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Feeding behaviour in the bumble bee *Bombus terrestris*

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ABSTRACT. Bumble bees (*Bombus terrestris*) are social insects that live in one-year colonies. Larvae are fed progressively by the workers up until the moment they pupate and transform into adults. An elaborate hypothetical scheme concerning the dynamics of feeding behaviour is presented. The central hypothesis is that larvae emit a hunger-signal that can inform workers about their nutritional status and thereby influence the feeding behaviour of the workers. In addition it is hypothesized that the receptivity of the workers for the hunger signal and their motivation also influence their decision whether or not to go and feed larvae. This contrasts with the prevalent view that workers impose a feeding regime on larvae, who passively undergo their rearing. Worker (feeding) behaviour in a number of colonies was recorded and experiments were conducted in order to elucidate several aspects of the dynamics of feeding behaviour. In the experiments presented here the strength of the hunger signal was manipulated in various ways: by starving larvae, feeding artificial food to larvae in vivo, and varying the number of larvae. The results of the experiments indicate that indeed in *B. terrestris* larvae emit a short-range hunger-signal that can be perceived by workers and that can trigger worker behaviour such as "long pollen eating" (which usually precedes feeding) and feeding larvae. This strongly suggests that a feeding regime is not simply imposed on larvae by workers. However, the motivation of workers also plays a decisive role in feeding behaviour.

KEY WORDS: *Bombus terrestris*, bumble bee, feeding behaviour, pollen eating, larvae, hunger signal, caste determination.

INTRODUCTION

Bumble bees are social insects that live in one-year colonies. The queen lays the eggs and the workers perform all necessary duties, among others foraging and feeding larvae. In *Bombus terrestris* (Latreille) larvae are fed progressively with a mixture of pollen and nectar plus some glandular secretions (PEREBOOM, 2000). At the colony level the feeding rate seems to be well regulated (PENDREL & PLOWRIGHT, 1981). At the level of individual larvae, however, regulation of the feeding rate appears relatively poor; the time between successive feedings of a larva varies considerably (PENDREL & PLOWRIGHT, 1981; RIBEIRO, 1999).

RÖSELER & RÖSELER (1974) refer to several authors who reported that in *Bombus* species some larvae are fed more than others, e.g. because of their position in the brood clump, and that this determines their final size. The prevalent view is that the workers impose a feeding regime on the larvae who passively undergo their rearing (RÖSELER, 1970, 1991; see also PLOWRIGHT & JAY, 1977). Contrasting with this view is evidence from PEREBOOM (1997) that workers are able to perceive the nutritional status of larvae and adjust their behaviour accordingly: He showed that starved larvae are fed more often than non-starved larvae. In addition, PEREBOOM (1997) found that larvae ingest food on their own account, and are capable of refusing food. These findings suggest a more active role of the larvae.

Before being able to feed, a worker needs to drink nectar and eat pollen. DUCHATEAU (unpublished data) found that workers who fed larvae after pollen eating ate pollen significantly longer than workers that did not feed after pollen eating (workers were observed for 30 minutes after they had stopped eating pollen). She also found that workers who were going to feed larvae spent more time on the

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broodnest and more time manipulating the larvae's wax envelopes than workers who were not going to feed.

The work of Pereboom (1997) and Duchateau (unpublished data) strongly suggests that workers initiate pollen eating and feeding in response to information they perceive concerning the nutritional status of larvae. Both Pereboom (1997) and Ribeiro (1997) suggested that larvae produce some kind of stimulus that elicits feeding behaviour. On the basis of their work we hypothesized that larvae emit a signal that can inform workers about their nutritional status ("hungryness"). The fact that there is considerable variation in the characteristics of the feeding behaviour of an individual worker (Pendrel & Plowright, 1981; Ribeiro, 1997, 1999; Pereboom, 1997) is an indication that receptivity for the hunger signal and the motivation to feed of the workers also play a role in feeding behaviour. In addition, many other factors may influence the dynamics of feeding behaviour. On the basis of literature and our hypotheses we made a schematic representation of the dynamics of feeding behaviour (Fig. 1). Several experiments were conducted to test the validity of this scheme. In this paper, experiments investigating the presence of the hunger signal are presented. This was done by starving larvae, manually feeding larvae in vivo and varying the number of starved larvae.

