as though their hive contained only worker comb. That is, I expected the colonies to build the same relative amount of drone comb in treatment Drone Comb as in treatment No Drone Comb. Furthermore, I expected this proportion of drone comb to be large. If tactile contact is not necessary, then I expected to obtain the same results seen in experiment 1: larger relative amounts of drone comb in treatment No Drone Comb than in treatment Drone Comb.

All colonies built comb throughout the experiment, and there was again considerable variation among colonies in total comb built and in drone comb proportion (Table 2). However, I found no significant difference in drone comb proportion between treatments for either of the collection periods, or for both collection periods combined (Fig. 2B). Furthermore, the mean drone comb proportion in each treatment was high and similar to that of treatment No Drone Comb in experiment 1, suggesting that the colonies built as though they had no drone comb. Evidently the bees need direct physical contact with the drone comb in their hives in order to assay its presence when building new comb.

Two of the colonies (III and IV) lost their queens at an unknown time during the experiment. Omitting these colonies from the statistical analysis did not change the results. Colonies III and IV built the largest relative amounts of drone comb of any colonies in the experiment. This might suggest that the presence of a queen somehow inhibits drone comb production or stimulates

Table 1 Total area of comb constructed and percentage of that comb comprised of drone cells in experiment 1 (see text and Fig. 1 for detailed descriptions of the treatments). Combs were removed and measured on three occasions: 2, 4, and either 7 or 8 days after the start of the experiment

Colony	Treatment	Day 2		Day 4		Day 7/8		All days	
		Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone
I	Drone	412	5	455	1	813	0	1680	1
	No drone	137	82	207	89	487	99	831	94
II	Drone	290	0	229	0	295	0	814	0
	No drone	151	0	154	0	583	18	888	12
III	Drone	448	0	453	0	879	0	1780	0
	No drone	235	8	320	58	505	51	1060	44
IV	Drone	396	0	313	0	446	0	1155	0
	No drone	397	26	390	95	774	52	1561	56
V	Drone	342	0	288	0	640	0	1270	0
	No drone	353	6	283	25	570	9	1206	12
VI	Drone	228	0	229	0	207	0	664	0
	No drone	104	0	113	25	645	36	862	30
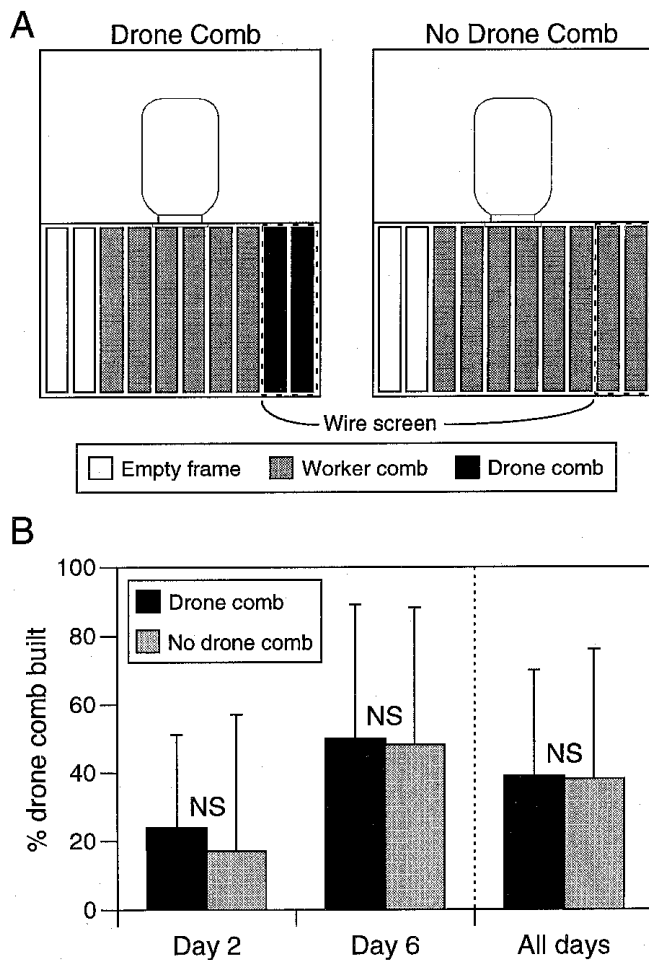
Fig. 2A,B Design and results of experiment 2 testing the necessity of the bees' having direct contact with the comb for proper regulation of drone comb construction. **A** The design was identical to that shown in Fig. 1A, except that the bees were separated from the drone combs by a wire mesh screen. As a control, two worker combs in the No Drone Comb treatment were similarly sealed off. **B** The amounts of drone comb built under each treatment were compared by one-tailed paired *t*-tests. See the legend to Fig. 1B for further details

worker comb production, and that the large amounts of drone comb built by these two colonies were an artifact of queenlessness. This idea is contradicted by observations made after the screens were removed at the end of the experiment, allowing the workers free access to all of the comb. When the colonies were allowed to build for 3 days under these conditions, colony III, which had ample drone comb, built nothing but worker comb, while colony IV, which had only worker comb, built nearly exclusively drone comb. Both colonies were still queenless, indicating that the bees can regulate construction type normally even without a queen. The next experiment tested more rigorously the role of the queen in the regulation of comb type.

