Research article

Feeding frequency and caste differentiation in *Bombus terrestris* larvae

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Summary. The frequency with which bumble bee larvae are fed during their development was studied using video-recordings. The behaviour of the workers while feeding worker, male and queen larvae of *Bombus terrestris* was recorded. At the beginning of development, female larvae of both castes were fed at a similar frequency. However, during their last phase queen larvae were fed much more often than worker larvae. Despite the differences in frequency, both queen and worker larval feeding followed a similar pattern. Male larvae were fed more often than worker larvae, but less often than queen larvae. They also differed from the female larvae in the way their feeding frequency increased during development. This suggests that the process of feeding male larvae occurs in a different way.

The time intervals between feedings were very variable for all larvae: from a few seconds up to 3 h. Although there was a general tendency for the intervals to decrease in duration with larval development, the irregularity was always present.

The differences in feeding frequency found at the individual level for larvae of the same age and the irregularity of the feeding process can be explained by the variation in the amount of food per feeding.

Finally, our data suggest that larvae play an active role in the regulation of the feeding process. This subject is discussed and compared to the situation in honey bees.

Key words: Bombus terrestris, bumble bee, feeding frequency, caste differentiation.

Introduction

As shown by Röseler (1970) and Röseler and Röseler (1974) caste in bumble bees may be determined at different phases during larval development, depending on the species. In *B. terrestris* the determination occurs early (first 3.5 days of larval development) through the influence of a queen pheromone. The larvae under the control of this pheromone become workers, while the ones which escape from this influence may develop into queens. The differentiation of these queen larvae occurs later, as a consequence of abundant feeding in the last instar. So far, it is not known precisely how the queen pheromone functions. It may be transmitted directly to the young larvae when the queen feeds them, or it may have an indirect influence on the worker's feeding behaviour (Röseler, 1970, 1975, 1976, 1991). Investigations into the interactions between workers and larvae could therefore shed more light on the subject.

Katayama (1973, 1975) observed the behaviour of nurse bees in *B. ignitus* and *B. hypocrita*. He found that, after ingesting food, a worker feeds 4 to 15 larvae, with one crop load. Therefore, it seems likely that the amount of food a larva receives varies, depending on the amount present in the crop of the worker at that moment. Up to now no attempt has been made to find out how this amount varies.

Röseler and Röseler (1974) found that in *B. terrestris*, in the last phase of the larval development, queen larvae are fed more often than worker larvae. But they did not have precise data for both kinds of female larvae in the beginning of development, since they were unable to count the number of feedings that occurred per larva when they were still together in a common cell.

Pendrel and Plowright (1981) investigated the larval feeding of two closely related bumble bee species (*B. terrestris* and *B. terricola*) at individual and colony levels. They found a strong correlation between the feeding rates and the larval biomass. However, their observations of individuals (of *B. terrestris*) did not cover the total period of larval development.

Therefore, we were interested in obtaining more detailed and accurate information about larval feeding in *B. terrestris*, mainly at individual level. Our objectives were to find out: a) how feeding frequency varies over time, b) whether the

female castes and the males differ in this respect, c) whether there is any periodicity in the feeding of larvae, and d) how much food is provided by adult workers to a larva per feeding event. The results concerning the workers' behaviour while feeding the larvae is presented in another paper (Ribeiro, 1999).

Finally we also discuss the possible role of larvae in the regulation of the feeding process.

Material and methods

Recording and collection of data

We reared *B. terrestris* colonies in the laboratory, under controlled conditions of humidity (60%) and temperature (28°C). (For details see Duchateau, 1985; Duchateau and Velthuis, 1988).

We monitored the colonies' development daily (up to 4 times a day) in order to register the appearance of new egg cells constructed by the queen. We made video-recordings of some egg cells that were clearly visible. The egg cells lie very close to each other, and in the third brood they may contain 8–10 eggs (Duchateau and Velthuis, 1988). During development, due to their increase in size, the larvae move away from their original site. With time they also separate from each other and do not longer occupy the same common wax involucrum. Thus, in a late stage of the colony development it may be difficult to distinguish between the larvae of two neighbouring egg cells. When this happened and the larvae differed in age by less than 12 hours, all individuals were considered as belonging to one group. In other cases, a new egg cell was chosen. Selected egg cells of first, second and third broods were recorded daily during their entire development, i.e. from the first day up to the last day of the larval period. Video-recording was made once a day, for 4 h, at the same hour of the day. The day when the egg was laid was considered to be day 1, and consequently the day of larval eclosion was day 5. In this paper we refer to the "total period of development" as the larval development until the prepupal stage (including the egg stage). We also monitored the emergence of the adults to obtain information about their caste. The development of larvae of 15 egg cells was recorded in a total of 664 hours of observation. We studied 169 individuals: 81 workers (12 of the first, 16 of the second and 53 of the third broods), 45 males and 43 queens from a total of 8 colonies.