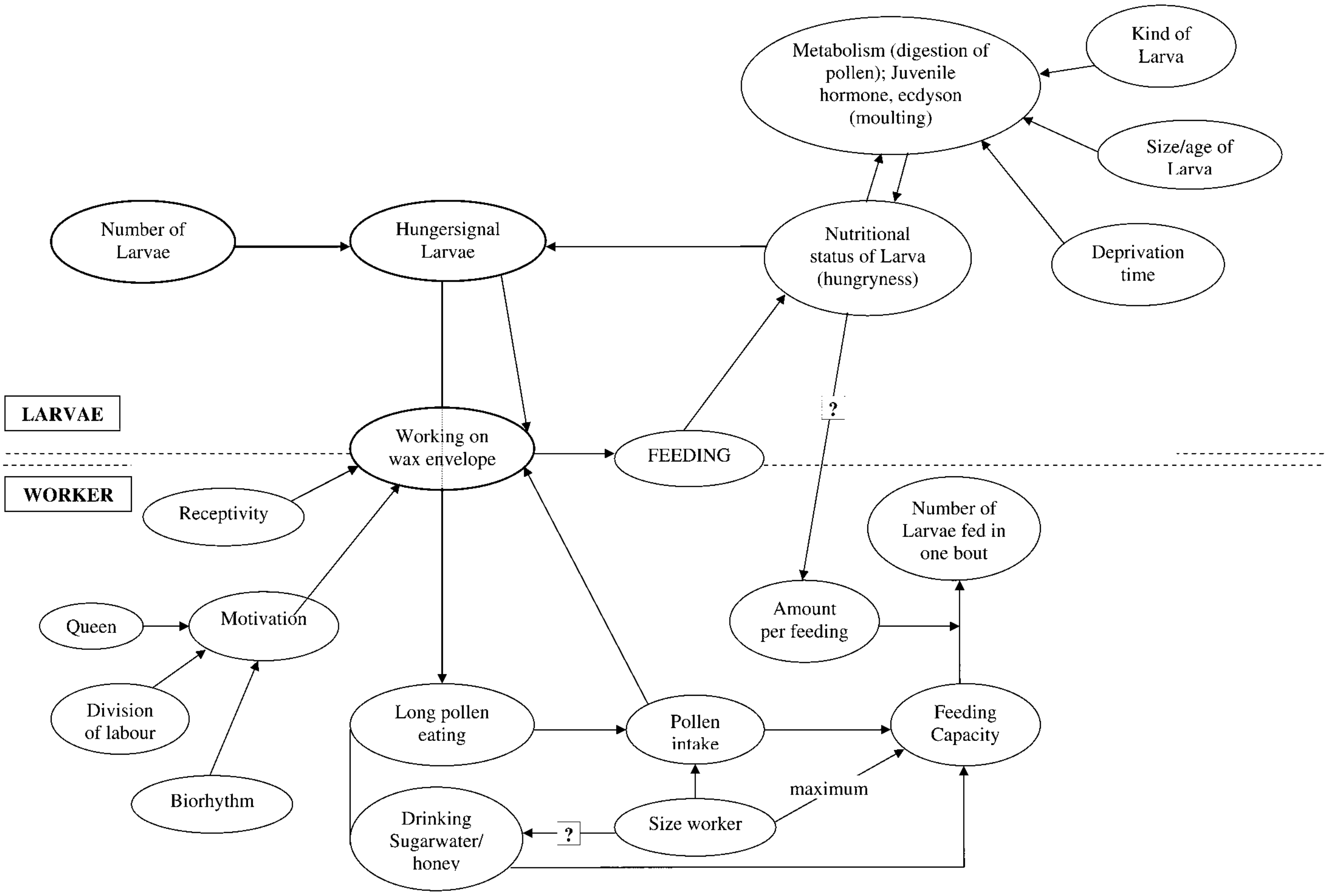

Fig. 1. – Hypothetical schematic representation of the dynamics of feeding behaviour.