Experiment 3: does the queen need to be in direct tactile contact with the drone comb in order for the workers to properly regulate drone comb quantity?

Since tactile contact appears to be necessary for proper regulation, this experiment asked whether it is the queen whose contact is necessary, or only the workers. The same design as experiment 2 was used, except that the wire screen was replaced by a queen excluder, a sheet of zinc perforated by holes large enough to admit a worker but too small for a queen. I used more comb than in the previous experiments, because the colonies had grown larger. Each colony in treatment No Drone Comb had twelve worker combs and each colony in treatment Drone Comb had nine worker combs and three drone combs. I also added an additional empty frame to the upper part of the hive (Fig. 3A). Combs were collected on days two, five and seven of each replicate. Four of the six colonies used in experiments 1 and 2 were again used in this experiment; because colonies III and IV had lost their queens they were replaced by new colonies of the same size.

If the queen is responsible for evaluating comb type, then I expected the bees in treatment Drone Comb to

Table 2 Total area of comb constructed and percentage of that comb comprised of drone cells in experiment 2 (see text and Fig. 2 for detailed descriptions of the treatments). Combs were removed and measured on two occasions: 2 and 6 days after the start of the experiment

Colony	Treatment	Day 2		Day 6		All days	
		Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone
I	Drone	473	35	845	38	1318	37
	No drone	562	0	815	0	1377	0
II	Drone	340	0	729	2	1069	1
	No drone	337	3	611	51	948	34
III	Drone	170	38	538	100	708	85
	No drone	151	0	387	90	538	65
IV	Drone	609	4	525	90	1134	44
	No drone	148	98	438	100	586	99
V	Drone	354	66	624	53	978	58
	No drone	326	0	818	15	1144	11
VI	Drone	350	0	837	15	1187	11
	No drone	367	0	454	35	821	19
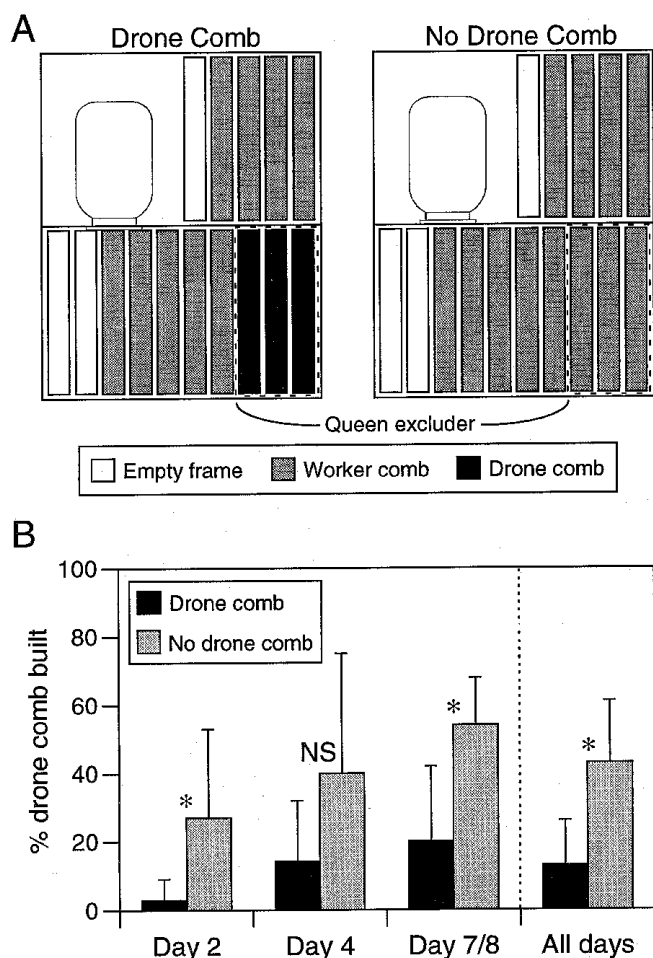
Fig. 3A,B Design and results of experiment 3 testing the queen's role in the regulation of drone comb construction. **A** The design was similar to that shown in Fig. 1A, except that additional frames were added to the upper super to accomodate increased colony populations. Also, the drone combs were surrounded by queen excluders, allowing direct contact by the workers but not by the queen. As a control, three worker combs in the No Drone Comb treatment were similarly sealed off. **B** The amounts of drone comb built under each treatment were compared by one-tailed paired *t*-tests. See the legend to Fig. 1B for further details

behave as though their hive contained only worker comb. That is, I expected the colonies to build the same relative amount of drone comb in treatment Drone Comb as in treatment No Drone Comb. Furthermore, I expected this proportion of drone comb to be large. If the queen is not responsible for assaying comb type, then I expected the same results obtained in experiment 1: larger relative amounts of drone comb in treatment No Drone Comb than in treatment Drone Comb.