During the analysis of video-tapes we registered all feeding events for each larva, as well as the time at which they occurred. The contraction and/or elevation of the abdomen of the feeder (a worker or the queen) was used to identify feeding behaviour.

We analysed all the tapes starting from the last one so we could follow the changes in the location of the larvae. In this way we easily obtained data for all larvae from the moment they could be identified individually. Before this time (i.e., from day 5 up to day 8–9) the larvae are not conspicuous, and are still inside a common wax involucrum. In a few cases, when the egg cells contained a small number of larvae, the larvae did not disperse much and we also were able to estimate their position and to collect data for each individual from the first day of their development.

In cases where egg cells rolled down (due to the larval growth) and, consequently, the larvae did not occupy the same position as before, it was not possible to obtain data per individual. In those cases we could record only the total number of feedings per egg cell. This drastically reduced the information at the individual level for the first days of larval development.

In order to obtain more information about the regularity of the feeding frequency, we analysed the intervals between feedings. We also made additional recordings of 24 h duration for three egg cells which contained in total nine last-instar queen larvae. They belonged to three different age groups: 13 days (larvae A, B), 14 days (larvae C, D, E) and 15 days (larvae F, G, H, I). As before, we recorded the number of feedings and the time when they occurred. We also recorded the number of inspections received by the five younger larvae (A to E). The oldest larvae (F to I) were not used in the analysis because we suspect that the inspections at the last moment of development may have functions other than feeding the larvae (as for example cleaning them). We recorded an inspection every time a worker put her head or part of it (mandibles, antennae) into the hole of the larval wax envelope. In this case, no contraction or elevation of her abdomen was observed and therefore, we concluded that no food was discharged. We used the term "total visits" to refer to all visits a larva received, being the sum of inspections and feedings. A rough estimation of the size of these larvae was done by measuring their diameter with a ruler on the monitor, at beginning and end of the 24 h video-registration.

Finally, to estimate the amount of food given per feeding we collected some last-instar larvae (many queens and a few males and workers) and placed them individually in cups made of honey bee wax. These larvae were then weighed and put in small observation boxes containing food (sugar-water solution and pollen), young workers, and a queen. After being fed by the workers within 10 min, they were taken away immediately and weighed again. The differences in weight indicated the amount of food they had received.

Corrections and data analysis

In preparation for their pupation, larvae stop eating and for the several individuals this occurs at different moments. Because the recordings were always made at the same time of the day, the data obtained from the last tape was not equivalent for all larvae. Some of the larvae were recorded until about 1 h before pupation and others up to almost one day before pupation. This caused considerable variation in the number of feedings in the last days. Therefore, a correction had to be made. We decided to exclude the data for all larvae which were already receiving far fewer feedings in the last one or two days of larval development. As a result most of the data we used corresponded to the period up to and including the day on which the larvae were fed with the maximum frequency.

To compare the development time for worker larvae, we initially divided them into three groups according to the brood they belonged to (first, second and third broods, respectively). Afterwards, and for other comparisons, we considered instead the two different phases of colony development which may have affected the feeding pattern. The first phase concerns the solitary phase of colony development in which worker larvae are fed only by the queen. This corresponds to the larvae of the first brood. The second phase is the social phase, when queen and adult workers feed together the worker larvae. This phase starts when the first worker emerges, e.g., during the development of the larvae of the first egg cell of the second brood and comprises also worker larvae reared in the third brood.

Worker larvae (only those of the third brood) and queen larvae were compared using larvae from those egg cells which produced either only workers or queens ("unmixed") or both kinds of females simultaneously ("mixed"). However, because the larvae of the unmixed egg cells were so different from the larvae of the mixed egg cells we decided not to use them in other comparisons. The possible reasons for these differences are discussed later. Thus, unless stated otherwise, worker and queen larvae were compared using larvae of mixed egg cells only.

We also compared worker and queen larvae in two periods of development: 5 to 9 days and from 10 days onwards. The reason for choosing this division was the "change-point" (10 days) which reflects a change in the growth of queen larvae (Ribeiro, 1994). Male larvae were compared to worker larvae and queen larvae of mixed egg cells only.