MATERIAL AND METHODS

All observations and experiments were conducted under standard laboratory conditions in a climate-controlled room (28°C, 60% rH), illuminated by red light. Colonies of *Bombus terrestris* were reared and kept in the laboratory under the same conditions and provided with ad libitum pollen and sugar water (Duchateau & Velthuis, 1988). Data were transformed and analyzed using Microsoft Excel 97 and SPSS 9.0.0 for Windows.

Observations of feeding behaviour in a colony

Young colonies were placed in an observation room (28°C, 60% RH) and connected to a small flight cage (40∗50∗66 cm) with a plastic tube (inner ∅ 15mm). The flight cage was covered by a non-transparent cloth and illuminated by a UV-lamp (TL/05 40 Watt) from 9.30 hours till 21.30 hours. A container with sugar water (1:1) was provided in the flight cage, ad libitum pollen in the nest box. Observations started as soon as the bees had learned to forage for the sugar water. Non-foraging workers were selected and marked by glueing a small numbered tag on their thorax. Marked workers were followed for at least 15 minutes until one of them started eating pollen. From that moment on all behaviour of this worker was continuously recorded for at least 90 minutes according to an elaborate ethogram using The Observer version 3.0 (Noldus Information Technology 1994). For the sake of simplicity, only one behavioural state was active at a time. If, at the end of 90 minutes of observation, the worker had just eaten pollen or was in the middle of a feeding bout the observation was continued up to 120 minutes. In this way 13 different workers from six colonies were observed. One worker was observed ten times, the others one, two or three times depending on whether or not they were the first of the marked workers in a colony to start eating pollen. The total number of observation sessions was 29.

From the observational data, the duration of each behavioural element was calculated. Later, several intervals related to feeding behaviour, such as the time between the end of a Pollen Eating (PE) session and the first feeding, and the time between the last feeding in a bout and the next PE-session, were calculated. If the time between two instances of pollen eating (PE) was smaller than 30 s, the durations of the two PE-sessions were lumped and counted as one PE-session. If the time between two instances of pollen eating exceeded 20 minutes (1200 s) and no larvae were fed, the two PE-sessions were considered separate sessions. If the time between two instances of PE was between 30 s and 20 minutes (1200 s) and no larvae were fed, the durations of PE were lumped but counted as two sessions (of one "pollen-eating bout").

Feeding behaviour has the following characteristics: a worker (or queen) manipulates the wax envelope surrounding (a clump of) larvae and makes a small opening with her mandibles, if necessary. Then she inserts her mandibles, antennae, and part of her head, and after 0.5 up to about 10 seconds of "positioning" she sits motionless for a short time (0.5-5 seconds) and subsequently regurgitates a droplet of food from her honey stomach onto the ventral side of the larva by contracting and/or elevating her abdomen. After that, she either closes the orifice or manipulates it for some time (see also KATAYAMA, 1973, 1975; RIBEIRO, 1999). The feeding behaviour as described above is usually repeated several times in a short period of time, comprising a feeding bout (KATAYAMA, 1973, 1975 for *B. ignitus* and *B. hypocrita*; PENDREL & PLOWRIGHT, 1981).

Choice experiment starved/non-starved larvae

A standard observation box (20x30x7 cm) was divided into three compartments by two pieces of metal grid. In one of the grids there was a small flexible piece allowing the experimenter to open and close a small door. In the middle compartment sugar water and pollen were available.

Larvae of roughly similar size (aged 4-7 days) were taken from several colonies and placed in groups of ten to 12 in flat cups constructed from bee wax. The cups were covered with a thin layer of the larvae´s own wax envelope and, if there was not enough of that, with involucrum, in order to mimic a natural group of larvae. The cups were placed two by two in boxes with five workers that were seen to manipulate wax in a colony, allowing the workers to "remodel" the wax covering of the cups. In the first trial, half of the cups were put separately in a box after several hours, starving them overnight (14-17 hours). In the second trial, sugar water and pollen were provided initially but the pollen was taken away from half of the boxes for the night to prevent the workers from feeding larvae. The next day a group of starved and a group of non-starved larvae were placed in one of the outer compartments of the observation box, alternately left and right of the small door, about 2 cm from the metal grid.

