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Effects of colony food shortage on behavioral development in honey bees

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Abstract Three experiments were conducted to explore the effects of severe food shortage on the control of two important and interrelated aspects of temporal division of labor in colonies of the honey bee (*Apis mellifera*): the size and age distribution of a colony's foraging force. The experiments were conducted with single-cohort colonies, composed entirely of young bees, allowing us to quickly distinguish the development of new (precocious) foragers from increases in activity of bees already competent to forage. In experiment 1, colony food shortage caused an acceleration of behavioral development; a significantly greater proportion of bees from starved colonies than from fed colonies became precocious foragers, and at significantly younger ages. Temporal aspects of this starvation effect were further explored in experiment 2 by feeding colonies that we initially starved, and starving colonies that we initially fed. There was a significant decrease in the number of new foragers in starved colonies that were fed, detected 1 day after feeding. There also was a significant increase in the number of new foragers in fed colonies that were starved, but only after a 2-day lag. These results suggest that colony nutritional status does affect long-term behavioral development, rather than only modulate the activity of bees already competent to forage. In experiment 3, we uncoupled the nutritional status of a colony from that of the individual colony members. The behavior of fed individuals in starved colonies was indistinguishable from that of bees in fed colonies, but significantly different from that of bees in starved colonies,

in terms of both the number and age distribution of foragers. These results demonstrate that effects of starvation on temporal polyethism are not mediated by the most obvious possible worker-nest interaction: a direct interaction with colony food stores. This is consistent with previous findings suggesting the importance of worker-worker interactions in the regulation of temporal polyethism in honey bees as well as other social insects.

Key words Temporal polyethism · Behavioral development · Behavioral plasticity · Foraging behavior · Division of labor

Introduction

Social insects are distinguished from nearly all other animals by their complex social life, which includes a division of labor among workers (Wilson 1971). Division of labor among workers is associated with differences in worker age (Hölldobler and Wilson 1990; Robinson 1992), genotype (Page and Robinson 1991; Moritz and Southwick 1993), and morphology (reviewed in Oster and Wilson 1978; Hölldobler and Wilson 1990). Division of labor is thought to contribute greatly to the growth, development, and reproductive success of insect colonies (Wilson 1985).

Age-related division of labor in insect colonies is based on a stereotyped pattern of worker behavioral development (Free 1965; Winston 1987). Worker honey bees typically perform in-hive duties such as tending brood, storing food, and maintaining the nest during the first 2–3 weeks of adult life, and then forage and protect the nest for the remainder of their 5- to 7-week life (Winston 1987). But there is great plasticity in this pattern. In response to environmental cues, individual workers may accelerate, retard, or even reverse their pattern of behavioral development (reviewed by Robinson 1992), resulting in tasks being performed at

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atypical ages. This developmental plasticity in behavior, which also occurs in other species (Calabi and Traniello 1989), is one component of a colony's ability to respond to changing conditions.

Short-term changes in worker task performance also contribute to flexibility in colony division of labor (reviewed by Robinson 1992; Gordon 1996). Workers change their activity levels in response to changing colony demography (Pendrel and Plowright 1981; Kolmes and Winston 1988), or switch between states of activity and inactivity depending on the need for labor in certain tasks (Wilson 1983; Breed et al. 1990). In some ant species, workers quickly switch tasks in response to disruptions of colony demography (Gordon 1989).

Plasticity in division of labor in insect colonies begins with the perception of colony needs, a process that is only beginning to be understood. The assessment of changing colony conditions by workers resulting in behavioral plasticity can take two forms: (1) a direct assessment of colony conditions by workers through interactions with the contents of the nest, as suggested by Lindauer (1952) and (2) an indirect assessment of colony conditions via worker-worker interactions, as suggested by Free (1965) and Ribbands (1952). Although these two mechanisms are not mutually exclusive, current evidence supports the hypothesis that worker-worker interactions mediate both short-term and long-term behavioral plasticity in colonies of honey bees. A forager honey bee gains information about its colony's carbohydrate store status by interactions with younger, "food-storer", bees as she unloads what she has just collected; she does not assess the food reserves of the colony herself (Seeley 1995). Information gained during these social interactions influences the subsequent short-term recruitment behavior of the forager. Jeanne (1996) reported that the coordination of colony foraging efforts for food and nest material in the tropical wasp, *Polybia occidentalis*, is achieved by a similar mechanism of worker-worker interactions.