To compare the data statistically we used: Pearson correlation coefficient, Student's t-test, Chi-square test and ANOVA (Sokal and Rohlf, 1981). Multilevel analysis of variance (Goldstein, 1995; computer program by Rasbash and Woodhouse, 1995) was used to handle the problem of having a different number of dependent observations per individual larva. The number of feedings was assumed to follow a Poisson
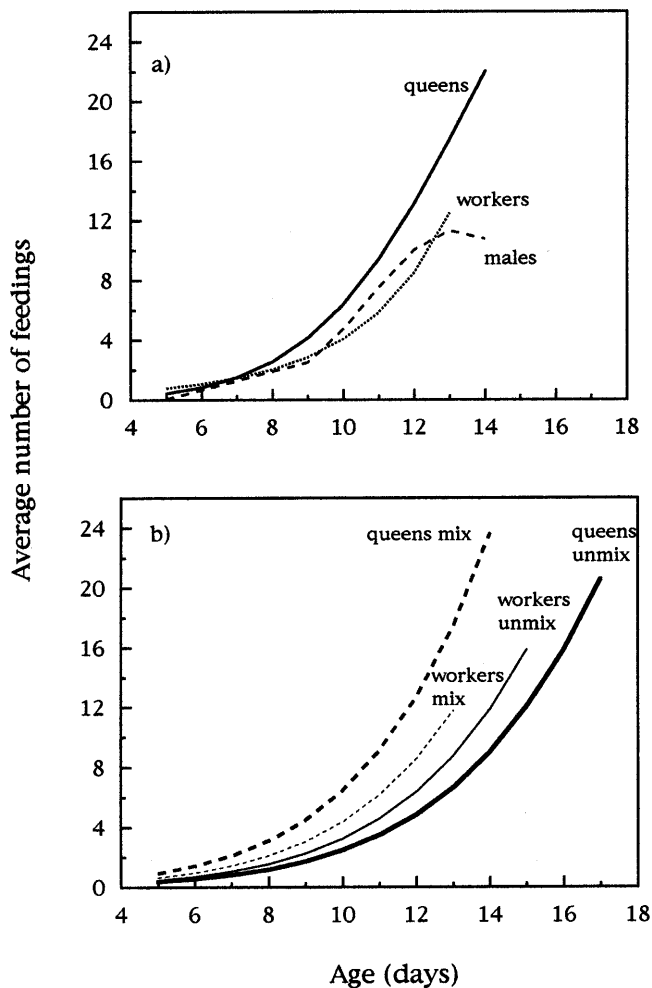
Figure 1. Average number of feedings received by larvae (third brood) of *B. terrestris*, during their entire development: (a) worker, male and queen larvae; (b) queen larvae and worker larvae from mixed and un-mixed egg cells.
Legend: mix = mixed egg cells; unmix = unmixed egg cells.

distribution. The relation between the logarithm of the number of feed-ings (y) and development time (x) was considered to be polynomial not higher than second degree ($y = a + bx + cx^2$). For each individual larva a second order polynomial was fitted. Then these polynomials were averaged per group and compared between the larval groups. This resulted in the models presented here (Fig. 1). We considered this the best way of solving the problem of the large differences found among individuals. Due to the difficulties in obtaining data at the individual level in the beginning of larval development (as already explained), for some larvae we had only a few data. These larvae were not used in the models because it was necessary to have more than two observations at different time points to define a curve for the feeding frequency of each individual. Data for the larval development are also presented as mean and standard deviation ($X \pm SD$). Results with a p-value smaller than 0.05 were considered significant.

To compare feeding intervals with inspection intervals and the total visits, we calculated their coefficients of variation (V), using the for-mula: $V = SD/X$ (*100), where SD = standard deviation and X = mean. The intervals were not normally distributed.

Results

Larval development time (from egg to prepupa)

Worker larvae from the first and second broods took on aver-age more time (about two days more) to develop than those from the third brood: 14.25 ± 1.48 (N = 12); 14.38 ± 1.31 (N = 16) and 11.58 ± 0.63 (N = 43) days, respectively (p < 0.05, ANOVA). Worker larvae of the last egg cell of the second brood needed more time to become prepupae (15.2 ± 0.92 days, N = 10) than the first worker larvae produced in the third brood (13.2 ± 1.23 days, N = 10), as indeed found pre-viously by Duchateau and Velthuis (1988). The last mentio-ned egg cell produced only workers. When we compared the worker larvae fed only by the queen and the ones fed by queen and workers we found the following averages for the development time: 14.25 ± 1.48 (N = 12) and 12.34 ± 1.52 (N = 59), respectively (p = 0.0002; t-test). Male larvae devel-oped on average in 13.33 ± 0.71 days (N = 45) and queens took longer: 14.58 ± 0.71 (N = 33). The difference between the development time of queen larvae and that of male and worker larvae was also significant (p < 0.05; ANOVA). One queen egg cell even had an extremely long development: 17.5 ± 0.53 days (N = 10). This egg cell produced only queens. Queen and worker larvae of unmixed egg cells had a significantly longer development than queen and worker larvae of mixed egg cells (p = 0.0001 and p < 0.01, respec-tively; t-test).

The feeding behaviour

The behaviour of workers while feeding larvae was similar to what has been described formerly (Katayama, 1973, 1975). The worker approaches the egg cell and opens it with her mandibles (when the larva is older there is a permanent opening in the individual wax envelope). Then she puts her head into it, stays motionless for a moment and contracts her abdomen, discharging the food. The droplet of liquid food is deposited on the ventral part of the larva. Because of the cylindrical shape of its body and its curled position, the larva is capable of holding the food and starts to eat it immediate-ly. In the case the larvae are still in a common envelope, the worker closes the orifice in the wax and leaves (see also Frison, 1928; Pendrel and Plowright, 1981).

