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Protein trophallaxis and the regulation of pollen foraging by honey bees (*Apis mellifera* L.)

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Abstract – Pollen foragers quickly sense increases in colony pollen stores, and modify their foraging activity appropriately. In association with these changes in foraging behavior, nurse bees transfer a larger portion of newly synthesized ^{14}C-phenylalanine-labeled protein to the foragers. These findings support the hypothesis that trophallactic interactions between nurse bees and pollen foragers may serve as a cue apprising pollen foragers of the colony's need for pollen. © Inra/DIB/AGIB/Elsevier, Paris

pollen forager / protein trophallaxis / nurse bee

1. INTRODUCTION

1.1. Information acquisition and colony-level regulation

Social insects precisely regulate colony-level processes critical for their survival. For example, honey bees modulate their intensity of nectar and pollen foraging in accordance with colony needs (Lindauer, 1952; Free, 1967; Cale, 1968; Barker, 1971; Free and Williams, 1971; van Laere and Martens, 1971; Al-Tikrity et al., 1972; Moeller, 1972; Seeley, 1989; Seeley et al., 1991; Fewell and Winston, 1992; Seeley and Towne, 1992). Fire ants apportion foraging efforts among carbohydrates, pro-

tein and lipids (Sorensen et al., 1985). They also regulate the rate of egg laying by the queen (Tschinkel, 1988). In response to changing environmental conditions, honey bees carefully control the temperature of their nest or swarm (Lindauer, 1954; Heinrich, 1981a, b, c) and termites also exhibit sophisticated regulation of the temperature, carbon dioxide level and humidity of their nest (Lüscher, 1961).

How do social insects accomplish these tasks? All regulatory mechanisms require acquisition of information about the state of the system and the implementation of appropriate regulatory responses. Multicellular organisms such as humans use 'centralized' homeostatic mechanisms to regulate physiological variables such as body temperature and glucose level. By means of a dense network of circulatory and neural pathways that permeate the body, information from throughout the organism can be acquired and processed centrally, by the brain for example, allowing the execution of the appropriate behavioral or physiological responses.

But the mechanisms used by a single organism are not the same as those used by a colony of many organisms. A colony of social insects may consist of thousands or even millions of autonomous individuals. It is unlikely that an individual colony member could acquire detailed information about the state of the entire colony. Therefore one would not expect specific individuals to play central roles in directing the activity of other colony members. In lieu of centralized mechanisms of colony organization, social insects have evolved efficient decentralized mechanisms based upon each individual's response to information acquired in its local environment. As such, to understand the regulatory mechanisms underlying colony-level processes, one must determine what information individuals acquire about colony needs, and how they use that

information to modulate their behavior. This study addresses this question in the context of the regulation of pollen foraging by honey bees.

1.2. Protein balance in honey bee colonies

Protein for honey bee larval development is derived from the digestion of pollen. Adult bees also require protein (Crailsheim, 1986, 1990a). However, not all bees consume and metabolize pollen equally well. Workers approximately 8 days old are the colony's primary pollen processors and distributors. They act as nurses, feeding proteinaceous hypopharyngeal gland secretion (jelly) to the larvae. They also feed jelly to other colony members (Crailsheim, 1990a, b, 1991). The gastrointestinal tract of these pollen processor bees has the highest pollen content and their midguts have the highest proteolytic activity of all bees in the colony (Moritz and Crailsheim, 1987; Crailsheim et al., 1992). In addition, their hypopharyngeal glands are especially well developed (Moritz and Crailsheim, 1987; Crailsheim and Stolberg, 1989). Throughout this paper we refer to these bees interchangeably as nurse bees or pollen processors.

In contrast, pollen foragers themselves consume little pollen, have little enzymatic capability to digest pollen, and have atrophied hypopharyngeal glands (Moritz and Crailsheim, 1987; Crailsheim, 1990a). Thus, the pollen processors serve as the colony's consumers and distributors of protein, while the foragers act as the pollen collectors.

1.3. The regulation of pollen foraging

A previous study (Camazine, 1993) showed that pollen foragers can quickly

acquire information about the colony's need for pollen, and that the foragers do not need 'direct' contact with the supplemented pollen to detect the colony's change of state. Instead pollen foragers are able to obtain their information about colony pollen need 'indirectly' from other bees in the hive. It was suggested that a likely source of information was the pollen processors. As the major consumers of pollen and distributors of protein, these bees are in a pivotal position to integrate as well as disseminate information concerning the supply and demand for pollen within the colony.

This raises the question of what information the pollen foragers are using and how they obtain information.