All colonies built comb throughout the experiment, and there was again considerable variation among colonies in total comb built and in drone comb proportion (Table 3). At every collection period the mean proportion of drone comb was higher in treatment No Drone Comb than in treatment Drone Comb, and significantly so in all but the second period (Fig. 3B). Thus, proper regulation of comb type does not depend on the queen's tactile contact with drone comb already present in the nest.

Experiment 4: must a colony's drone comb contain drone brood in order to be treated as drone comb by the bees?

Given that detection of the presence of drone comb requires direct contact by the workers with the comb, I performed this experiment to determine whether the cue is present in the comb itself or in the brood it contains. In experiments 1–3, the drone comb provided to the colonies contained, or had recently contained, drone brood. Thus the bees may have perceived the presence of this brood, rather than the comb itself. To test this possibility I duplicated the setup of experiment 3, except that I used drone combs which had never contained any brood (Fig. 4A). I obtained these combs by having bees build comb on commercial drone comb foundation placed in a hive body separated from the brood comb and queen by a queen excluder. The design was otherwise identical to that of experiment 3, except that data
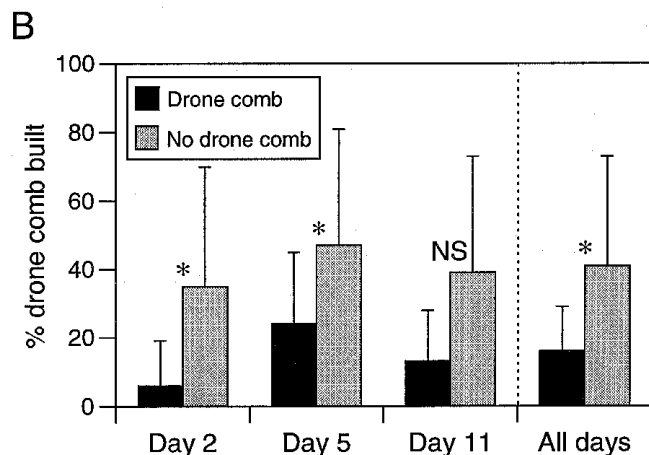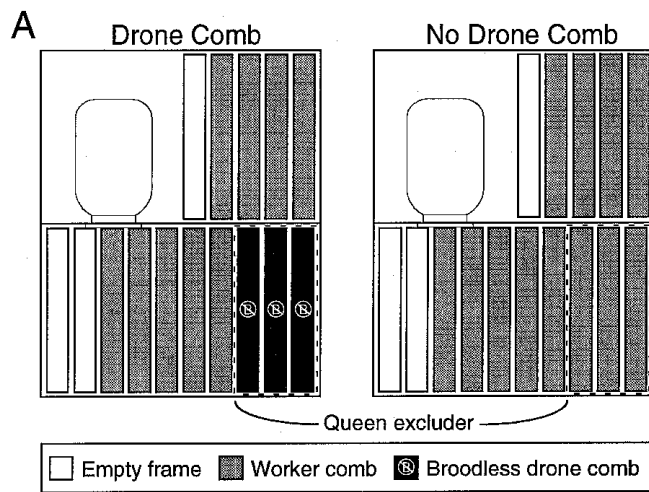
Table 3 Total area of comb constructed and percentage of that comb comprised of drone cells in experiment 3 (see text and Fig. 3 for detailed descriptions of the treatments). Combs were removed and measured on three occasions: 2, 4, and either 7 or 8 days after the start of the experiment

Colony	Treatment	Day 2		Day 4		Day 7/8		All days	
		Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone
I	Drone	647	0	566	0	892	1	2105	0
	No drone	348	51	501	25	740	70	1589	52
II	Drone	481	0	392	46	647	25	1520	23
	No drone	564	11	562	42	955	63	2081	43
V	Drone	424	15	393	7	579	57	1396	30
	No drone	313	55	323	99	557	66	1193	72
VI	Drone	687	3	826	20	1099	29	2612	19
	No drone	1241	2	1032	15	1397	52	3670	25
VII	Drone	862	0	753	0	1061	0	2676	0
	No drone	455	44	442	60	615	36	1512	45
VIII	Drone	593	0	935	9	1164	5	2692	5
	No drone	226	0	285	2	756	39	1267	24

Fig. 4A,B Design and results of experiment 4 testing the role of drone brood in the regulation of drone comb construction. **A** The design was identical to that shown in Fig. 3A, except that the drone combs contained no brood and had never contained any brood. **B** The amounts of drone comb built under each treatment were compared by one-tailed paired *t*-tests. See the legend to Fig. 1B for further details

were collected on days two, five and eleven of each phase.