Effects of worker-worker interactions on long-term developmental changes in worker behavior also have been reported in honey bee colonies. In "single-cohort colonies," small colonies composed initially of entirely young bees, some bees show accelerated behavioral development and become precocious foragers (Robinson et al. 1989). Huang and Robinson (1992) transplanted older foragers into single-cohort colonies and found that they inhibit the production of precocious foragers. This inhibitory effect was also seen when soldiers, older bees involved in colony defense (Breed et al. 1990), were transplanted into single-cohort colonies. Furthermore, when young bees are raised in isolation and then transferred into a single-cohort colony, a significantly larger number of them become foragers compared to sisters raised in typical colonies (Huang and Robinson 1992). Huang and Robinson (1996) also showed that when a colony is depleted of its foragers, precocious foraging is induced. Conversely, when foragers in colo-

nies are confined by artificial rain, the normal development of younger bees into foragers is delayed. These results indicate that worker-worker interactions are playing a role in regulating worker behavioral development.

There are other influences of colony food stores on short-term changes in the division of labor that relate to foraging activity, but it is not known whether these are mediated by worker-nest or worker-worker interactions. Colony food stores are potentially a potent source of cues that may regulate worker behavioral development. While not all species store food, for those that do it can be a valuable source of information regarding the nutritional state of the colony and hence colony foraging needs. Short-term changes in foraging behavior are known to occur in response to changes in food storage levels in bumble bees (Cartar and Dill 1990) as well as honey bees (Rinderer and Baxter 1978; Fewell and Winston 1992; Camazine 1993).

Changes in honey bee colony food stores influence short-term changes in worker behavior, but it is not known whether this factor also influences temporal polyethism. Colonies that experience a shortage of food perhaps cannot rely solely on increases in the activity of the existing foraging force. There are times when the existing foraging force is relatively small, such as in late spring in temperate climates when the older bees that survived the winter begin to die (Fukuda and Sekiguchi 1966). It seems reasonable to hypothesize that colony food shortage can cause an acceleration of behavioral development to provide the colony with new foragers. Although Huang and Robinson (1992, 1996) have shown that temporal polyethism is not mediated by direct assessments of the nest, but rather via worker-worker interactions, these studies were not conducted under conditions of food shortage. Testing this hypothesis thus allows us to learn more about how a bee colony functions when it is threatened by starvation, and explore the generality of the conclusions of Huang and Robinson (1992, 1996).

We determined the effects of colony starvation on the number and age distribution of foragers. After detecting a starvation effect, we explored whether it reflects short-term behavioral activation of bees that are already competent to forage, or an acceleration of behavioral development. Finally, we determined whether starvation-induced changes in behavioral development are mediated by the perception of empty comb in the bee hive. If workers are responding directly to the absence of colony food stores, this would be evidence that worker-worker interactions are not solely responsible for the control of temporal polyethism. If workers do not respond directly to empty comb, starvation-induced changes in behavioral development could be mediated instead by changes in social interactions, or the nutritional status of individual bees, or both.

Methods

General methods

This section provides details of methods common to all three experiments. Additional details for each experiment are given in the sections that follow.

Experimental colonies

Experiments were performed during the summers of 1995 and 1996 using honey bees (*Apis mellifera*) maintained by the University of Illinois Bee Research Facility. "Single-cohort colonies" were used throughout this study. A single-cohort colony is a small colony established entirely with 1-day-old adult bees. Single-cohort colonies allow one to distinguish easily between effects on behavioral development and short-term regulation of the activity of foragers. This is because in a single-cohort colony, behavioral development must occur first. Single-cohort colonies develop foragers in a precocious manner because of the lack of an existing foraging force, an example of plasticity in behavioral development (Robinson et al. 1989; Huang and Robinson 1992). Single-cohort colonies also afford two logistical advantages. Foragers develop within only 10 days of colony establishment, 1–2 weeks earlier than in more typical colonies, allowing more efficient replication of experiments. It is also easier to precisely control and manipulate colony population, genotypic composition, and food stores in a single-cohort colony than in a more typical colony. The relevance of data obtained with single-cohort colonies is considered in the Discussion.

One-day-old bees were obtained by taking frames of honey comb containing old pupae from large colonies in our apiaries ("source colonies") and placing them in an incubator at 33 °C. Each single-cohort colony was made up from bees from three to eight different source colonies. This is because each trial of each experiment required that three or four single-cohort colonies be established at the same time, and it is not possible to obtain the necessary number of bees from a single source colony. One-day-old adults from the different source colonies were thoroughly mixed prior to colony establishment so that all colonies in a given trial of an experiment were composed of a roughly equivalent mixture of worker genotypes. Bees were marked on the thorax with a spot of Testor's enamel paint, a different color for each single-cohort colony.