In the majority of cases the number of orifices in the wax envelope made by the queen and/or workers when feeding the larvae corresponded to the number of larvae present in the egg cell. The orifices were also made at different places in the wax envelope, suggesting that instead of receiving feedings communally, as it has been supposed previously (Hobbs, 1964; Katayama, 1973, 1975), the larvae were fed individu-ally right from the beginning of their development. This is in agreement with the findings of van den Toorn and Pereboom (1996). Using coloured sugar-water solution they showed that larvae within the same egg cell were fed individually by the workers at least from the second larval instar (6th day of development). This could happen even in the first instar, al-

though a drop of food was generally shared by more than one larva. Due to the extremely low number of feedings observed on day 5 of the development this factor was certainly not a large source of error in our data.

Frequency of feedings throughout larval development

At the beginning of their development all larvae received food at a very low frequency (on day 5, almost no feeding occurred in the 4 h of observation). In the following days the frequency increased and reached a peak close to the end of larval development. Finally, just before pupation, all larvae were fed much less frequently.

There was a large variation in the number of feedings per day and in the total number of feedings recorded for each larva within the same caste and larval clump. Analysing the feeding frequency throughout larval development we found that worker larvae, when in the presence of the queen and adult workers, were fed at a higher frequency than worker larvae fed only by the queen (p < 0.001, Multilevel analysis). The interaction between these two types of larvae and their larval development time was also significant (p < 0.001, Multilevel analysis). This means that worker larvae were not identical in the way they were fed over time, when reared in different colony phases.

In the third brood, comparison between worker larvae and queen larvae in the first period of their development showed that there was no statistically significant difference in the number of feedings, maybe due to a small amount of data. However, in the second period of their development queen larvae were fed with a much higher feeding frequency than worker larvae of the same age, i.e., irrespective of the feeding period (p < 0.001, Multilevel analysis). No significant interaction between caste and larval development time was found, i.e., the ratio between feeding frequencies of worker and queen larvae was more or less constant over time.

Comparing the entire period of development of all larvae of the third brood (Fig. 1a), we found that the average frequency with which the queen larvae were fed was significantly higher than the average frequency with which worker larvae and male larvae were fed (p < 0.01, for both comparisons; Multilevel analysis). However, the shape of the curves for the female larvae was similar. In other words, worker larvae and queen larvae, although receiving in total a different number of feedings, were fed according to a similar pattern over time. Male larvae, however, differed not only in the frequency with which they were fed but also in their feeding pattern (p < 0.01, Multilevel analysis). Their number of feedings increased throughout time in a different way from queen and worker larvae, especially in the last days of development.

Mixed and unmixed egg cells

From the egg cells of the third brood selected for video-recording four produced female larvae of both castes. Two others produced a single kind of larvae: either workers or queens. We decided to analyse the larval clumps separately. The results are presented in Figure 1b. Both queen and worker larvae from the unmixed egg cells differed significantly in the frequency with which they were fed in comparison to the queen and worker larvae from the mixed egg cells, respectively (p < 0.001, Multilevel analysis). Queen and worker larvae received a much lower number of feedings (especially the queens) in unmixed egg cells. But it is doubtful whether the fact of being in mixed or unmixed egg cells is an important factor because the groups also showed other differences. For example, the larvae from unmixed egg cells (as already mentioned) needed a much longer time to develop (this may be a consequence of the lower number of feedings). Another fact is that the situation in the colonies at the moment these larvae were raised was very different. The colony containing worker larvae in an unmixed egg cell did not produce any queen at all. Queen larvae from the unmixed egg cell were also reared in the total absence of worker larvae in that colony. This colony produced many queens (more than 200) and the egg cell recorded was one of the last ones produced before the adult workers started to lay eggs (for details about colony development see Duchateau and Velthuis, 1988). Finally, for most of the queen larvae of the unmixed egg cell the feeding frequency increased slower than for queens of mixed egg cells. This probably occurred because of competition with other larvae in the colony. At the moment of our recordings there were many old queen larvae present which probably monopolised the attention of the adult workers (the worker : larva ratio was about 1 : 2). This might have caused the delay in their development.

The pattern of feedings

As already mentioned, we found a large variation in the number of feedings each individual larva received in 4 h of observation. One could expect a kind of regulation to occur with time in the number of feedings received by all larvae. Thus, individuals that received a small number of feedings recently would be likely to receive a larger number in the future, and vice-versa. Our data are certainly not sufficient to allow us to draw any firm conclusion, but they indicate that even in a 24 h period there is a variation in the number of feedings each larva receives (Fig. 2).