If drone brood is the source of cues telling the bees that drone comb is present, I expected colonies with broodless drone comb to behave like colonies with no drone comb at all. That is, I expected them to build similar and large proportions of drone comb in both treatments. If drone comb itself is a direct source of cues indicating its presence, I expected the colonies to build significantly less drone comb in treatment Drone Comb than in treatment No Drone Comb.

All colonies built comb throughout the experiment, and there was again considerable variation among colonies in total comb built and in drone comb proportion (Table 4). However, at every collection period the mean proportion of drone comb was higher in treatment No Drone Comb than in treatment Drone Comb. This difference was statistically significant in every period except the third (Fig. 4B). These results indicate that the comb itself is an adequate source of cues indicating the presence of drone comb.

Experiment 5: Combined analysis

In this experiment I repeated the tests made in experiments 1, 3 and 4 on the existence of regulation, the role of the queen, and the role of drone brood. I introduced a few methodological improvements described below, and ran all treatments simultaneously, allowing more comparisons among treatments than were possible in the earlier experiments.

In the first phase of the experiment eight colonies each received one of four treatments (Fig. 5A). (These colonies were not the same ones used in the earlier experiments.) Colonies in treatment No Drone Comb received eight frames of worker comb and two empty frames in which to build. Colonies in treatment Drone Comb received six frames of worker comb, two frames of drone comb containing brood and two empty frames. Colonies in treatment No Queen received the same comb

Table 4 Total area of comb constructed and percentage of that comb comprised of drone cells in experiment 4 (see text and Fig. 4 for detailed descriptions of the treatments). Combs were removed and measured on three occasions: 2, 5, and 11 days after the start of the experiment

Colony	Treatment	Day 2		Day 5		Day 11		All days	
		Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone
I	Drone	564	0	1290	32	2554	35	4408	30
	No drone	871	27	1077	43	927	35	2875	36
II	Drone	169	0	651	10	2064	8	2884	8
	No drone	645	27	784	39	1217	5	2646	20
VII	Drone	1113	26	1266	51	1915	10	4294	26
	No drone	704	84	1380	95	2295	86	4379	89
VIII	Drone	998	0	1734	5	2825	0	5557	2
	No drone	823	0	1833	13	2841	31	5497	20

setup as those in treatment Drone Comb, except that the drone combs were separated from the remainder of the hive by a queen excluder. As an improvement over the similar treatment in Experiment 3, the excluder was placed between two worker combs. This removed the possibility that the queen was directly acquiring information about the presence of drone comb by partially crossing the queen excluder and antennating or otherwise touching the drone comb. The colonies in treatment No Brood had the same setup as those in treatment No Queen, except that the drone comb contained no brood. It was obtained in the same way as the similar comb used in experiment 4.

As in experiments 1–4, I left the colonies in their treatments for several days and periodically collected the combs. I then switched the treatments among colonies and repeated the process. After four such phases, each colony had received each treatment exactly once. The data collection schedule varied across the four phases due to bad weather and other contingencies.

Based on the results of experiment 1, I expected the colonies to build significantly more drone comb when in treatment No Drone Comb than when in treatment Drone Comb. If the queen's tactile contact with the comb is not necessary for the workers to properly regulate their construction, then the colonies were expected to build similar drone comb proportions in treatment No Queen as in treatment Drone Comb, and significantly less drone comb in treatment No Queen than in treatment No Drone Comb. If drone brood is necessary to cue the bees to the presence of drone comb, then colonies were expected to build significantly more drone comb in treatment No Brood than in treatment Drone Comb and the same amount of drone comb in treatment No Brood as in treatment No Drone Comb.

As in the other experiments, colonies varied considerably in area of comb built and proportion of drone comb (Table 5). Two colonies (D and F) built little or no comb for one or more treatments and were excluded from the analysis. The observed drone comb proportion for colony B in treatment No Drone Comb was replaced with an estimate interpolated from the other observed values (Sokal and Rohlf 1981), because the colony was queenless during all or part of this treatment. (The de-

grees of freedom in the analysis of variance were reduced by 1 to account for this estimation.) When the mean proportion of drone comb built within each treatment was compared, the only significant differences were between treatments No Drone Comb and Drone Comb and between treatments No Drone Comb and No Queen (Fig. 5B). The difference between No Drone Comb and Drone Comb duplicated the result of experiment 1, confirming the ability of colonies to regulate their construction in response to perturbation of the proportion of drone comb. The difference between No Drone Comb and No Queen duplicated the result of experiment 3, confirming that this regulation does not depend on the queen's access to the comb.