Each colony in a trial was established with an identical number of 1-day-old bees (1000–1250, depending on their availability), a single frame of food, and a caged mated queen. Queens were caged to prevent inter-colony variation in the amount of brood, which could influence division of labor (Free 1965; Y. LeConte and G.E. Robinson, unpublished work). Caging the queen also eliminated the possibility that workers in starved colonies could cannibalize the brood.

The purpose of this study was to determine the effects of carbohydrate shortage on division of labor. No pollen was given to any colony; "fed" or "starved" colonies in our experiments refer to only the presence or absence of carbohydrate. No pollen was used because it is difficult to set up colonies with precisely controlled amounts of nutritionally equivalent pollen. The nutritional content of pollen is difficult to measure; stored pollen in colonies consists of a honey-pollen mix referred to as "bee bread", and artificial pollen supplements are administered as mixtures with sugar to be palatable to bees. Both of these extra sources of carbohydrates could potentially confound the goals of this study. Raw dry pollen collected from different floral sources contain different levels of nutrients, which could be a further source of variation between colonies (Loper and Cohen 1987).

Behavioral observations

Foraging behavior for all colonies in an experiment was observed by a single individual (D.J.S.) for five to eight periods throughout

the day, from the beginning of foraging in the morning until it subsided at dusk. Each period consisted of 5 min of observation per colony, in succession, repeated three times, totaling 15 min per colony per period. Each colony in a trial of an experiment was observed for the same amount of time and within 15 min of the other colonies. The hive entrance was blocked for 10 min prior to observations, and returning foragers were identified by brightly colored pollen loads on their corbiculae or abdomens obviously distended by a full crop. Pollen foragers also were included in this study because some pollen foragers also collect nectar (Calderone and Page 1988). Each forager was marked with a spot of Testor's enamel paint on the abdomen as it attempted to enter the (blocked) hive entrance. Returning foragers were then counted and allowed to return to the hive. Two trials of experiment 1 and one trial of experiment 2 were performed blind with respect to colony type.

Colony census

At the end of each experiment, a census was performed to determine the precise number of non-foragers in each colony. The colonies were anaesthetized with CO₂, killed by freezing, and then each bee was examined for the appropriate thoracic and abdominal paint marks.

Statistical analyses

For each trial of an experiment, colony differences in the distributions of foragers and non-foragers were determined with 2 × 2 contingency table analyses. Colony differences in the age distributions of foragers were determined with Mann-Whitney *U*-tests. Bonferroni corrections were performed to eliminate possible effects of multiple comparisons.

Experiment 1: effect of colony food shortage on precocious forager development

A set of four single-cohort colonies was established, each containing 1250 bees from the same group of source colonies. Three colonies were starved and one was a fed control colony. Each starved colony was given a single frame which contained an amount of honey that was estimated to be sufficient to sustain it for either two, three or four days (based on a consumption rate of 1.1 g sugar/day/100 bees; Z.-Y. Huang, unpublished work). A minimum of 2 days' worth of food was given to assure survival to an age at which the bees are able to fly (2–3 days old). The fed colony was given a frame with much more honey than was necessary for survival. The food frame in each starved colony was removed on either day 2, 3 or 4, all bees on it brushed back into the hive, and replaced with a completely empty frame. The fed colony was treated similarly by brushing off all of the bees from the food frame and simply replacing the frame into the hive. Foraging observations were performed on all colonies for 4 days after foraging began by any colony. Two trials of this experiment were performed initially, and additional trials were included as part of experiments 2 and 3.

Experiment 2: short-term regulation of foraging activity or long-term development of foragers?

Four single-cohort colonies, containing 1000 bees each, were established as in experiment 1, two as starved and two as fed. In the evening of day 4, after the first day of foraging by any colony, we removed the now empty food frame from one of the starved colonies, and replaced it with a frame full of honey. This colony is subsequently referred to as the starved-to-fed ("S→F") colony. At the same time, we removed the food frame from one of the fed colonies and replaced it with an empty frame. This colony is subsequently referred to as the fed-to-starved ("F→S") colony. The other two colonies, one starved and one fed, both were sham manipulated as in experiment 1. Foraging observations were

performed as in experiment 1, and proceeded for 4 days after the above described food frame switch. Three trials of this experiment were performed.

Fisher's exact tests were used for intra-colony comparisons of the proportion of foragers and non-foragers on each of the first 3 days of foraging following the frame switch. Because the colony censuses conducted after the completion of the experiment revealed low mortality (Table 1B), and non-forager population of the colonies could not be determined while the experiment was running, the number of non-foragers used for these comparisons was the initial population of the colony minus any previously observed foragers.

Experiment 3: do bees assess colony food shortage by perceiving empty comb in the hive?