In the first trial, one worker that was observed to feed larvae was taken from a colony and put in the middle compartment. After 15 minutes of habituation the small door was opened allowing the worker to access the compartment containing the two groups of larvae. For the following 10 minutes it was recorded on which group of larvae the worker was present and for how long. Also the occurrence of feeding and the first choice (the group of larvae she walked on first) of the worker was recorded. Then the observation box was cleaned with wet tissue to remove possible scent marks. After 3-4 hours the group of non-starved larvae was replaced by a new one. This procedure was repeated 25 times in 3 days.

In the second trial a group of five workers that were observed pollen eating or sitting on the main pollen store (located in a petri dish 5 cm in diameter) were taken from a colony and placed in the main compartment of the observation box. After the small door was opened, the occurrence of feeding and the first choice of workers were recorded. This procedure was repeated 30 times in 3 days.

Manually feeding a group of larvae in vivo

Twelve queen larvae in two different colonies were used. Six randomly chosen queen larvae were manually fed artificial food during 2 hours. This was repeated three times. During the experiment feedings by workers to these larvae and six control larvae were recorded. The artificial food consisted of a mixture of 10 g glucose and 40 g fructose filled up to 100 ml with tap water plus 1/3 volume of pollen (PEREBOOM, 1997). On two days the manual feeding regime was 19µL every 20 minutes, on one day 9µL every 10 minutes for 1 hour and then 3.5µL every 5 minutes for the second hour.

Varying the number of larvae

Larvae aged 5-7 days were taken from colonies and put into flat cups constructed of bee wax in groups of approximately five or approximately 15 larvae using the same procedure as in the choice experiment described before. When larvae had recuperated sufficiently, the cups were put apart from the workers and starved overnight (16-20 hours). Then, a feeding worker was obtained from a colony. Her abdomen was pressed gently so as to remove the food store in her honey stomach. Subsequently she was placed in a standard observation box with a cup of starved larvae and provided with sugar water. After 1.5-2 hours of habituation, pollen was provided and the pollen dish and the larvae were videotaped for 6-9 hours using a Euromex tablecamera. Afterwards the tapes were analyzed recording all instances of Pollen Eating and Feeding larvae. In this way 11 sessions were done with groups of about five larvae of which ten sessions were used for further data analysis (one session yielded no data). Thirteen sessions were done with groups of about 15 larvae of which 11 sessions were used for further data analysis.

RESULTS

Feeding behaviour in a colony

In order to get a detailed impression of feeding behaviour, individual workers in a colony were observed continuously for 90 minutes or longer. Here, only the duration of Pollen Eating (PE) and the number and timing of feedings will be presented.

PE not followed by Feeding (FE) consisted of one eating session in seven out of nine cases (78%). The mean frequency of PE not followed by feeding was 0.24 ± 0.51 times per hour. The mean duration of PE not followed by feeding was 72 ± 79s.

PE followed by FE was much more frequent, on average 1.85 ± 1.53 times per hour and consisted of one eating session in 27 out of 44 cases (61%). In the other cases (39%) PE followed by FE consisted of more than one eating session. Thirteen out of these 17 “eating bouts” (76%) consisted of two sessions (30% of the total, 30s<interval time<1200s). The mean duration of PE followed by FE was 287 ± 187s. This was significantly longer than the mean duration of PE not followed by feeding (Mann Whitney U test $p<0.01$, n=9 and n=48 respectively). This confirms the finding of DUCHATEAU (unpublished data) that on average workers eat pollen significantly longer before feeding larvae.