The effect of drone brood was not as clear. Colonies in treatment No Brood built less drone comb than did colonies in treatment No Drone Comb and nearly as little as colonies in treatment Drone Comb, the expected result if drone brood is not necessary to inform the builders of the presence of drone comb. However, the difference between No Brood and No Drone Comb was not statistically significant. This equivocal result, combined with the result of experiment 4, suggests that ample drone brood contributes to but is not necessary for the inhibition of further drone comb construction. Even in the absence of drone brood, the bees are capable of detecting the presence of drone comb and reducing their subsequent construction of drone comb.

►

Fig. 5 Design and results of experiment 5 examining the importance to proper regulation of drone comb construction of the presence of drone brood and the opportunity for direct contact with the comb by queens. **A** Each colony received four treatments: (1) Drone Comb: the bees were provided with ample brood-filled drone comb to which the queen had free access; (2) No Queen: the bees were provided with ample brood-filled drone comb, but the queen was separated from it by queen excluders; (3) No Brood: the bees were provided with ample brood-less drone comb from which the queen was separated; (4) No Drone Comb: the bees were not provided any drone comb. **B** The amounts of drone comb built under each treatment were compared by repeated measures ANOVA and multiple *F*-tests. Treatment means not significantly different are joined by *horizontal lines*. *Bar heights* show the mean percentage drone comb across colonies; *error bars* show the standard deviations

The analysis yielded the same result when the data were based on cell counts rather than area measurements (Fig. 6). Because cell counts were not available for every colony-treatment combination, the analysis was performed only on later collections (that is, comb built on days 3–7 of each replicate). Also, one drone comb proportion for colony E had to be estimated because no cell count data were available for its No Brood treatment (Snedecor and Cochran 1967). A total of 6175 cells were measured, ranging from 58 to 590 for each colony-treatment combination (658–1485 per colony). The correlation coefficient between measurements by the two techniques was 0.96 ($P \ll 0.0001$).
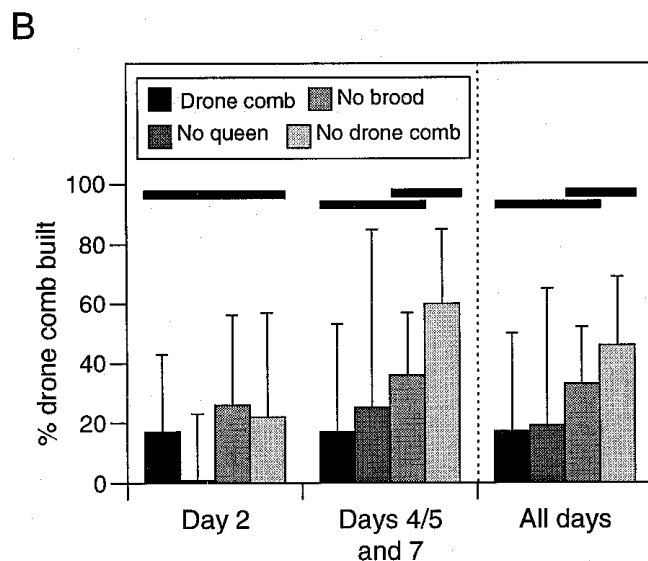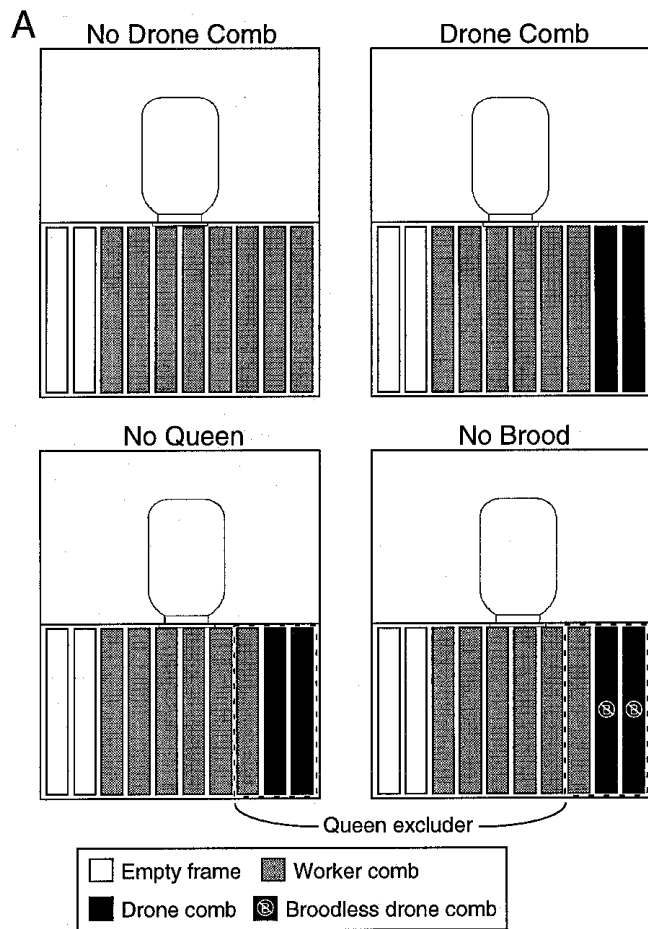
Discussion