1.4. An hypothesis for the regulation of pollen foraging

How might pollen processors communicate information about the colony's need for pollen? We focus on the possibility that trophallactic interactions between pollen processors and pollen foragers provide automatic and reliable information concerning the colony's need for pollen. When the colony has ample pollen stores, pollen processors may have large amounts of protein-rich jelly available to distribute to the pollen foragers. The consumption of sufficient jelly by the pollen foragers may inhibit their foraging. In contrast, under conditions of pollen dearth, the pollen processors may have less protein available for the foragers, thereby stimulating them to forage for pollen.

In this paper we address the question of whether pollen processors provide information to pollen foragers concerning the status of the colony's proteinaceous pollen stores. In particular, we address the following two questions: 1) How do changes in the colony's pollen stores affect the behavior of the pollen foragers? 2) How do changes in colony's pollen stores affect the trophallactic behavior and physiology of the pollen processors?

2. MATERIALS AND METHODS

2.1. Study sites and honey bee colonies

For each of the three experiments, we prepared a pair of colonies matched for adult population; amount and age of brood; and honey and pollen stores. The two colonies were arranged so that their entrances were 2 m apart. Each colony had two frames containing brood and honey. The contents of the third frame varied with each experiment, as described below. Experiment 1 was conducted in Urbana, Illinois, using Italian honey bees (*Apis mellifera ligustica*) kept in observation hives with three full-depth Langstroth frames, and internal dimensions of $78 \times 46.5 \times 4.5$ cm. Experiments 2 and 3 were conducted in Graz, Austria, using carnica honey bees (*A. m. carnica*). These colonies were kept in observation hives with internal dimensions $78 \times 44 \times 4$ cm, containing three 42×22 cm frames. The colonies were set up 2 weeks before the start of each experiment.

The colonies for experiments 1, 2 and 3, contained approximately 8 500, 6 800 and 7 500 bees, respectively. Population counts were made by marking off a 5 cm square grid on the glass walls of the observation hive, and counting the number of bees in each grid square.

2.2. Experimental design

The experimental design for these experiments involved setting up two colonies in parallel, depriving the bees of pollen for several days, and then supplementing one colony with pollen, while keeping the other colony pollen deprived. During the treatment period, analyses were made of the behavior of the pollen foragers, trophallactic interactions between pollen processors and pollen foragers, and physiological changes of the pollen processors.

2.2.1. Introduction of newly-emerged, marked bees

Seven days before day 1 of each experiment, 200 newly emerged bees were marked and placed into each colony. These bees had been taken from an unrelated colony. On day 1 of the experiment, these bees were 8 days old, the age which corresponds to the approximate peak of their pollen consumption and nursing activity (Crailsheim, 1991).

2.2.2. Pollen deprivation

For 5 days prior to the start of each experiment, both colonies were deprived of pollen. Each colony had approximately equal amounts of brood located on the upper and middle frames of the observation hive. A strip of queen excluder material was placed between these two upper frames and the lower frame, restricting the queen to the upper portion of the hive. As a result, the two upper frames became nearly full of brood, except for a small rim of honey. There was little pollen on the upper frames; at the end of the day, fewer than 20 pollen cells were found, and by the next morning, little, if any, pollen was seen. The lower frame in each hive was removed at the end of each day (between 1700 and 1900 hours) and replaced with an empty frame, or a frame with some honey, depending upon whether the colony had sufficient honey stores. Since almost all the pollen collected by the foragers was deposited on the lower frame, and since this frame was removed each evening, the colony was relatively starved for pollen during this preparatory period.

2.2.3. Experimental day 1

On the first day of each experiment, we treated both colonies identically: each had an empty frame in the lower position of the observation hive, placed in the colony the evening before as a part of the pollen deprivation protocol. Starting at approximately 0800 hours, when the first foragers began to return to the hive, each returning pollen forager was identified by the presence of pollen loads in her corbiculae. The pollen foragers from each of the two hives were marked with a different color paint, spotted on the thorax and abdomen with a fine paintbrush. Paints were mixed using shellac, dry pigments and sufficient 96 %

ethanol to obtain the proper consistency. Over the next 6–8 h, every unmarked pollen forager was marked as she returned to the hive, and a record was kept of the number of bees marked during each 15-min period. We stopped marking bees between 1400 and 1645 hours when the number of returning pollen foragers fell below approximately five bees per 15-min period. Then, the observation hives were opened, the queen excluder screens removed, and each colony was given a treatment. One colony was randomly selected as the experimental colony, and its lower frame was replaced with a frame approximately half-full of pollen. (The remainder of the frame was empty.) The other colony was designated the control colony, and it was given an empty frame. These frames remained in the colony overnight and during the next day, experimental day 2.

2.2.4. Injection of marked bees with radioactive amino acid

On the evening of day 1, at approximately 2100 hours (between 4 and 5 h after the experimental colony received supplemental pollen), 50 of the marked 8-day-old bees were taken from each colony, injected with 1 μL ^{14}C-phenylalanine (18.4 GBq/mmol, 3.7 MBq/mL, 0.033 mg/mL, from NEN), and returned to their colony. A second batch of 12 marked nurses was killed after 1.5 h and 1 μL of hemolymph was taken from each bee. This hemolymph sample was frozen at –20 °C, and kept for amino acid analysis. The gastrointestinal tract (GI) of these bees was also removed for measurements of midgut weight and protein content.