We explored this issue by separating two factors that are usually coupled in nature: the nutritional state of the colony and that of its individual workers. This was accomplished by allowing well-fed bees to develop in a starved colony, i.e., a colony completely devoid of any food stores. Unfortunately, we were not able to perform the converse experiment with starved bees in well-fed colonies; this would require surgical manipulations of bees that could also have independent effects on foraging ontogeny.

Starved colonies with fed bees were established as follows. The colony was provided *ad lib* with a feeder containing 50% sugar syrup, dyed with a blue food coloring. Food from a feeder is known

to be spread throughout a colony via trophallaxis (Ribbands 1952; Free 1956). The colony's sole frame was modified so that it was only one-sided, that is, only one side had storage cells. We drilled a hole at the base of each of the approximately 2000 cells of the frame, and a low-power suction device was used to remove any stored sugar syrup through the holes at the bottom of each cell (Fig. 1). This was done, whenever food stores were detected, multiple times each hour from approximately 0700 to 1800 hours, with roughly half-hourly checks continuing from 1800 to 0200 hours. No more than 100 cells at one time were ever found to contain food stores, even after the period between 0200 and 0700 hours when no vacuum removal occurred. Sham manipulations were not performed for the starved and well-fed colonies, as the procedure did not cause a disturbance in the colony. The bees were well-fed, as determined by the regular depletion of sugar syrup from the feeder and the appearance of the colored syrup in the bees (see below), but the colony had virtually no food stores. These colonies are subsequently referred to as "fed bees, starved colony" (FBSC) colonies.

Three single-cohort colonies were used for each trial, one FBSC colony, one fed colony, and one starved colony; all were established with 1000 bees. To standardize experimental conditions, starved and fed colonies also were supplied with dyed 50% sugar syrup in place of honey (in the same amounts as in experiment 1). Bees in all three colonies were examined for the presence of dyed food in their alimentary canals by casual observation. The contrast of the blue color of the syrup and the light color of honey bee cuticle allowed us to easily determine with the naked eye the presence or absence of

Fig. 1 Schematic diagram of the frame of modified (single-sided) honey comb designed to create colonies with fed bees but little or no food stores (FBSC colonies). **A** The magnified area shows comb with holes drilled in the back of each cell where stored sugar syrup was removed. **B** Cross-section of the frame, demonstrating how stored sugar syrup was removed via suction

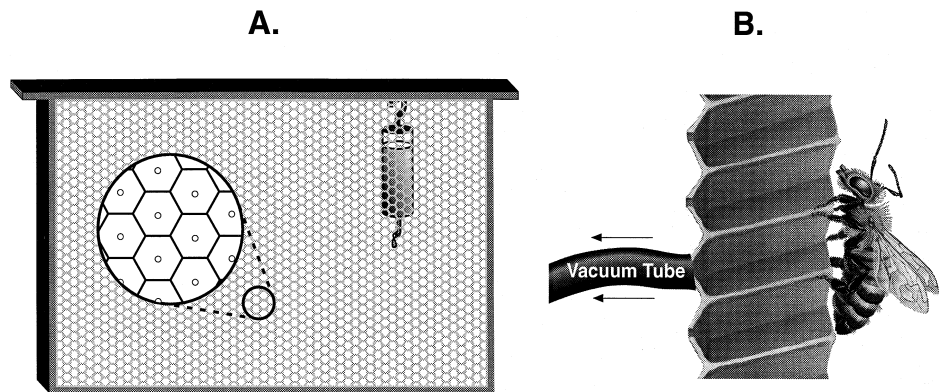


Fig. 2 Number of new foragers each day in starved and fed colonies during the first 4 days of foraging after colony establishment in **A** experiment 1 and **B** experiment 2. Letters next to data points indicate significant differences ($P < 0.02$) in the age distribution of foragers between colonies (Mann-Whitney *U*-tests)