The results of this study confirm earlier reports (Free 1967; Free and Williams 1975; Page et al. 1993) that the amount of drone comb in a honey bee nest is governed by negative feedback from drone comb already constructed (experiments 1 and 5). They further show that this inhibition depends on the workers having direct contact with the drone comb in their nest, but does not depend on the queen's contact with the comb (experiments 2, 3 and 5). Finally, they show that the comb itself, rather than the brood within it, is sufficient to produce the negative feedback, although the brood may also contribute to the effect (experiments 4 and 5). These findings are consistent with a mechanism for drone comb regulation based on decentralized control by the builders rather than centralized control by the queen.

Although the above results are apparent in the mean effect of each treatment on building behavior, the colonies within each treatment showed marked variation in the amount of drone comb constructed. Some of this variation reflected differences in the overall tendency of each colony to build drone comb, regardless of treatment. For instance, colony I generally built greater proportions of drone comb than colony VI, although their differential responses to the experimental treatments were similar. In other cases, particularly in experiment 5, the responses of some colonies to certain treatments differed from the mean response to those treatments across all colonies. For example, colonies E and H showed little difference in how much drone comb they built in treatments Drone Comb and No Drone Comb. The meaning of these departures is unclear, but they could reflect the influence of unknown and uncontrolled factors affecting comb type regulation. Nonetheless, the mean effects clearly demonstrated in these experiments allow conclusions to be drawn about the factors which were directly tested.

The dependence of regulation on the comb itself, rather than on the drone brood whose quantity is presumably the ultimate target of regulation, makes sense given that significant comb construction may occur when little or no drone brood is being reared. Drone rearing occurs over a relatively limited 2- to 3-month season (Allen 1965; Page and Metcalf 1984), while comb construction may extend over 5 or more months (Pratt 1997). Dependence on drone brood to properly regulate construction might induce excessive drone comb construction during parts of the year, creating persistent inappropriate comb type proportions. Nonetheless, the

A

No Drone Comb   Drone Comb

No Queen   No Brood

Queen excluder

Empty frame   Worker comb
Drone comb   Broodless drone comb

B

Drone comb   No brood
No queen   No drone comb

% drone comb built

Day 2   Days 4/5 and 7   All days

Table 5 Total area of comb constructed and percentage of that comb comprised of drone cells in experiment 5 (see text and Fig. 5 for detailed descriptions of the treatments). Combs were removed and measured on three occasions: 2, either 4 or 5, and 7 days after the start of the experiment. The notation *nc* in the Area column indicates that no collection was made for the given colony-treatment combination. A dash in the % Drone column indicates that no percentage could be calculated because the bees built no comb or because no collection was made

Colony	Treatment	Day 2		Day 4/5		Day 11		All days	
		Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone	Area (cm^2)	% Drone
A	Drone	420	0	462	4	617	10	1499	5
	No queen	555	0	873	0	nc	–	1428	0
	No brood	501	0	729	34	nc	–	1230	20
	No drone	493	91	492	80	717	82	1702	84
B	Drone	298	0	272	0	214	0	784	0
	No queen	287	0	382	0	nc	–	669	0
	No brood	317	32	516	0	nc	–	833	12
	No drone	139	40	208	70	494	100	841	83
C	Drone	136	0	225	31	nc	–	361	19
	No queen	131	0	123	100	350	100	604	78
	No brood	216	0	377	48	585	68	1178	49
	No drone	186	3	346	94	nc	–	532	62
D	Drone	49	0	39	0	131	0	219	0
	No queen	0	–	0	–	nc	–	0	–
	No brood	13	0	0	–	nc	–	13	0
	No drone	69	0	127	37	348	14	544	18
E	Drone	430	55	374	79	856	12	1660	38
	No queen	372	0	672	7	nc	–	1044	5
	No brood	352	0	674	24	nc	–	1026	16
	No drone	256	2	285	42	406	41	947	31
F	Drone	22	21	0	–	nc	–	22	21
	No queen	191	3	243	81	404	63	838	55
	No brood	244	0	401	83	669	41	1314	46
	No drone	138	36	73	82	nc	–	211	52
G	Drone	519	4	736	0	nc	–	1255	2
	No queen	515	0	625	10	919	23	2059	13
	No brood	567	65	623	75	958	27	2148	51
	No drone	91	0	199	40	nc	–	290	27
H	Drone	295	42	429	31	nc	–	724	35
	No queen	344	3	446	22	668	23	1458	18
	No brood	469	58	428	42	607	54	1504	52
	No drone	399	15	489	66	nc	–	888	43