2.2.5. Experimental day 2

In experiments 2 and 3, each colony was opened at approximately 0700 hours, and all the radiolabeled nurse bees were removed for analysis of radioactive label. In addition, 12 non-injected, marked nurses were killed for hemolymph amino acid analysis, and GI tract weight and protein content to compare with the samples taken on the evening of day 1. Twelve pollen foragers and 12 switchers (bees that were pollen foragers on day 1, but switched to nectar foraging on day 2) were also prepared for analysis of midgut weight and protein content as they returned from foraging on the

morning of day 2. As on day 1, starting at approximately 0800 hours, when pollen foragers began to return to the hive, each bee that had been marked as a pollen forager on the previous day, was again marked with colored paint as she returned to the hive. The bees were marked with a different color depending upon whether they returned to the hive with pollen or without pollen. This resulted in two categories of bees: 1) bees that had foraged for pollen on day 1, and that continued to forage for pollen on day 2 (hereafter called 'continuing pollen foragers'), and 2) bees that had foraged for pollen on day 1, but that had not foraged for pollen on day 2. (These bees are foragers that presumably switched from pollen foraging on day 1 to nectar foraging on day 2, and are hereafter called 'switchers'.) In addition, at the end of the day, the observation hive was opened, and all the bees were collected which had foraged for pollen on day 1, but had not left the hive on day 2. (These bees were easily identified because they had a paint mark from having collected pollen on day 1, but had no paint mark indicating that they had returned to the hive on day 2.) These bees made up a third category of foragers: 3) bees that had foraged for pollen on day 1, but had not foraged at all on day 2, and are hereafter called 'quitters'.

2.2.6. Analysis of the levels of radioactivity in the nurse bees and foragers

The nurse bees and foragers were kept frozen until analysis. They were then burned in an Packard Oxidizer where CO_2 was trapped with Carbosorb that was mixed with Hionic Fluor, a liquid scintillation cocktail (both from Packard). Counts of ^{14}C activity were carried out with a Packard 1900CA Tri-Carb liquid scintillation counter. Results are given as DPMs (disintegrations per minute).

2.2.7. Analysis of midgut weight and protein content

Honey bees were individually dissected, and the midgut along with its contents was removed and weighed to the nearest 0.1 mg. The protein content of the midgut was then determined according to the method of Lowry et al. (1951), with bovine serum albumin (from Sigma: fraction V) as a standard. The tissues were put into 0.5 mL of a 1 M NaOH solution and homogenized for 30–45 s with an ultrasonic homogenizer. Then, an additional 0.5 mL of NaOH was added. The homogenate was kept at room temperature for 1–1.5 h. Thirty microliters of this solution were used for Lowry protein determination. After 45 min the absorption was measured with a Beckman spectrophotometer at a wavelength of 750 nm against a blank of NaOH solution.

2.2.8. Location of nurse bees and foragers

On the morning of day 2 of the experiment prior to the onset of foraging, the location of the marked nurse bees and pollen foragers was noted. Over the course of several minutes, the hive was scanned systematically, and the location of each bee was indicated with a symbol on the glass wall of the observation hive. The brood areas were also outlined. This tracing was then transferred onto another pane of glass, and photographed against a white background. A figure was prepared from the photograph by scanning it into a Macintosh computer.