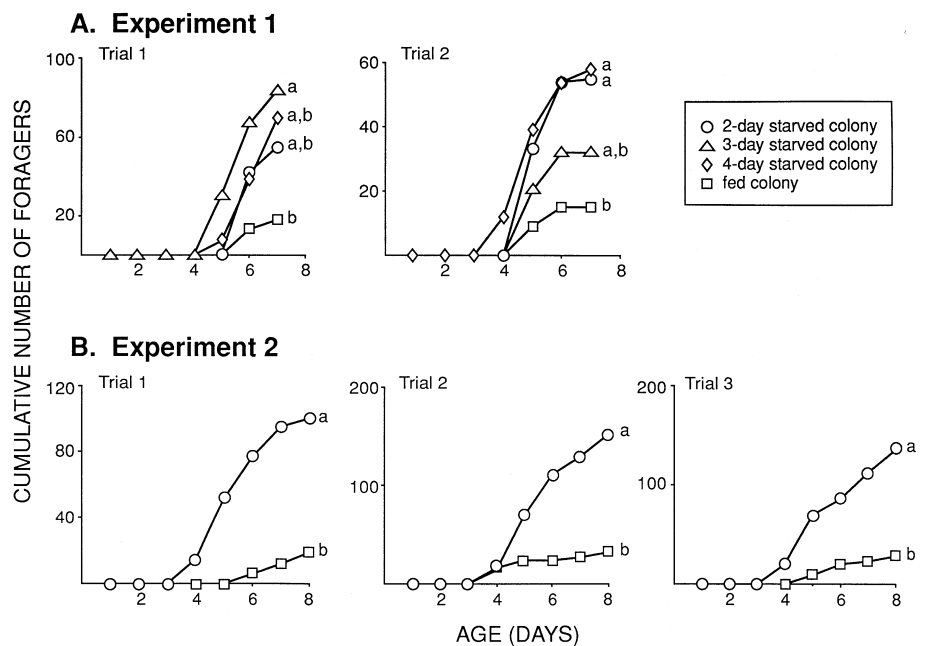


Table 1 Comparisons of the proportion of foragers and non-foragers of starved and fed colonies throughout the three experiments. Significant differences are reported for 2×2 contingency table analyses

	<i>Colony</i>	<i>Foragers</i>	<i>Non-foragers</i>	<i>Significant difference (vs. fed)</i>
A. EXPERIMENT 1				
Trial 1	2-Day-starved	55	993	$P < 0.01$
	3-Day-starved	84	1177	$P < 0.01$
	4-Day-starved	70	1183	$P < 0.01$
Trial 2	Fed	18	1216	
	2-Day-starved	55	1124	$P < 0.01$
	3-Day-starved	32	1163	$P < 0.02$
	4-Day-starved	58	1173	$P < 0.01$
	Fed	15	1202	
B. EXPERIMENT 2				
Trial 1	Starved	100	1024	$P < 0.01$
	Fed	19	1110	
Trial 2	Starved	161	1039	$P < 0.01$
	Fed	32	1168	
Trial 3	Starved	135	1065	$P < 0.01$
	Fed	28	1172	
C. EXPERIMENT 3				
Trial 1	Starved	54	920	$P < 0.01$
	Fed	17	1021	
Trial 2	Starved	148	330	$P < 0.01$
	Fed	40	500	
Trial 3	Starved	78	420	$P < 0.01$
	Fed	50	721	
Trial 4	Starved	79	557	$P < 0.01$
	Fed	42	779	

sugar syrup in bees. Bees from the fed colony and FBSC colony were confirmed to contain blue dye while the starved bees were almost entirely lacking. Foraging observations were performed for 4 days after the onset of foraging. Four trials were performed.

Results

Experiment 1: effect of colony food shortage on precocious forager development

In both trials, there were significantly more foragers observed for starved colonies than the fed colony (Table 1A). The age distributions of foragers from starved colonies also were significantly younger than for the fed colony in three out of six comparisons (Fig. 2A). There were no significant differences among the three starved colonies, for either the number or age distribution of foragers (consequently starved colonies in subsequent experiments all were given 2 days' worth of food).

Experiment 2: short-term regulation of foraging activity or long-term development of foragers?

Results from experiment 1 suggest that food shortage induces an acceleration of behavioral development, which results in a larger force of precocious foragers. Alternatively, starvation may increase the intensity of foraging behavior in bees already competent to forage (Rinderer and Baxter 1978). Even though we detected foraging as early as day 3 in experiment 1, it is not known when foraging competence actually begins. Ex-

periment 2 was performed to determine whether starvation influences behavioral development.

As in experiment 1, there were significantly more foragers observed for the starved colony than the fed colony in three out of three trials (Table 1B). The age distribution of the foragers from the starved colony also was significantly younger than for the fed colony in three out of three trials (Fig. 2B).

There was a relatively quick effect of feeding a starved colony; the number of new foragers dropped dramatically within 1 day of the frame switch (Fig. 3A). The number of new foragers was significantly ($P < 0.01$) lower for each of the 2 days immediately following feeding compared to the day before feeding in three out of three trials. Casual observations revealed that the activity of previously marked foragers also was reduced; no foraging activity, by new or previously marked foragers, was observed on the day immediately after feeding in two out of three trials.

Starving a fed colony caused an increase in the number of new foragers, but this was not evident until 2 days after the frame switch (Fig. 3B). There was no significant ($P > 0.25$) increase in forager number on the day immediately following the frame switch in three out of three trials. There was a significant ($P < 0.01$) increase on the second day in three out of three trials.