Choice experiment starved/non-starved larvae

To investigate whether or not workers are able to discriminate between starved and non-starved larvae from a distance of 2 cm, two choice experiments were conducted. One using one worker and one using a group of five workers. Table 1 shows the first choice of workers in both experiments. Clearly, the first choice of workers is not biased. Also, the mean time workers spent on the broods in the one worker experiment did not differ between the two broods (starved: mean 167 ± 199s, non-starved: mean 241 ± 214s, paired samples t-test, n=25, p=0.334). A similar result was obtained for the five worker experiment (scan sampled, paired samples t-test, n=30, p=0.365). From this it can be concluded that workers did not prefer one or the other brood. However, in the five worker experiment nine feedings were observed, all involving starved larvae. This suggests that workers were unable to distinguish between broods of starved and non-starved larvae from a distance, but that they were able to do so when they had access to the broods.

TABLE 1

First choice of workers that were alone or in a group of five and were given access to a brood of starved and a brood of non-starved larvae. First choice indicates the brood that was visited first.

Experiment	First Choice		χ^2	p-value
	starved brood	non-starved brood		
1 worker	12	13	0.040	0.841
5 worker group	24	23	0.021	0.884

Manually feeding a group of larvae in vivo

To study in vivo whether or not workers respond to the nutritional status of larvae and the corresponding strength of their hunger signal, in a colony six out of 12 queen larvae were selected and manually fed artificial food in order to saturate them. The other six larvae served as a control. The experiment lasted two hours, during which all feedings by workers were recorded.

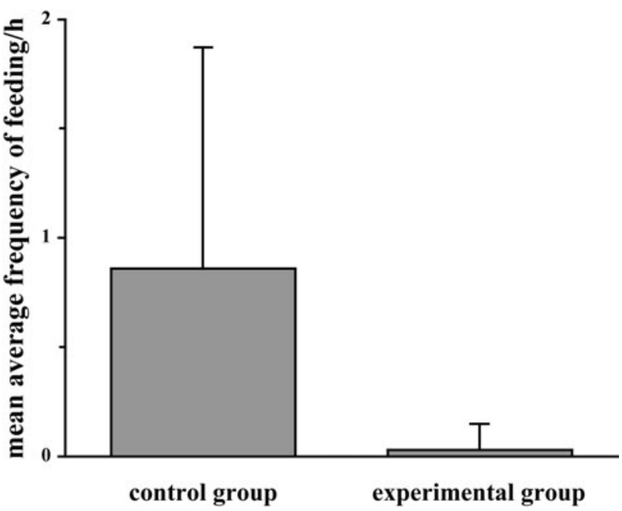

Fig. 2. – Mean average frequency/hour at which a control (n=18) and an experimental group of larvae (n=18) in a colony were fed by workers. Larvae in the experimental group were fed manually with artificial food. The frequency of feeding differs significantly between the two groups (t-test p=0.003).

Fig. 2 shows the mean average frequency at which larvae in the two groups were fed by workers during the experimental period. The control group was fed more by workers than the group that was fed manually (p=0.003). In fact, in the experimental group only one larva was fed once. Apparently, workers were able to perceive the nutritional status of larvae and they adjusted their feeding behaviour accordingly.

During the experiment a novel behaviour was observed: workers were seen to suck away the artificial food given to the larvae of the experimental group (mean frequency=2.17±1.61 times/hour/larvae, n=18). This "sucking away" occurred at least sometimes while larvae were still eating, which rules out the possibility that workers removed the artificial food because larvae were saturated.

Varying the number of larvae

In order to investigate the effect of the strength of the larval hunger signal on the feeding behaviour of individual workers, the pollen eating and feeding of workers confronted with broods of five or 15 starved larvae was observed. In addition, the data from the observations done in a colony were used (see before).

It was assumed that under the experimental condition a measure for the strength of the hunger signal, which triggers workers to feed larvae, is the duration of the adding pollen-first PE interval. Therefore the mean duration of this interval in the two experimental groups was compared. There was no difference between the five and 15 starved larvae groups (five starved larvae: mean 6271±5303s, 15 starved larvae: mean 4073±3529s, Mann Whitney U test, n=10 for both groups, p>0.10).