3. RESULTS

3.1. Behavioral response of pollen foragers to pollen supplementation

Figure 1 shows the total number of pollen foragers marked on day 1 and day 2 in the pollen-supplemented and the pollen-deprived colonies in each experiment. The graphs show that pollen supplementation on day 1 results in a decrease on day 2 in the number of foragers foraging for pollen. The graphs along with *table 1* indicate that in the presence of supplemental pollen, a significant proportion of the pollen foragers that foraged on day 1 do not forage on day 2 (in each experiment, $P < 0.01$ by a G-test of independence). Note that the pollen foragers on day 2 consist of two subgroups: 1) those that foraged for pollen on day 1 and which continued to forage for pollen on day 2, and 2) new pollen foragers that did not
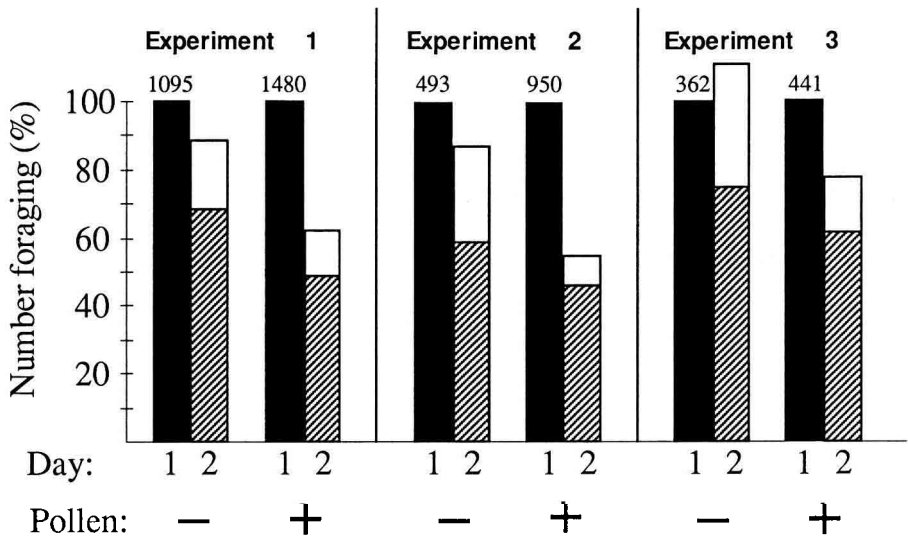
Figure 1. Number of pollen foragers marked on day 1 and day 2 under conditions of either colony pollen deprivation or pollen supplementation. For each of the three experiments, the data are expressed as percentages, with the day 1 total as 100 %. The stacked bars for day 2 consist of the sum of the foragers that collected pollen on day 1 and also collected pollen on day 2 (continuing pollen foragers, shown as cross-hatched bars) plus pollen foragers that did not collect pollen on day 1 but were newly marked as pollen foragers on day 2 (new pollen foragers, shown as open bars). The numbers above each bar on day 1 (solid bars) represent the total number of bees marked on that day. Colony pollen status: pollen deprivation (—) or pollen supplementation (+).

Table I. The number of pollen foragers marked on day 1 that either continued to forage for pollen on day 2 or did not forage for pollen on day 2 with respect to the treatment (pollen supplemented versus pollen deprived).

	Continuing to forage	Not continuing to forage
Experiment 1		
Pollen supplemented	740 (71 %)	309 (29 %)
Pollen deprived	746 (82 %)	168 (18 %)
Experiment 2		
Pollen supplemented	439 (52 %)	404 (48 %)
Pollen deprived	291 (67 %)	142 (33 %)
Experiment 3		
Pollen supplemented	269 (72 %)	107 (38 %)
Pollen deprived	269 (81 %)	62 (29 %)

Proportions are indicated in parentheses. In each of the three experiments there are significant differences in the proportions ($P < 0.01$) based upon the G-test of independence.

forage for pollen on day 1. In the pollen-deprived colonies, the proportion of pollen foragers on day 2 compared to day 1 is nearly 1 (0.93, 0.87 and 1.10 in experiments 1, 2 and 3, respectively). In contrast, in the colonies given supplemental pollen, many fewer bees foraged for pollen on day 2 so that the proportion of pollen foragers on day 2 compared to day 1 was considerably less than 1 (0.62, 0.55 and 0.77 in experiments 1, 2 and 3, respectively). In the pollen-supplemented colonies, two factors account for the decrease in the number of pollen foragers. First, there are fewer pollen foragers that continued to forage for pollen on day 2 after having foraged for pollen on day 1. Second, there is less recruitment of new pollen foragers. The second factor plays the greater role in the decrease in the number of pollen foragers marked on day 2.

3.2. The effect of pollen supplementation on trophallactic transfer within the colony

We wondered whether the behavioral differences between the pollen foragers in the pollen-supplemented and the pollen-deprived colonies were correlated with differences in trophallactic transfer of protein between pollen processors and pollen foragers. In all three experiments, the colony that was treated with pollen had a greater amount of ^{14}C-phenylalanine transferred to the pollen foragers marked on day 1 than the colony which was pollen deprived *(figure 2)*. In the three experiments, the amount of label transferred (measured in DPMs) in the colony given supplemental pollen was 2.25, 6.27 and 1.58 times, respectively, the amount transferred in the pollen deprived colony.