Pendrel and Plowright (1981) found that workers do not feed larvae at random, but in "bouts". They also concluded the larvae have a "rather unpredictable regime of feedings" and are "not likely to be fed more than once in a short time interval". But in our data we quite often saw larvae being fed a few times within seconds or a couple of minutes. In order to know how unpredictable the feeding rate could be we looked at the feeding intervals with more detail. The time intervals between two feedings did indeed vary greatly for all larvae during their entire development: from a few seconds up to more than 3 h. A frequency distribution showed that intervals of up to 20 min were predominant. They were observed more frequently for queen larvae (approximately
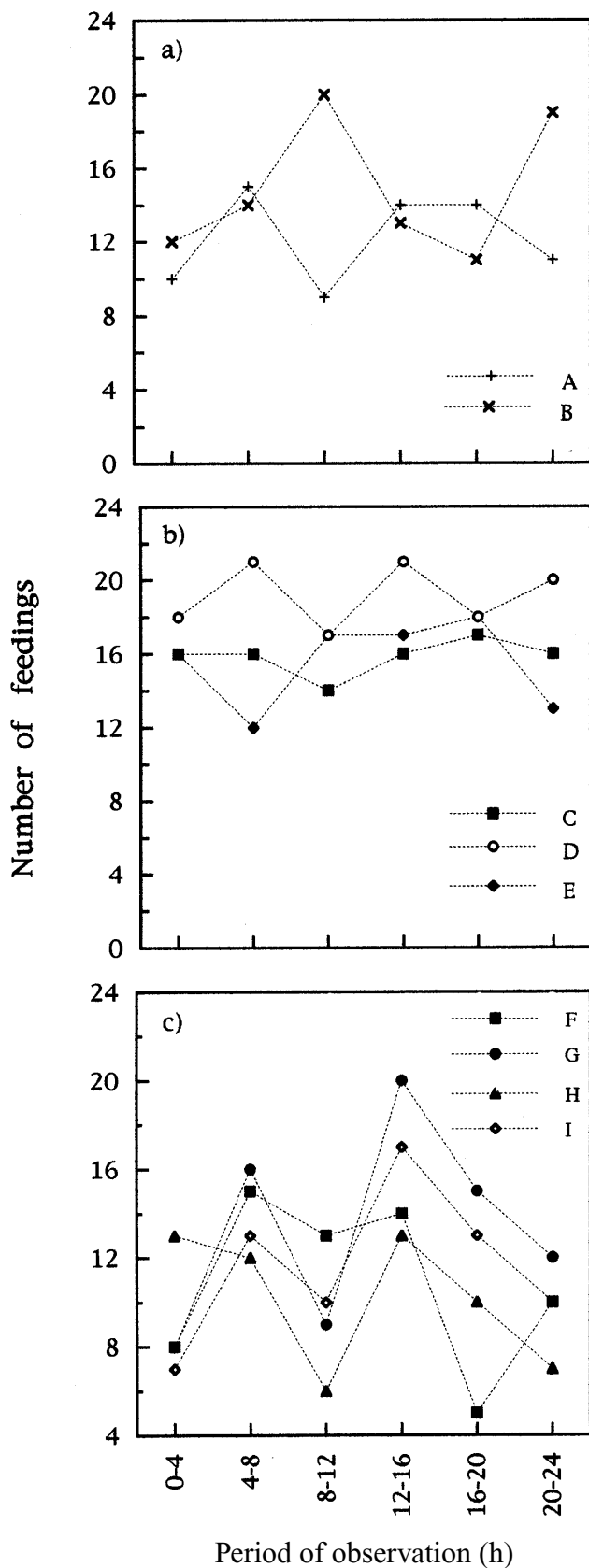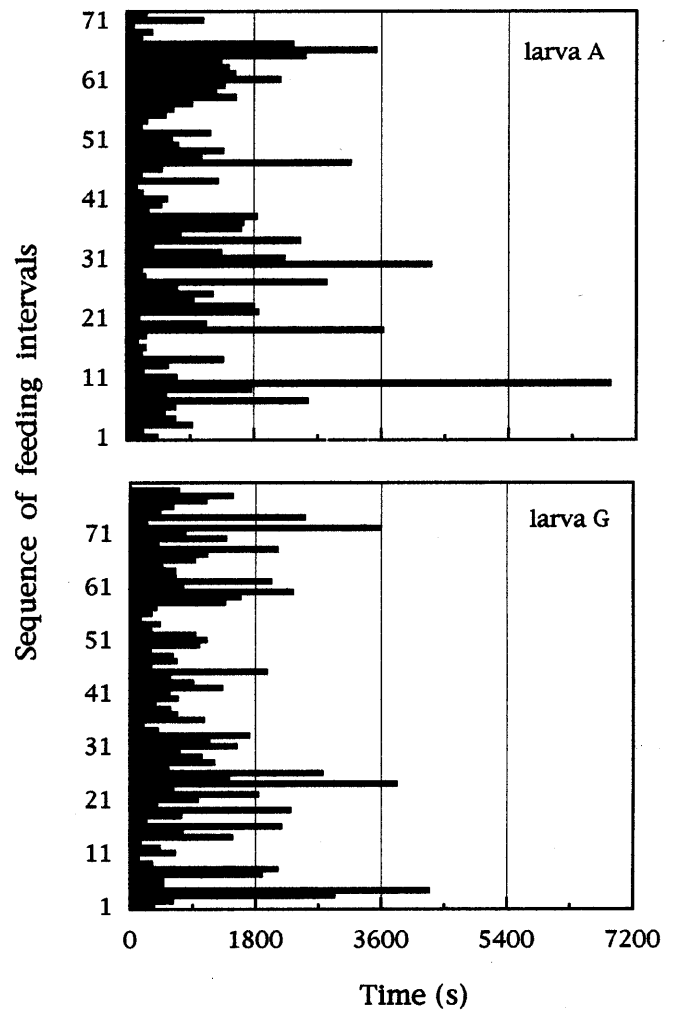
Figure 3. Sequence and duration of intervals (s) between feedings received by two last-instar queen larvae of *B. terrestris*.