Experiment 3: do bees assess colony food shortage by perceiving empty comb in the hive?

Honey bees are a hoarding species, and empty comb has been shown to have an influence on the intensity of

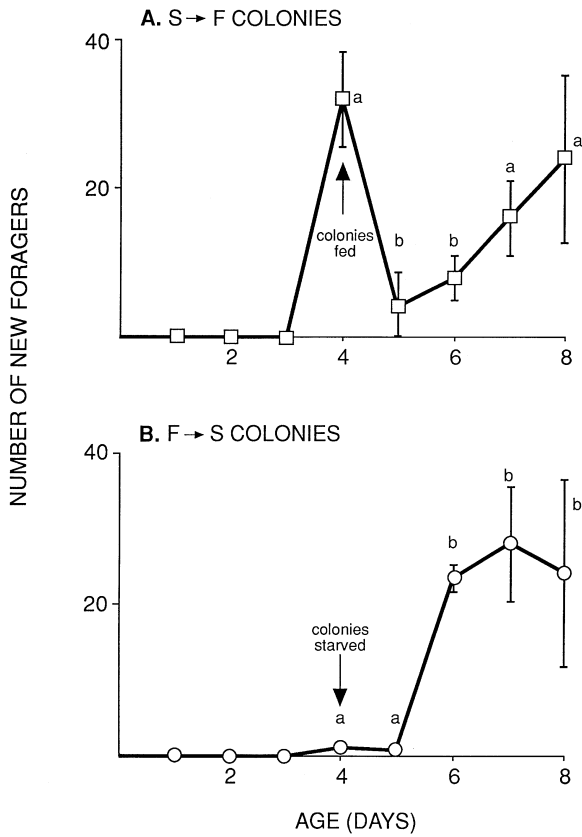


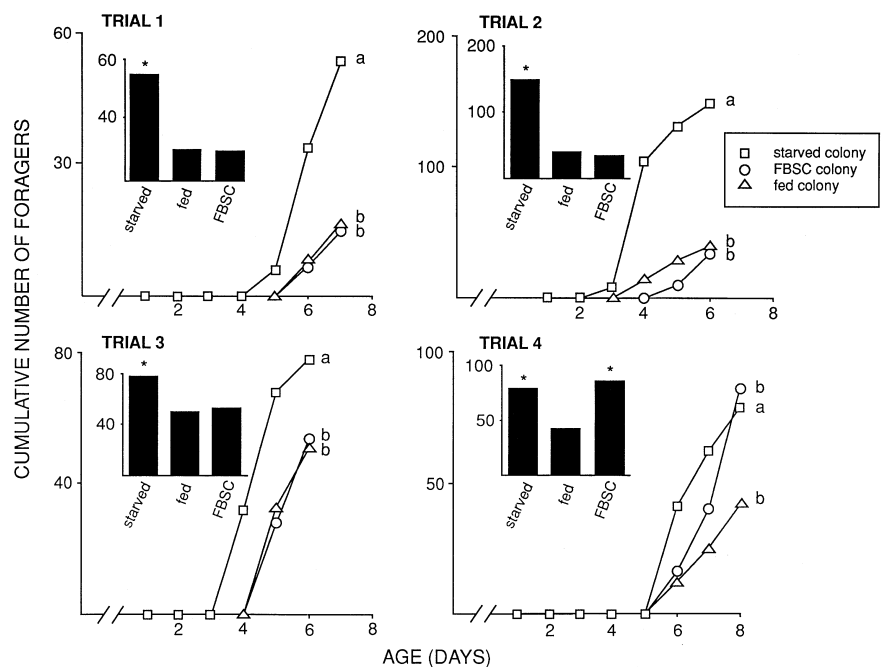
Fig. 3 Number of new foragers (\pm SE) produced each day by **A** S→F and **B** F→S colonies. Food frame switches occurred between days 4 and 5 (marked by arrow). Letters indicate significant differences in the distribution of foragers and non-foragers (~1100) on each day relative to the distribution on day 4 within the same colony (Fisher's exact test). Statistical tests were performed on each individual colony in each of the 3 trials; results of these tests were identical for all three trials, and so are pooled here for conciseness

foraging behavior by already committed foragers (Rinderer and Baxter 1978). However, it was not known whether empty comb mediated the starvation effect on behavioral development detected in experiments 1 and 2. This question has important implications for understanding social regulation of foraging (Seeley 1995) and temporal polyethism (Huang and Robinson 1992, 1996), and so was examined in experiment 3.