3.3. Contact between nurses and pollen foragers

Of course, in order for trophallactic interactions to occur between pollen foragers and pollen processors, the two groups of bees need to have contact with one another. Therefore, we documented the locations of the marked pollen foragers and the marked pollen processors on the morning of day 2. *Figure 3* shows the results for experiment 3; it provides a
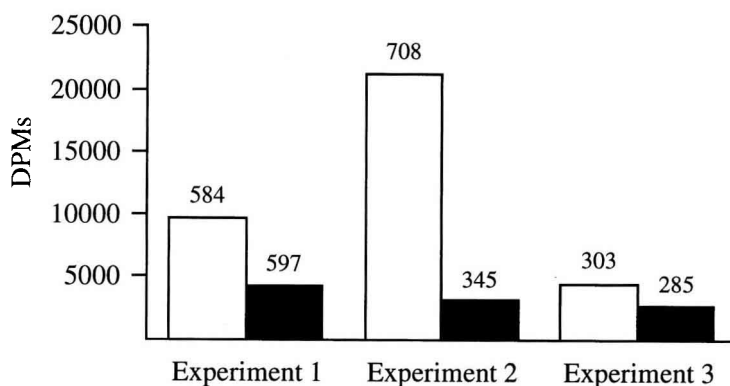
Figure 2. The total amount of ^{14}C-phenylalanine label transferred by the injected nurse bees to the pollen foragers marked on day 1. Open bars indicate the colony given supplemental pollen on the evening of day 1, and solid bars indicate the colony that was deprived of pollen. The number of bees examined in each experiment is indicated above the bar. Measurements are in DPM (disintegrations per minute).
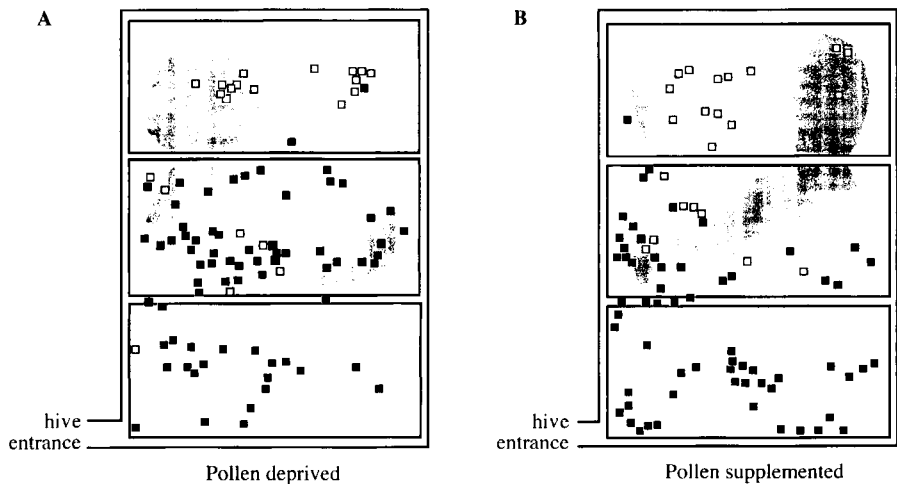
Figure 3. The locations of the marked pollen foragers (solid black squares) and pollen processors (8-day-old nurse bees) (open squares) on one side of the observation hive on the morning of day 2 of experiment 3 prior to the start of foraging. The brood areas are indicated by shading. (a) Colony that was pollen deprived; (b) colony that was pollen supplemented.

momentary 'snapshot' of the potential interactions between these two groups of bees. Since the results from experiment 1 were similar, only a representative figure from experiment 3 is presented. (Data were not available for experiment 2.) As seen in the figure, most of the marked pollen processors were found on the upper and middle frames which contained the brood; although many pollen foragers were on the lower broodless frame, many are also on the middle frame interspersed among the pollen processors. In addition, in the pollen-deprived colony, a greater proportion (63 %) of the pollen foragers are on the two upper brood frames than in the pollen supplemented colony (37 %). This is a significant difference in proportions ($P < 0.01$) based upon the G-test of independence. The effect was equally pronounced in experiment 1, where 25 % of the pollen foragers were seen on the two upper brood frames in the pollen deprived colony versus 0 % in the pollen supple-

mented colony. Clearly, the pollen foragers and pollen processors are in proximity with one another, permitting trophallactic interactions to occur.

3.4. The effect of pollen supplementation on midgut weight and midgut content of foraging bees

We suspected that the behavioral differences between the pollen foragers from the pollen-supplemented and the pollen-deprived colonies might be correlated with physiological differences between the bees in the two treatment groups. Therefore, a sample of bees marked as pollen foragers on day 1 were collected on the morning of day 2 of the experiment as they were returning to the observation hive. Bees returning with pollen (pollen foragers) as well as bees returning without pollen (nectar foragers) were collected. There were

Table II. The mean midgut weight and protein content measured in mg.

	Experiment 2		Experiment 3	
	Pollen added	Pollen deprived	Pollen added	Pollen deprived
Nectar foragers midgut weight	9.89 ± 2.05	9.28 ± 1.97	10.57 ± 2.29	10.48 ± 1.78
Pollen foragers midgut weight	8.77 ± 2.2	9.03 ± 1.78	9.57 ± 1.83	11.76 ± 1.59
Nectar foragers midgut protein	0.96 ± 0.09 a	0.86 ± 0.06 a	0.96 ± 0.09 b	0.72 ± 0.07 b
Pollen foragers midgut protein	1.00 ± 0.12 c	0.86 ± 0.09 c	0.94 ± 0.16	0.85 ± 0.07

Sample size was between 10 and 12 bees in each group. In comparisons (Mann-Whitney U-test) between colonies deprived of pollen or given supplemental pollen, there are significant differences ($P < 0.05$) between the two means marked with the same letters.