70%) than for male larvae (around 57%) and worker larvae (about 42%). As expected, with the increase in the number of feedings with development, there was a tendency for the long intervals to become less frequent and to decrease in average duration. However, the irregularity was always present. Long intervals were found even for larvae of the last instar which were observed for 24 h. Figure 3 illustrates this for two larvae.

If there were some kind of regularity in the feeding process we would expect a high correlation between consecutive feeding intervals. In order to study this possibility we plotted each feeding interval against its successive interval. The results were similar for all larvae, of the three types: very low correlation coefficients were found between the feeding intervals. For the 24 h-recording of the last-instar queen larvae the same variation in feeding intervals was found: from a few seconds until almost 1 h 20 min. There was no pattern discernible in the feeding of the larvae.

Figure 2. Number of feedings received by nine last-instar queen larvae of *B. terrestris* during 24 h.

The regulation of the feeding process

The relation between inspections and feedings

Like Pendrel and Plowright (1981) we used the term "inspection" only when the worker introduced mandibles and/or antennae inside the orifice of the larval wax envelope without feeding her. However, probably every feeding is preceded by an inspection because this behaviour serves as a feed-back mechanism to the worker and as a check on whether the larva is ready to eat (Lindauer, 1953; Pendrel and Plowright, 1981). We observed that usually inspections did not last long, but could vary from a fraction of a second to some minutes. Probably these long inspections were, as mentioned before, related to other factors like cleaning, and not to larval feeding.

We did not find any regularity in the frequency of inspections either. The intervals between two inspections varied from a few seconds up to 2 h 30 min, and there were no high correlation coefficients between successive intervals.
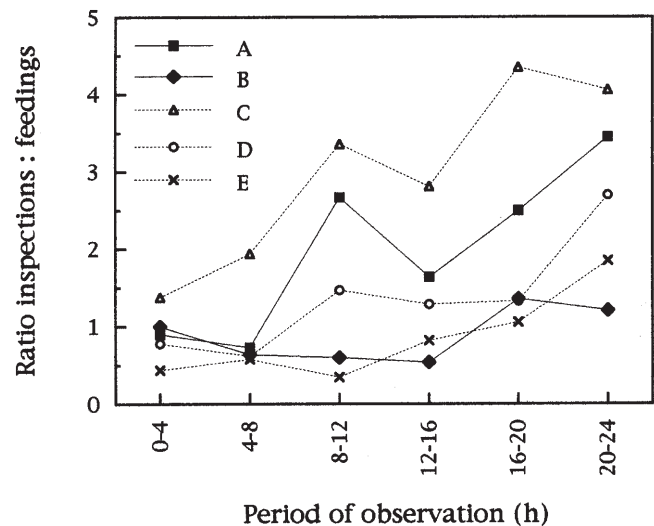
The results related to the coefficients of variation of feeding and inspection intervals were similar to those found by Pendrel and Plowright (1981) for *B. terricola*. The coefficients for feeding-feeding intervals were smaller than inspection-inspection intervals and than total visits intervals (Table 1). The results indicate that also for *B. terrestris* feeding intervals, though irregular, are more regular than inspection intervals.

Pendrel and Plowright (1981) observed that in a colony of *B. terrestris* the ratio of inspections to feedings given to larvae dropped after an experimental removal of some of the adult workers. To find out how this ratio would be in a normal situation (i.e., without worker number manipulations), we plotted the ratio of inspections to feedings throughout the 24 h period (Fig. 4). Even for larvae of the same age (A and B, and C, D and E respectively) this ratio varies with time. It is also interesting to see that while some larvae have a similar number of feedings and inspections (low ratios) for others (A, C) the ratio is much higher.