Another measure of the effect of the hunger signal on worker behaviour is the average frequency of feeding during the experimental sessions. This is shown in Fig. 3, in which the relationship between the average frequency of feeding in a session and the duration of the adding pollen-first PE interval for the two groups of workers is plotted. For both groups the correlation is not significant. However, an adding pollen-first PE interval longer than 4000s clearly corresponds with a low or zero frequency of feeding. The mean feeding frequency of the five starved larvae group tends to be higher than that of the 15 starved larvae group (Mann Whitney U test, p=0.081), contrary to the expectation. Interestingly, the average feeding frequency of a single worker in a colony is significantly higher than that in both experimental sessions (Kruskal Wallis p<0.05; Mann Whitney U test natural (n=29)-five starved larvae p=0.029, natural-15 starved larvae p<0.001, means±SD: natural 4.2±2.4, five starved larvae 2.3±2.1, 15 starved larvae 0.76±0.78 times/hour). This suggests that both experimental settings had an effect on worker feeding behaviour and indicates that the motivation of workers also plays a role.

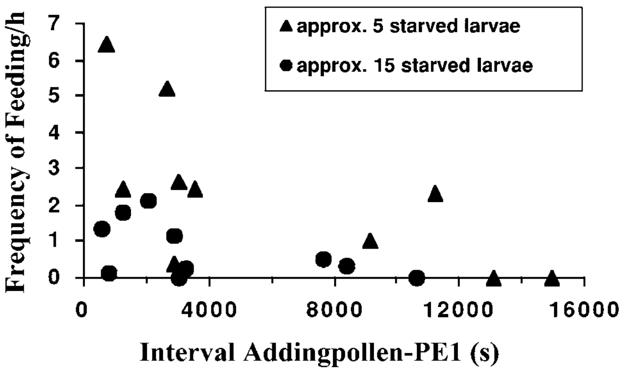

Fig. 3. – Feeding frequency after the adding pollen-PE1 interval versus the duration of the adding pollen-PE1 interval for the 5 (n=10) and the 15 (n=10) starved larvae group. For both groups there is no significant correlation (5 starved larvae r=-0.456, p=0.185, 15 starved larvae r=-0.398, p=0.255, combined (n=20) r=-0.236, p=0.317)).

Another measure of the strength of the hunger signal is the time between PE and the first feeding following that PE. A short PE-first feeding interval is assumed to reflect that a worker is reacting on the hunger signal. In Fig. 4 the mean duration of the PE-FE1 interval is shown for the three groups. The "natural" group does not differ from the five starved larvae group (Mann Whitney U test p=0.698) and both these groups have a shorter mean PE-FE1 interval than the 15 starved larvae group (Mann Whitney U test 15-natural p<0.001, 15-5 starved larvae p=0.001). Again, workers in the 15 starved larvae group appear to be less motivated by the hunger signal than workers in the five starved larvae group, contrary to the expectation. Thus, measuring the strength of the hunger signal is complicated by the effect of worker motivation.

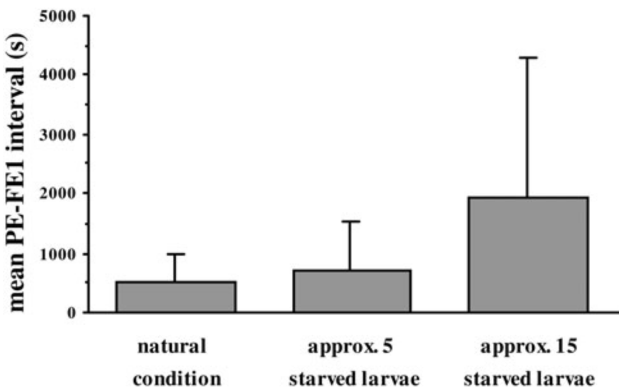

Fig. 4. – Mean time ±SD (s) between Pollen Eating (PE) and the following (first) feeding (FE1) for workers under natural condition (in a colony, n=48) and single workers confronted with 5 (n=45) and 15 (n=16) starved larvae. The mean duration of the PE-FE1 interval differs significantly between the 15 starved larvae group and the other two groups (Man Whitney U test 15 starved larvae-5 starved larvae p=0.001, 15 starved larvae-natural p<0.001, 5 starved larvae-natural p=0.698).