As in experiments 1 and 2, there were significantly more foragers observed for the starved colony than the fed colony in four out of four trials (Table 1C). The age distribution of the foragers from the starved colony also was significantly younger than for the fed colony in four out of four trials (Fig. 4). Colony censuses revealed that population losses were high in trials 2, 3 and 4, which were the only experiments performed in the summer of 1996. Similar mortality occurred in other experiments performed at our laboratory at this time. However, the consistency of the results from starved and fed colonies in these trials relative to those in experiments 1 and 2 suggest that the high mortality did not influence the results.

Individuals in FBSC colonies acted more like those in fed colonies, and different from those in starved colonies. There were no differences in the numbers or age distributions of foragers for FBSC and fed colonies in three out of four trials (Fig. 4). In contrast, there were significantly less foragers observed for FBSC relative to starved colonies in three out of four trials (Fig. 4). The reason why the fourth trial yielded a different result is not known. However, the age distribution of the foragers from the FBSC colony was significantly older than for the starved colony in all four trials (Fig. 4). These results demonstrate that the presence of empty comb alone is not sufficient to accelerate worker behavioral development.

Fig. 4 Cumulative number of foragers in starved, fed, and FBSC colonies. Letters indicate significant differences in age distributions (Mann-Whitney *U*-tests). Insets show the total number of foragers after 4 days of observations; *indicates a significant difference ($P < 0.02$) in the proportion of foragers and non-foragers (~1000) relative to the fed colony (2×2 contingency table analyses)



Discussion

The principal significance of these results is that they demonstrate that a shortage of colony carbohydrate stores can affect temporal polyethism in honey bee colonies, but not because workers sense the shortage directly, via worker-nest interactions. Previous studies have demonstrated a short-term effect of colony food stores on behavioral regulation, as did the present study. Free (1955) showed that the addition of food to storage pots of bumble bees reduced the number of foraging trips made by committed foragers. Conversely, Rinderer and Baxter (1978) reported that the addition of empty comb to honey bee colonies increased hoarding behavior, and depletion of pollen stores in honey bee colonies increased the rate of pollen collection by competent foragers (Fewell and Winston 1992; Camazine 1993). Previous studies also reveal that one reaction to food shortage is cannibalism, either of adults as in termite soldiers (Su and LaFage 1986) or of brood as in honey bees (Woyke 1976, 1977). This is the first demonstration that changes in colony carbohydrate stores can affect temporal polyethism in an insect colony.

Because removing food stores from fed colonies did not produce an immediate response, the 2-day delay involved in the onset of foraging in these colonies suggests that the young bees required a developmental period before they were physiologically capable of foraging. Alternatively, the onset of foraging in these colonies could have been delayed by the bees being sustained by the food they had stored in their crops, and only when this reserve was depleted did they begin to forage. However, adding food to a starved colony also decreased the production of new foragers, further suggesting a developmental effect. Thus carbohydrate shortage appears to accelerate forager development.

But it is not the presence of empty comb that accelerates behavioral development in single-cohort colonies. Since this is the most likely cue in the nest associated with food shortage, it indicates that the effects of starvation on temporal polyethism are not mediated by direct worker-nest interactions. The possibility exists that the used comb itself contains some cue that food is present in the colony. However, it would not be adaptive for bees to use comb in this way because it could mask a possible food shortage. Furthermore, Rinderer and Baxter (1978) reported that adding used empty comb to mature colonies *increased* foraging intensity. Finally, in our experiments, if used comb contained a cue indicating the presence of food, then no starvation effect would have been seen at all.

These new results are consistent with and extend those of Huang and Robinson (1992), which show that social interactions mediate the age at which foraging begins. Huang and Robinson (1992, 1996) formulated a model which can explain how social interactions mediate development of temporal polyethism. This model contains two elements: (1) an activator (juvenile hormone)

which induces bees to forage when present in high levels in the blood, and (2) an inhibitor (as yet unidentified) which decreases the rate of behavioral development. The inhibitor is hypothesized to be produced and/or transferred in greater amounts by older bees. According to this model, the rate of long-term behavioral development depends to a large extent on the amount of exposure to the worker inhibitor. Recent results indicate that older bees must be able to antennate and feed younger bees in order to inhibit their endocrine and behavioral development, implicating trophallaxis as a route of inhibitor transfer (Huang et al., in press). Perhaps starved bees develop faster because they engage in less trophallaxis relative to fed bees. If the inhibitor is transferred during these social interactions, then there would be reduced transfer of the inhibitor among starved bees. Reduced inhibitor transfer in starved colonies would be consistent with the observed starvation-associated acceleration of behavioral development. Conversely, increased inhibitor transfer in FBSC and fed colonies would explain their relatively fewer numbers of precocious foragers.