One would think that the rate of inspections could be influenced by size, position or age of the larvae. However, at least for larvae D and E, other factors must be involved. These two larvae were the same age, were similar in size and occupied similar positions, very close to each other. Even so, in 24 h the ratio of inspections to feedings for larva D was

Figure 4. Ratio between inspections and feedings received by five last-instar queen larvae of *B. terrestris*, during 24 h

about 1.6 times higher than for larva E (the first received many more inspections in relation to feedings than the latter: 2.99 and 1.36, respectively). This demonstrates clearly that a high number of inspections is not necessarily followed by a large number of feedings.

In addition Figure 4 shows that although the larvae showed a large variation in the ratio over time, there was a common upward trend. This would suggest that the number of inspections increase with larval size: larger larvae receive more inspections than smaller ones. Indeed, the larvae were from 7 to 13% larger at the end of the 24 h. Furthermore, larva C was the largest of all larvae and received the highest number of inspections (284 inspections in 24 h).

The interactions between larvae and workers

We observed much variation in the amount of attention (inspections and/or feedings) the larvae (of similar age, size and position) received from workers. A worker could be busy for some minutes feeding several larvae of the same larval clump. Or she could feed only one larva and then move and feed larvae (of other age/size) from a neighbouring larval clump. A possible reason is that some larvae are more attractive to workers than others. But one may think that inspections and feedings occur at random. In this case all larvae would have the same probability to be visited by workers. However, this was not always observed. Among larvae of the same caste and age, some received more visits than others (Figs. 2, 4). This difference was significant for larvae C, D and E (p ≤ 0.001, Chi-square test), but not for larvae A and B (p > 0.05, Chi-square test). This might be related to the size of larvae. Larvae A and B were of similar size, while larvae C was bigger than larvae D and E.

One might argue that often a larva was not fed after being inspected because the feeder (worker) did not have enough

Table 1. Coefficients of variation for feeding-feeding intervals; inspection-inspection intervals and "total visits" intervals for queen larvae of *B. terrestris* in 24 h.

Larvae	Feeding-feeding intervals	Inspection-inspection intervals	"Total visits" intervals
A	97.07	130.15	127.35
B	91.89	140.56	104.27
C	75.54	143.83	104.34
D	77.89	117.35	116.68
E	77.42	109.58	98.85

food in its crop. But many times we observed that after inspecting a larva (and not feeding her), the same worker inspected and fed (immediately thereafter) one to several other larvae. It is possible that the first larva was not fed because she was not hungry.

In other few cases, a larva was not fed by the first worker but by a second one, which came along soon after. This suggests that workers also present individual differences when evaluating larval needs. Individual differences in larval feeding activity were indeed found to be pronounced among workers (Ribeiro and Velthuis, 1997).

The amount of food per feeding

In our experiment, the food amount given to queen larvae varied considerably in each feeding event. The median was 5.6 mg, but the values varied from 0.6 to 40.2 mg (N = 82). Since the workers did not have to feed many larvae (they were given the opportunity to feed only one per time) and had abundant food, it is possible that the amount provided to a larva does not depend only on the amount present in the crop of the feeder. Probably the worker evaluates the larval needs at that particular moment and adjusts the amount of food accordingly.

Discussion

Our results, based on the model for feeding frequency ($y = a + bx + cx^2$), showed that the frequencies with which all larvae were fed increased with age. However, differences were observed in relation to the total number of feedings they received during the entire period of development. Worker larvae that were fed only by the queen received a much smaller number of feedings per 4 h period (which may have caused their significantly longer development) than worker larvae fed by the queen and workers. This was expected since at the beginning the queen feeds all larvae on her own and must do all others tasks besides feeding larvae. Moreover, the ratio between feeders and larvae is very different in these phases: the queen when still alone, may have 8–10 larvae simultaneously to take care of, while in the third brood the average worker often has a much smaller number of larvae to feed (Duchateau and Velthuis, 1988). But it was surprising to find that the way the number of feedings increases also differs for worker larvae in the two situations. This may indicate that the feeding pattern is affected by the colony phase, possibly due to other factors that change with the increased number of workers, e.g., the temperature of the nest that could affect larval metabolism.

Comparing female and male larvae from the third brood, we found differences in the way in which their frequency of feedings increased (Fig. 1 a). This suggests that the male larvae have different needs which are reflected in the way they are fed. They could provide other stimuli to the workers than female larvae. In fact, larvae of *B. terrestris* are said to have sex-specific cuticular hydrocarbon profiles; these might be used by the adult bees to discriminate among them (Greenwood, in Fisher and Pomeroy 1990).