Fig. 5 shows that under a natural condition there is a low, but significant, negative correlation between the number of feedings and the PE-FE1 interval (r=-0.373, p=0.019), indicating that the duration of this interval is indeed a possible measure of worker motivation. Interestingly, for the two experimental groups there is no significant correlation between the duration of the PE-FE1 interval and the number of feedings. This, once more, suggests that the experimental setting was too different from the natural condition, resulting in abnormal feeding behaviour of the workers.

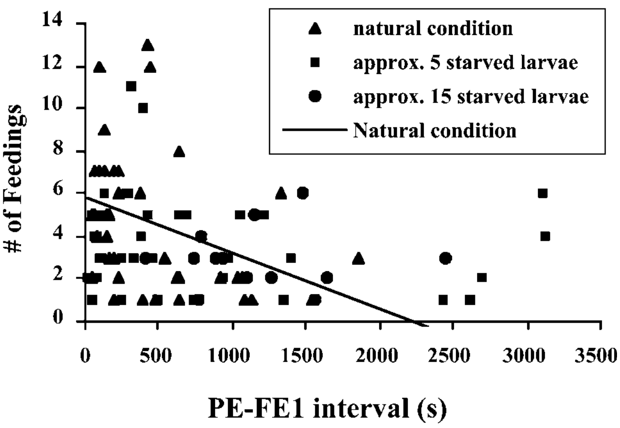

Fig. 5. – Number of feedings after Pollen Eating (PE) versus the time between PE and the first feeding for the three groups (n=39. n=39 and n=14 respectively). Only for the natural group there is a significant negative correlation between the PE-FE1 interval and the number of feedings after PE (r=-0.373, p=0.019. 5 starved larvae: r=-0.170, p=0.300, 15 starved larvae: r=0.195, p=0.504).

That worker motivation played a significant role in these experiments is further supported by the fact that there were big differences between the sessions of each group of experiments (using 5-15 starved larvae) in the amount of data obtained, due to differences in worker activity; some workers ate pollen and fed larvae a lot and seemed very "dedicated". Others kept on walking around and ate pollen and fed very little, seemingly not at ease and not paying as much attention to the larvae.

DISCUSSION

Feeding behaviour in general and the presence of a larval hunger signal in particular was studied in the bumble bee *Bombus terrestris*. We replicated the finding of Duchateau (unpublished data) that the duration of PE followed by feeding is on average longer than that of PE not followed by feeding. This in spite of the high degree of variation characterizing other aspects of bumble bee behaviour (Pendrel & Plowright, 1981; Pereboom, 1997; Ribeiro, 1997). We also found considerable variation in the duration and frequency of all aspects of feeding behaviour (standard deviations are usually close to the mean or even larger).

Results of the choice-experiment with a group of starved and a group of non-starved larvae show that workers are unable to perceive the difference in nutritional status between the larvae from a distance of about two centimeters. The fact that only starved larvae were fed shows that, after given the opportunity for closer examination, workers are able to distinguish between starved and non-starved larvae, as has previously been reported by Pereboom (1997). Furthermore, this supports the idea that larvae somehow advertise their nutritional status to workers by emitting some kind of hunger signal.

Further evidence that workers are able to perceive the nutritional status of larvae and adjust their behaviour accordingly was provided by the experiment in which a group of larvae was manually fed in vivo: feeding by the experimenters drastically decreased the rate of feeding by workers. On two of the three days experimentally fed larvae were not fed at all, and on one day one larva was fed only once. Interestingly, workers were observed to suck away artificial food from the larvae, also when they were still eating. A possible explanation for this behaviour is that too much artificial food was provided in one "feeding" and that consequently the excess was removed. Possibly, it was also to prevent dehydration of the larvae, which might result from the high osmotic value of the food.