10–12 bees in each group. As seen in *table II,* in three out of four cases, the mean midgut protein content was significantly greater ($P < 0.05$ by the Mann-Whitney U-test) in foraging bees from the pollen-supplemented colonies. (Although the fourth case showed the same effect, the variance was too large for a significant effect.) These differences in midgut protein content can not be attributed to pollen consumption, however, since the midguts of bees in both groups contained little pollen , and the midgut weights were not significantly different for any pair *(table II).*

4. DISCUSSION

For a colony of honey bees to precisely regulate its pollen foraging, individual foragers must acquire information about the colony's nutritional needs. A potential source of information for the foragers is the nurse bees which act as the pollen processors in the colony. As the primary consumers and dispensers of both pollen and proteinaceous jelly derived from pollen, these bees automatically integrate information about the colony's supply and demand for pollen in the course of their daily activities: information about pollen supply is gathered as they search for, and consume pollen; information about pollen demand is available as they provide pollen and jelly to brood and adult bees which need proteinaceous nourishment.

The present study supports the hypothesis that the pollen processors (nurse bees) in the colony provide pollen foragers with reliable information concerning the colony's need for pollen which they use to modulate their level of pollen foraging activity. The evidence is as follows: there is contact between these two groups of bees allowing opportunity for trophallactic interactions to occur *(figure 3).* In addition, compared to conditions of pollen excess, under conditions of pollen deprivation, a greater proportion of foragers are found in the brood nest where they are more likely to encounter nurse bees *(figure 3).* Under conditions of pollen sup-

plementation, pollen processors distribute more labeled protein to foragers than pollen processors in colonies deprived of pollen *(figure 2)*. The greater amount of label transferred to the marked pollen foragers in the pollen supplemented colonies suggests that pollen processors either have more protein to dispense or that they are more willing to dispense that protein, or both.

We suggest that these differences in trophallactic transfer of jelly provide cues to the pollen foragers which are used to modulate their foraging activity, thus accounting for the observed differences in the foraging behavior of the pollen-supplemented versus pollen-deprived foragers *(figure 1)*. Foragers do not need to directly assess the pollen stores (Camazine, 1993), nor do they obtain information about the colony's nutritional status by consuming pollen themselves. Midgut weights did not increase significantly in the bees from the pollen-supplemented colonies *(table II)* as would be expected if they ingested more pollen. Furthermore, pollen foragers have been shown not to eat significant amounts of pollen (Crailsheim et al., 1992), correlated with a decrease in the proteolytic activity of their gut enzymes (Moritz and Crailsheim, 1987). Nonetheless, pollen foragers from colonies given supplemental pollen do show physiological changes indicating increased protein intake: the protein content of their gut is greater *(table II)* and they have higher body levels of radio-labeled protein than pollen foragers from colonies deprived of pollen *(figure 2)*. Presumably the additional protein was obtained through trophallactic interactions as jelly fed to the foragers by the pollen processors.

Why have we hypothesized this indirect mechanism of information acquisition rather than an apparently simpler, and equally reliable mechanism in which the pollen foragers themselves assess the colony's need for pollen? One argument is that direct assessment would presumably require extensive and ongoing surveys by each forager of both colony's supply (pollen stores) and demand (amount of brood and their state of nourishment). Such a feat of information collection would undoubtedly be time consuming, if not impossible, and would certainly detract from the foragers primary task of gathering pollen.

A second argument against direct assessment comes from previous experiments (Camazine, 1993) which showed that pollen foragers do not require direct contact with the colony's pollen stores to assess colony pollen needs. Pollen foragers separated from the colony's pollen stores by a double screen (which prevents trophallactic interactions between foraging bees and house bees) appeared to lack information about the colony's pollen status. In contrast, foragers separated by a single screen (which prevented access to pollen stores, but allowed trophallactic interactions across the screen) obtained accurate information about the colony's pollen status and foraged appropriately. These experiments suggested that a non-foraging class of bees was able to indirectly provide the foragers with reliable information concerning the colony need for pollen.

Such a system of indirect information acquisition may be both effective and efficient. All the pollen forager needs to do in order to accurately assess the colony's need for pollen is to sense her own hunger for protein. The system is similar to that for the regulation of nectar foraging (Seeley, 1989), where the nectar foragers modulate their foraging behavior based upon simple cues obtained from the nectar receiving class of bees. Here, too, the foragers do not, themselves, directly assess the colony's honey stores, but do so indirectly through information provided by other bees which automatically obtain that

information in the course of their nectar storing activities.