Another explanation exists to account for these results. The physiological effects of starvation on individual bees may somehow trigger the pathways associated with forager development independent of social interactions. It has been reported that starved bees have higher rates of juvenile hormone biosynthesis (Kaatz et al. 1994), but these bees were also placed in isolation, so the results may also reflect a lack of trophallaxis. The fact that only a portion of bees in a starved colony develop into foragers suggests that some social negative feedback system is operating, such as the one implicit in the activator-inhibitor model. As more foragers are developing in a starved colony, presumably their levels of inhibitor are increasing, preventing other bees from also developing precociously. Our results are thus consistent with the activator-inhibitor model in showing that the rate of behavioral development is not influenced by worker-nest interactions, but do not provide further evidence in support of it.

All of the experiments reported here were conducted with small, single-cohort colonies; are the results relevant to larger colonies of honey bees, with more typical age structures? For honey bees of predominantly European origin in North America, colonies newly founded from swarms can have populations smaller than 4000 bees, and even less for Africanized bees in the tropics (Winston 1987). Colonies also have comparably small populations when overwintering (Jeffrey 1955). Small colonies of honey bees show robust and highly consistent patterns of social behavior (von Frisch 1967; Seeley 1995). Moreover, studies of division of labor using smaller (Lindauer 1952; Winston and Punnett 1982) and larger (Seeley 1982; Robinson 1987; Calderone and Page 1992) colonies have revealed similar patterns of behavior, suggesting that the behavior of bees in small colonies is comparable to what occurs in larger colonies. The age demography of honey bee colonies in nature also varies

a great deal, especially under temperate conditions. For example, in late spring, as the first generation of the year begin to emerge as adults, many of the older bees that have lived all fall and winter die, leaving younger workers as the bulk of the worker force. Colonies in this state resemble single-cohort colonies, and premature foraging occurs (Robinson 1987). Giray and Robinson (1994) showed that genotypes of bees that develop more precociously in single-cohort colonies also develop slightly more precociously than do other genotypes in colonies with a more typical age demography. These results suggest that factors that influence precocious behavioral development also influence typical behavioral development. Single-cohort colonies of honey bees are very useful as experimental models, but it is likely that the effects of starvation on temporal polyethism observed in this study are probably much stronger than would be observed in larger colonies with more typical age structures. In such colonies increased foraging could be accomplished by under-employed foragers or middle-age bees that accelerate their rate of behavioral development slightly. Our results thus demonstrate that very young honey bees can respond to food shortage by accelerating their rate of behavioral development, but studies involving larger colonies with more typical age structures are necessary to determine how this capability fits into a colony's overall response.

Although we did not demonstrate that worker-worker interactions are the modality by which colony needs are perceived, our results further implicate such a phenomenon. These worker-worker interactions presumably involve trophallaxis. Trophallaxis has long been thought to play a major role in the social organization of insect colonies (Roubaud 1916; Wheeler 1918), providing a link both between adults and between adults and larvae. Trophallaxis may be one of the key extrinsic factors in the evolution of eusociality, as it provides a mechanism for acquisition of nourishment completely separate from that of solitary species (Hunt 1982). Trophallaxis may also have further evolved as the basis for communication to coordinate colony-wide activity. For example, there are changes in colony foraging due to changing trophallaxis patterns in colonies of honey bees (Seeley 1986), paper wasps (Jeanne 1996), and fire ants (Sorensen et al. 1984, 1985). Starvation changes the nutritional profile of food brought in by foragers of *Solenopsis invicta*, and is apparently regulated during trophallactic exchange between foragers and reserves (Sorensen et al. 1985). These results suggest that to better determine the mechanisms involved in increased forager production due to starvation in honey bees, future studies should examine the effects that colony food shortage have on trophallaxis.

The presence or absence of carbohydrate food stores affects both the development of new foragers and the activity of existing foragers in honey bee colonies. The adaptiveness of such a system is not a mystery, for any colonies that cannot respond appropriately to the threat of starvation will certainly perish. What is not clear is

precisely how the presence or absence of food acts indirectly to affect behavioral development. Perhaps by not relying directly on the nest for this information they avoid the need for all of a colony's workers to constantly be aware of dynamic food storage conditions. A worker need not know whether a distant comb contains food stores, or whether a particular cell is destined for honey or brood, to assess the need for food in the colony; it can rely solely on its personal nutritional status or its pattern of social interactions. Further investigations should reveal how the bees are affected by starvation conditions, both physiologically and socially, to further our understanding of the integration of behavior in honey bee colonies.

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