Comparison of the feeding behaviour of workers confronted with broods of five and 15 starved larvae yielded some unexpected results. In the case of 15 starved larvae, workers were clearly less motivated to feed than in the case of five starved larvae: the average feeding frequency was lower and the mean PE-FE1 higher for the 15 starved larvae group. In the case of 15 starved larvae, the broods that were constructed often suffered from the increased mobility of hungry larvae, requiring the workers to repair the wax envelope. Sometimes larvae were pulled from the brood cup by workers and discarded. The wax envelopes of the five starved larvae broods usually were in better "shape". Comparison of the feeding behaviour of workers in a colony with that of workers in an experimental set up with a brood of five or 15 starved larvae yields the impression that on average in a colony workers receive a hunger signal approximately equal to or stronger than that of five starved larvae. The PE-FE1 interval and the number of feedings after PE were similar for the natural and the five starved larvae condition. However, for workers in a colony there was a significant negative correlation between the duration of the PE-FE1 interval and the number of feedings after PE. For both experimental groups this correlation was absent, indicating that not only the 15 starved larvae group but also the five starved larvae group gave rise to some extent to abnormal feeding behaviour. Therefore, it is likely that the experimental conditions of the five and 15 starved larvae were such that workers did not perform normal feeding behaviour. The results of these experiments suggest that the combination of the number of feedings after PE and the time between the end of that PE and the first feeding (PE-FE1) is a rough indicator of a worker's motivation to feed. A worker that feeds many times shortly after eating pollen is considered more motivated than one that feeds only once, long after pollen eating.

On the whole, the data support the hypothesis that in *Bombus terrestris* larvae emit a signal allowing the workers to perceive their nutritional status. Furthermore, this signal can trigger a worker to eat pollen for a long time, and to subsequently feed larvae. That larvae actively solicit food in relation to their level of hunger has been reported in fire ants (CASSILL & TSCHINKEL, 1995, 1996, 1999a). There, larvae are even able to regulate their exact diet (CASSILL & TSCHINKEL, 1999b). Since in bumblebees all larvae basically receive the same food (PEREBOOM, 2000), it is unlikely that food soliciting by bumble bee larvae is as sophisticated as it is in fire ants.

Our results cast doubt on the hypothesis that workers impose a feeding regime on the larvae (RÖSELER, 1970; PLOWRIGHT & JAY, 1977). RÖSELER (1970) found that in *B. terrestris* caste is determined already in the first 3.5 days of larval development. With regard to caste differentiation he states that the queen pheromonally "instructs" the workers, who in turn regulate the rearing of the larvae into either workers or queens. RÖSELER (1991) elaborates on this by stating that last instar larvae respond to quantitative changes in nutrition (imposed by workers) by modulating their endocrine activity, which in turn triggers either the worker or the queen developmental pathway. Our results suggest that it is the larvae who, once determined to become either a worker or a queen, solicit food from the workers depending on their needs (among others related to their developmental stage).

The motivation of a worker also appears to play a role in the decision to feed (see e.g. the 15 starved larvae case). LINDAUER (1952) already suggested that bees (*Apis mellifera*), while patrolling in the nest, receive numerous signals, and on the basis of this information and their "Stimmung" ("mood", influenced by age and physiological state) devote themselves to a particular task. He adds that, in addition, signals of other bees could also influence their decision to feed or not. Furthermore, Lindauer reports that feeder bees inspect the larval cells, and he suggests that on the basis of the amount of food present in the cell or some other cue, they decide to feed or not. In bumble bees, as in bees, the division of tasks is also adapted to current colony needs (FREE, 1955). However, workers do not perform inspections in order to decide to feed larvae (no such behaviour was observed during this study, see also PEREBOOM, 1997; however see RIBEIRO, 1999). We suggest that workers perceive the nutritional status of larvae (by means of the larval hunger signal) during manipulation of the wax envelope that surrounds larvae.

From the above it follows that recruitment of workers to initiate feeding behaviour somehow needs to be regulated. It is plausible that workers have some threshold above which the larval hunger signal affects their behaviour, and that this threshold differs among workers depending on their physiological state, which in turn could depend on age, life history, food availability, temperature etc. (receptivity). If so, the signals of all larvae taken together will, through the effect they have on individual workers, eventually result in the regulation of feeding behaviour. In short, an individual worker needs to make an adaptive decision to go feeding or not depending on current larval and colony needs. The details of the interaction between larvae and workers and, more specifically, the effect of the larval hunger signal on worker behaviour require further research.

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