equivocal result of experiment 5 suggests that drone brood exerts some effect on building type. It may be that more than one cue is effective, including one or more associated with the brood and one or more with the comb itself.

Free (1967) suggested that workers determine how much drone comb is in their nest by detecting a pheromone worked into the comb as it is built. The necessity of direct worker-comb contact for effective inhibition appears to rule out a volatile pheromone as such a signal, but leaves open the possibility of a chemical cue requiring tactile contact for perception. A likely alternative is that the bees perceive the difference between worker and drone cells by directly assaying their size through mechanical sensors, since they presumably must be able to do so when regulating cell size during construction. They may, for example, use their forelegs as a pair of calipers to determine cell diameter. Queens who have had their forelegs amputated are no longer able to distinguish cell size and lay the appropriate egg type (Koeniger 1970). Workers may similarly depend on their legs as measuring instruments, although no experimental evidence has been presented for such a role. The trickier task for the bees is tallying the relative amounts of the two kinds of cells. An advantage of a volatile pheromone in this regard is that workers might readily determine drone comb quantity by simply noting the strength of the pheromone's concentration at any point, assuming that it is dispersed adequately throughout the nest. Detection mechanisms depending on direct contact with the comb, whether mediated by chemical or mechanical sense organs, would seem to require that bees wander throughout the hive, directly assaying the type of large samples of comb, and keeping track of the relative quantity of each type. Moreover, these independent assessments would be subject to sampling error and might produce highly variable estimates of drone comb amount. A volatile pheromone, in contrast, could provide all workers with identical, high-quality information.
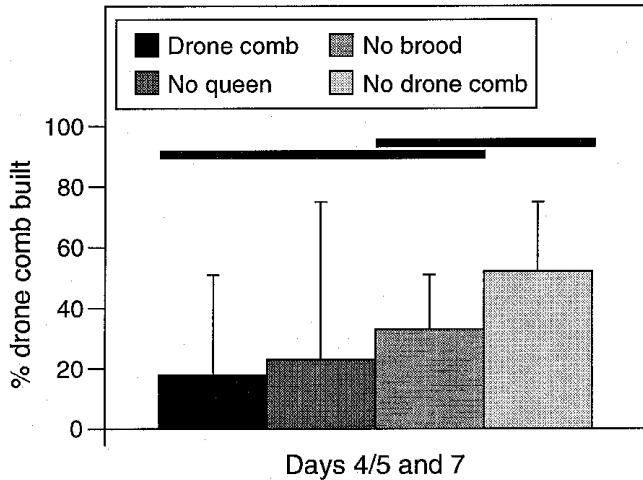
Fig. 6 Results of the same experiment shown in Fig. 5, except that the relative drone areas were measured by sampling cells rather than by tracing combs. These data were available only for comb collected on days 4, 5, and 7 of the experiment. Treatments were compared by repeated measures ANOVA and multiple *F*-tests. See the legend to Fig. 5 for further details

If the bees depended on the queen's assessment, the process might retain the advantages of simplicity and consistency provided by volatile pheromones, since thousands of builder bees would be spared the task of determining comb type proportions and would receive their information from a single source. However, the results of experiments 3 and 5 make clear that this is not the case. When the queen is prevented from coming into contact with the drone comb, the colony nonetheless regulates its construction normally, building relatively little new drone comb. Thus, even in circumstances conducive to the queen's acting as a central information processor, she does not play such a role. This finding is consistent with studies of other decision-making mechanisms in large-colony insect societies. Phenomena as diverse as the allocation of foragers among food sources (Seeley et al. 1991), the construction of complex nests (Franks et al. 1992; Deneubourg and Franks 1995), and the formation of species-typical recruitment patterns (Deneubourg et al. 1989; Franks et al. 1991) depend on decentralized control mechanisms. Consistent and coordinated colony-level phenomena arise from the separate actions of hundreds or thousands of rather poorly informed individuals, rather than through the centralized control of highly informed leaders.