Although the results presented here support the hypothesis that trophallactic interactions (transfer of proteinaceous jelly from pollen processors to pollen foragers) provide information used by the foragers to modulate their pollen foraging, several important questions remain. Foremost is an explanation of precisely how trophallactic interactions provide information to the pollen foragers. We can suggest several alternatives: 1) the 'amount' of protein received by a pollen forager from a pollen processor may affect the forager's likelihood of foraging, 2) The 'ease' with which a pollen foragers receives protein from a pollen processor may affect the foragers likelihood of foraging. Ease might be evaluated by the pollen forager as a function of the 'number' of interactions required to receive a specific amount of protein from a pollen processor, the 'time' required to receive a specific amount of protein, or the number ('proportion') of trophallactic encounters with pollen processors in which the pollen forager is able to receive jelly.

With each of these proposed mechanisms, under conditions of ample pollen reserves, a pollen forager would presumably find it easy to locate pollen processors able and willing to transfer large amounts of proteinaceous jelly. Despite the plausibility of this hypothesis, it still remains to be demonstrated that trophallactic interactions actually modulate the behavior of the forager. In addition, if this is the mechanism, there remains the question of whether it is the physiological effects of the transfer of jelly that is important, or whether associated behavioral interactions that take place during trophallaxis are crucial in affected subsequent foraging activity? It might be possible to answer these questions if one were able to artificially feed proteinaceous jelly or jelly components to cohorts of foragers under con-trolled conditions and observe their subsequent foraging behavior. Such an experiment would provide much-needed direct evidence for the role of protein trophallaxis in the regulation of pollen foraging.

ACKNOWLEDGMENTS

Résumé – Trophallaxie des protéines et régulation de la récolte du pollen par les abeilles (*Apis mellifera* L.). Une colonie d'abeilles régule de façon précise sa récolte de pollen. Pour ce faire, les butineuses de pollen doivent acquérir chacune des informations concernant les besoins nutritionnels de la colonie. Une étude antérieure (Camazine, 1993) a montré qu'il n'était pas nécessaire que les butineuses de pollen aient un contact direct avec les provisions de pollen pour connaître les besoins de la colonie. Au contraire, l'information semble être obtenue indirectement à partir des autres individus de la colonie. Les nourrices constituent une source potentielle d'information puisqu'elles sont les premières transformatrices du pollen en le consommant, en le convertissant rapidement en gelée (sécrétion protéinique de la glande hypopharyngienne) et en le distribuant aux adultes et aux larves dans toute la colonie. Nous avons étudié le

transfert des protéines (trophallaxie) par les nourrices, transformatrices de pollen, comme source d'informations pour les butineuses. Nous avons testé l'hypothèse selon laquelle des variations dans le flux de gelée protéinique en direction des butineuses de pollen serviraient d'indications utilisées par ces dernières pour estimer les besoins protéiniques de la colonie ; ces variations leur fourniraient des informations qui leur permettraient de décider si elles doivent ou non poursuivre la récolte de pollen. L'expérimentation a porté sur deux colonies en parallèle ; toutes deux ont été privées de pollen durant quelques jours, puis l'une a reçu du pollen tandis que l'autre en est restée privée.

Les résultats sont les suivants : les butineuses de pollen perçoivent très rapidement – en quelques heures – les changements dans les provisions de pollen de la colonie. Dans la colonie supplémentée, de nombreuses butineuses de pollen réagissent en ne poursuivant pas leur récolte. Soit elles se mettent à récolter du nectar, soit elles cessent toute activité de butinage (*figure 1* et *tableau I*). Par ailleurs, selon qu'elles viennent des colonies supplémentées ou des colonies privées de pollen, les nourrices n'ont pas le même comportement vis-à-vis des butineuses de pollen. Elles réagissent à la supplémentation en pollen en donnant aux butineuses une grande partie de la gelée nouvellement synthétisée. Les nourrices des colonies supplémentées, auxquelles on a injecté de la phénylalanine marquée au C^{14}, distribuent plus de protéines marquées aux butineuses que celles des colonies non supplémentées (*figure 2*). Dans les trois expériences, la quantité de nourriture marquée transferrée aux butineuses (mesurée en désintégrations par minute) dans la colonie supplémentée était respectivement de 2,25, 6,27 et 1,50 fois celle transferrée dans la colonie non supplémentée. Comme le montre le *tableau III,* dans trois cas sur quatre, la teneur

moyenne en protéines de l'intestion moyen était significativement plus élevée ($p < 0,005$ test-U de Mann-Whitney) chez les butineuses des colonies supplémentées. Ces différences ne peuvent être attribuées à la consommation de pollen par les butineuses de pollen elles-même puisque les intestins moyens des abeilles des deux groupes renfermaient peu de pollen et que le poids des intestins moyens ne différait significativement pour aucune des paires *(tableau II)*. Ces résultats suggèrent au contraire que les butineuses de pollen obtiennent des protéines en sollicitant de la nourriture auprès des nourrices. Ceci confirme l'hypothèse selon laquelle les interactions trophallactiques entre nourrices et butineuses de pollen servent d'indications informant ces dernières des besoins en pollen de la colonie. © Inra/DIB/ AGIB/Elsevier, Paris