Female larvae on the other hand, showed a similar pattern in the increase of the frequency of feedings. However, they showed significant differences in the total number of feedings they received. Analysing the development in two parts, we found that in the first period (5–9 days) worker larvae and queen larvae did not differ statistically in the frequencies with which they were fed. However, in this phase they already have different growth rates (Ribeiro, 1994). Thus, even being fed at similar frequencies, queen larvae grow more slowly than worker larvae. There are two possibilities which could explain this. If the average amount of food both types of female larvae receive is similar in this period, then queen larvae should have a different metabolism: with the same food they grow more slowly than worker larvae. This idea is indirectly reinforced by the fact that queen larvae remain longer in larval instars than do worker larvae (Cnaani et al., 1997).

The second hypothesis is that if the amount of food also varies in this initial phase of development, then queen larvae might receive less food than worker larvae, and therefore, they grow more slowly. The curve for the amount of pollen ingested by queen larvae indeed shows a lower slope than the one for worker larvae (Ribeiro, 1994; Fig. 3.2 b in Ribeiro, 1997). But we have no information about the variation of other food components (sugars and free proteins). Because we examined the amount of food per feeding for last-instar larvae only, we cannot discard any of these possibilities.

In the second part of the development, on average, queen larvae had statistically higher increase in the number of feedings than workers. However, as Figure 1 b shows, the feeding frequency does not determine caste. This agrees with the results of Röseler and Röseler (1974). We interpret the differences presented in Figures 1a and b as an indication that, once queen development starts, in most colonies the conditions for feeding larvae have improved in comparison to the earlier period during which workers were raised. This results in, on average, a steeper line for the queens. It seems that a low feeding frequency "forces" pre-determined queen larvae to complete their development in a longer time. This confirms the idea that food deprivation tends to lengthen the larval stage, as showed for *B. terricola* (Sutcliffe and Plowright, 1990).

In the present work, the variable amount of food provided to the larvae per feeding could explain the irregularity found in the feeding process (no correlation between feeding intervals, no clear pattern). Long intervals between feedings also occurred for the last-instar larvae (which had a high feeding frequency) possibly because they had received a large amount of food the previous time they were fed. Differences in the amount of food ingested by larvae at each feeding were also found by Plowright and Jay (1977) when rearing *B. rufocinctus* larvae in vitro. The amount of food ingested per time was determined by the frequency with which the larvae were fed. The variation in the amount of food per feeding brings a crucial consequence: knowing about the number of feedings

does not necessarily mean knowing about the final amount of food received by the larvae at the end of development.

Finally, our data on feedings and inspections and the irregularity of their occurrence indicate that queen larvae are able to affect (up to a certain point) the frequency with which they are fed. Worker and male larvae are probably able to do this as well. Up to now it has been assumed that larvae are passive and the rate with which they are fed is determined by the workers (Röseler, 1967, 1970; Röseler and Röseler, 1974; Plowright and Jay, 1977; Pendrel and Plowright, 1981). However, when only the workers would determine the feeding frequency one could expect that a larva which receives a high number of inspections would also receive a large number of feedings. Our data show that many times the situation is inverse: larvae which are inspected many times may receive a few feedings; probably they are able to refuse food. Although the inspections inform the worker of the state of the larval needs (Lindauer, 1953; Pendrel and Plowright, 1981), it is only after the larva "communicates" her appetite that she will be fed. Perhaps factors such as amount of food received in the last feeding and time spent on digestion are important and influence larval behaviour. As described by Katayama (1973), a bumble bee larva responds to the stimulus of being touched by moving her head and mandibles. This movement could be interpreted by the worker as the larva being ready to receive food. Therefore, larvae possibly do play an active role in the regulation of the feeding process. The frequency with which larvae are fed might be a consequence of individual decisions of both larva and adult.

Recently Pereboom (1996, 1997) demonstrated that the nutritional status of the larvae influences the probability of being fed. Comparing food-deprived larvae with a control group, he found that the starved larvae were fed earlier and with a higher initial frequency than the control group. This suggests that bumble bee larvae do emit "hunger signs" to adults. In other social insects such signs do exist: sound vibrations made with the mandibles in hornets (Ishay and Landau, 1972) and pheromonal signs in honey bees (Free et al., 1989; Huang and Otis, 1991).

However, no further experiments are known to prove the nature of these hunger signs in bumble bees.

The intensity of the "hunger sign" could also depend on size (and age) of the larva: bigger (older) larvae could produce more intense signs. According to our data the same larvae received more inspections after they became larger (and older). Indeed, in honey bees the brood pheromone which has been identified and found to be important for larval rearing (and brood capping) varies (in concentration and composition) with larval age (for reviews see Le Conte et al., 1990, 1995). A pheromone is also present in the brood of bumble bees (Heinrich, 1974; Gamboa et al., 1987), but it is not known whether it has an influence on larval feeding.

In conclusion, although the feeding frequency is a very relevant part of caste differentiation is certainly not the only factor. To understand more about the subject we need to know details on larval metabolism and on the interactions between workers and the larvae.

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