That centralized control is absent even in a case where circumstances would seem to favor its development may be taken as further evidence of the ubiquity of decentralized mechanisms of colony coordination. Why should centralized control be absent in this case, especially given the apparent drawbacks of requiring several thousand bees to independently evaluate drone comb quantity? For one thing, workers may have other reasons to inspect comb extensively. They may, for example, evaluate the degree to which the colony's comb is filling with honey, in order to know whether to build new comb for food storage (Pratt 1997). If this is the case, transferring the measurement of drone comb quantity to the queen may not markedly reduce the information-processing burden on the workers.

More generally, decentralized control mechanisms help a complex system to conserve the computational power of its subunits (Simon 1996). Provided the problem of coordination can be solved, reliance on thousands of decision-makers, each required only to assay its immediate surroundings, may be less taxing on an individual bee's capacities than single-handed evaluation by the queen. Moreover, spreading a decision process over several thousand workers may improve the reliability of the decisions made. In general, the use of many parallel behavioral pathways enhances the reliability of a social insect colony's performance (Oster and Wilson 1978). In this case, using many evaluators rather than one may take advantage of the reduction of statistical error provided by large sample sizes. We can suppose that the evaluations of a single insect are, on average, correct readings of the colony's need, but that they show some variance around this mean. If only one insect (the queen) makes the evaluation, then this variance will also be the variance of the colony's overall assessment. On the other hand, if thousands of workers independently evaluate drone comb quantity, and the colony's response approximates the mean of their assessments, the variance of the colony's performance will be reduced by a factor roughly equal to the number of evaluators.

This leaves unanswered the question of how a coordinated building decision actually emerges from the separate choices of thousands of bees using only local information to measure drone comb quantity. The problem can be broken into two parts. First, how does each bee come to her own decision about the colony's need for drone comb? While it is possible that she actually counts cells as she inspects them, keeping a tally of each kind, such counting abilities have not been demonstrated in bees. On the other hand, bees have a well-established capacity to measure time intervals. Foragers assay their colony's nutritional status by measuring the duration between their arrival in the nest with a load of nectar and their encounter with a house bee willing to receive this nectar (Seeley 1989). In similar fashion, a builder may initiate an inspection walk over the comb, measuring several cells and keeping track of the delay until she encounters a drone cell. On average, the length of this delay will be correlated with the rarity of drone cells. The bee's decision may be based on a simple threshold rule: below a certain delay build worker comb; above it build drone comb.

The second part of the problem is coordination of the separate decisions of thousands of builders. An advantage of the evaluation mechanism described above is that it requires the bees to sample only a small portion of the colony's comb. However, evaluations based on limited samples will be error-prone. A bee who happens to sample in a drone-cell-rich portion of a

colony which has little drone comb overall will incorrectly decide to build worker comb. Thus when a colony begins to build new comb, there will be differences among the builders in their preferred comb type. This presents an organizational challenge, because comb construction is a highly distributed process in which several builders contribute to the construction of each cell. The bees form a large cluster at the nest periphery, and builders scurry along the growing edge of the comb, shaping the cells, adding new wax in some places, and removing it in others. In such a cluster, drone-cell builders and worker-cell builders will work at cross purposes. However, a sort of voting process within the cluster may ensure that the appropriate type of comb is constructed. The members of each group will repeatedly encounter nascent cells produced by bees of the other group, and alter them to yield the type it is trying to build. If one group is larger than the other, its efforts will prevail, and the comb will emerge in its favored size. Moreover, members of the smaller group may, after repeated encounters with growing comb of the other type, switch their behavior and begin building the majority type. Thus the comb itself could serve as a conduit for communication among builders. A similar voting process allows certain ant colonies to choose the best of two food sources, the decision emerging from numerous foragers' combined contributions and responses to pheromone trails (Beckers et al. 1993; Stickland et al. 1995).

In addition to placing relatively light information-processing burdens on individual bees, this kind of voting mechanism may be more reliable than single-handed evaluation by the queen. This is because it prevents the evaluation errors of individual bees from becoming the errors of the entire colony. No such error reduction is possible if evaluation depends solely on the queen. Moreover, such a system need not require the colony to build only one type of comb at a time. If construction commences in several places, with different groups of bees working at each site, then each group could come to an independent decision on comb type. Thus a colony could simultaneously build drone comb in one part of the nest and worker comb in another. Such a split decision would be more likely if the numbers of bees favoring each type were nearly equal, which may be the case when the drone comb proportion is near its ideal value.

The mechanism outlined above is purely speculative, but it is open to empirical test, and could also usefully be modeled via computer simulation. Analysis of this and similar phenomena will contribute to a deeper understanding of how such distributed mechanisms coordinate activity in social insect colonies.

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