Apis mellifera / trophallaxie / protéine / nourrice / récolte pollen

Zusammenfassung – Proteintrophallaxis und die Regulation des Pollensammelns bei Honigbienen (Apis mellifera L.). Honigbienenvölker können den Eintrag von Pollen sehr genau regulieren. Hierzu müssen die einzelnen Pollensammlerinnen notwendigerweise Information über die Ernährungslage des Bienenvolkes erhalten. In früheren Untersuchungen (Camazine, 1993) konnte bereits gezeigt werden, daß die Pollensammlerinnen zur Abschätzung des Pollenbedarfs keinen direkten Kontakt zu den Pollenvorräten haben müssen. Stattdessen scheint die Information indirekt von anderen Arbeiterinnen im Volk zu stammen. Eine der möglichen Informationsquellen stellen hierbei die Ammenbienen als die primären Pollenverarbeiterinnen im Bienenvolk dar. Diese verzehren den Pollen und wandeln ihn sehr rasch in das proteinhaltige Sekret der Hypopharynxdrüsen um, das sie sowohl an die Larven als auch

an die ausgewachsenen Tiere im ganzen Volk weitergeben. In der vorliegenden Studie wird die mögliche Rolle des Transfers von Protein (Trophallaxis) durch diese Pollenverarbeiterinnen (Ammenbienen) als Informationsquelle für die Sammelbienen untersucht. Wir prüften die Hypothese, daß ein unterschiedlich starker Fluß von proteinhaltigem Drüsensekret von den Ammenbienen zu den Sammelbienen von diesen als Hinweis auf den Proteinbedarf des Volkes genutzt wird, auf Grund dessen sie entscheiden, ob sie weiterhin Pollen sammeln. Wir berichten hier über folgende Ergebnisse: Pollensammlerinnen nehmen eine Veränderung der Pollenvorräte innerhalb weniger Stunden, also sehr rasch wahr. In einem Volk, dem nach Pollendeprivation zusätzlicher Pollen gegeben wird, unterbrechen viele der Pollensammlerinnen den Polleneintrag . Sie gehen dann entweder zum Nektarsammeln über, oder sie stellen die Sammelflüge ganz ein *(Figure 1* und *Tabelle I)*. In Verbindung mit dieser Änderung des Sammelverhaltens zeigen die Pollenverarbeiterinnen in den mit zusätzlichem Pollen versorgten Völkern ein anderes Verhalten gegenüber den Pollensammlerinnen als in den pollendeprivierten Völkern. Sie reagieren auf die Zufügung von Pollen mit der Übertragung einer größeren Menge ihres frisch synthetisierten Futtersafteiweißes. Mit ^{14}C-phenylalanin injizierte Proteinverarbeiterinnen in den mit zusätzlichem Pollen versorgten Völkern verteilen mehr markiertes Eiweiß an Sammlerinnen als entsprechende Pollenverarbeiterinnen in Völkern ohne zusätzlichen Pollen *(Figure 2)*. In den drei Experimenten war die Menge des übertragenen Markers (gemessen in DPMs) in dem Volk mit zusätzlichem Pollen 2.25, 6.21 beziehungsweise 1.58 mal so hoch wie in den mit Pollen unterversorgten Völkern. *Tabelle II* zeigt, daß in drei von vier Fällen der mittlere Proteingehalt im Mitteldarm bei den Sammlerinnen in mit zusätzlichem Pollen versehenen Völkern signifikant

erhöht war ($P < 0.05$, Mann-Whitney U test). Diese Unterschiede können nicht auf den Verzehr von Pollen durch die Sammlerinnen selbst zurückgeführt werden, da in beiden Gruppen der Pollengehalt im Mitteldarm nur sehr gering war und die Gewichte des Mitteldarms bei keinem der Paare unterschiedlich war *(Tabelle II)*. Das Ergebnis weist darauf hin, daß die Pollensammlerinnen sich von den Pollenverarbeiterinnen mit Protein versorgen lassen. Diese Befunde unterstützen daher die Hypothese, daß trophallaktische Interaktionen mit den Pollenverarbeiterinnen den Pollensammlerinnen als Hinweis zur Einschätzung der Pollenversorgung des Bienenvolkes dienen. © Inra/DIB/AGIB/ Elsevier, Paris

Apis mellifera / **Pollensammlerinnen / Proteintrophallaxis / Ammenbienen**

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