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Research article

The composition of larval food and the significance of exocrine secretions in the bumblebee *Bombus terrestris*

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Summary. This paper describes a study on the relation between the composition of larval food and the development of female castes in bumblebees. The first aim was to evaluate the significance of glandular secretions in the larval diet as a possible factor involved in larval feeding and caste differentiation. Small amounts of proteinaceous secretions were found to be added during the ingestion of sucrose but not while discharging food to the larvae. It is discussed that these secretions are digestive in function rather than food additives that would possibly play a role in the process of caste differentiation.

Secondly, a comparative analysis was made of the general composition of food samples obtained from larvae of different castes and ages and from various periods in the social development of the colony. No significant differences in the total amount of pollen, sucrose and protein were detected between the castes or different age groups. Unlike honeybee workers, individual bumblebee workers did not change the composition of the diet they supplied to the brood in relation to their own age, nor to the social development of the colony. These findings suggest that all larvae receive the same nourishment during their total development and indicate that differences in development between queen larvae and worker larvae are neither caused by variations nor by a qualitative modification of their food with respect to the amount of pollen, protein and carbohydrates.

*Key word*s: Bumblebees, caste development, larval development, nutrition, glandular secretions.

Introduction

Queen-worker differentiation in social insects depends primarily on nutritional and social factors (De Wilde and Beetsma, 1982; Wheeler, 1986). In honeybees *(Apis mellifera)*, for example, queen larvae are fed a special brood-food which plays an important role in the process of queen differentiation (Haydak, 1943; Brian, 1965; Dietz, 1972; Rembold, 1974, 1976). Developing queen, worker and male larvae receive their proteins, lipids and vitamins primarily with the food produced by the nurse bees; more in particular by their hypopharyngeal glands, mixed with honey. Prospective queens are fed with a qualitatively richer nutrition than workers; the royal jelly. Although both royal jelly and worker jelly seem to have a particular nutritional composition, specific factors leading to queen determination have never been found. However, the quantity and the biochemical balance of the glandular food that is consumed by a larva, is crucial in the determination of its developmental fate (reviewed by Moritz, 1994).

The mechanisms regulating the rearing of new queens in bumblebees, as well as the process of developmental differentiation into queens and workers are not yet fully understood. The production of new bumblebee queens has been related to factors such as the availability of food, the relative number of workers involved in foraging and nursing and the presence of the queen, which in turn are strongly associated with the colony cycle (Cumber, 1949; Free and Butler, 1959; Pomeroy and Plowright, 1982; Duchateau and Velthuis, 1988). Queens are normally produced towards the end of colony development. According to early studies by Röseler (Röseler, 1970; Röseler and Röseler, 1974), queen rearing could be inhibited by the queen by means of a pheromonal control of the workers' feeding behaviour. His assumption was that with a waning influence of queen pheromones as colony development proceeds, workers change their nursing behaviour and start to rear new queens.

This might be achieved by a nutritional switch, either qualitative or quantitative, linking nutrition and larval development (Wheeler, 1986). The nature of this switch could be a change in the composition of the larval food or in the actual feeding behaviour of the workers. Larvae of the pollen-storing bumblebee species (e.g., *B. terrestris, B. hypocrita)*

are fed progressively by the workers with a liquid mixture of pollen and honey (Sladen, 1912; Michener, 1974). Immediately after the intake of honey and pollen from the storage pots, a worker discharges a small droplet of liquid food to a larva by a short contraction of the abdomen, after which it is all eaten within a few seconds. The total amount of food ingested during larval development determines adult size (Plowright and Jay, 1968; Sutcliffe and Plowright, 1988, 1990). Given that bumblebee queens are larger than workers, it seems obvious that queen larvae receive more food. Indeed, prospective queen larvae are generally provisioned more often than workers during the last instar (Röseler and Röseler, 1974; Ribeiro et al., 1999). However, Cnaani et al. (1997) and Pereboom (1997) pointed out that, due to physiological differences, queen larvae in *B. terrestris* have a longer development time during which they continue to eat, and, as a result, attain a higher body size.

Little, however, is known about the possible role of the food quality in caste development. Ribeiro (1994, 1999) suggested that queen and worker larvae receive a different diet during the last instar, and that glandular secretions fed by the workers play an important role in caste differentiation since they might make queen larvae grow "more efficiently". Most authors, however, seem to agree on the absence of glandular secretions (Bailey, 1954; Free, 1955; Katayama 1975; Plowright and Jay, 1977), although there are hardly any data available on the composition of larval food. Indirect data suggest that differences in the larval diet are probably not involved in caste development in bumblebees. Katayama (1973, 1975), for example, supposed that larval food is just a simple mixture of pollen and honey, which does not contain pre-digested products. He reasoned that the short time elapsed between the intake of pollen and the discharge of the food, renders a real digestive processing of the pollen by the workers improbable. Furthermore, since workers can feed different types of larvae in rapid succession during one feeding bout with the same crop content, queen, worker, and male larvae probably receive exactly the same diet. Unfortunately, Katayama did not rule out the possibility that workers might add glandular secretions selectively while supplying food to the larvae, thereby making a distinction between queen, worker, and male larvae. Pre-digested products originating from pollen contents, could still be admixed to the food as secretions from exocrine worker glands.

Apart from glandular additives, queen-worker dimorphism may also be induced by pronounced dietary differences in, for example, the concentration of carbohydrates. In honeybees, sugars in the food seem to act as phagostimulants (Asencot and Lensky, 1976). Royal jelly which is fed to queen larvae contains about 12% sugars on the first days of their development, while worker jelly comprises only 4% sugars (Shuel and Dixon, 1959). Due to the high sugar content, a high intake of food during these early days results in the juvenile hormone mediated development of queens (Shuel and Dixon, 1959; Asencot and Lensky, 1976; Goewie, 1978). Later, the food for worker larvae is changed by adding honey and pollen to the worker jelly, whereas the food for queen larvae remains unaltered throughout the total development.

In bumblebees also, caste determination might be induced by such a nutritional switch at the beginning of larval development, which is when the developmental fate is assumed to be determined (cf. Röseler, 1975). But, since major differences in worker and queen development become most apparent during the last larval instar, an altered composition of the diet at this specific period could be held responsible for the differentiation of the two castes.

To assess the significance of the processing and the composition of larval nutrition in caste formation, a comparative analysis was made of worker crop samples and different samples of larval food. These samples were obtained from larvae of different castes and ages during various periods in social development of the colony. All analyses were made with respect to the total amount of pollen, protein and carbohydrates in the food only!

Methods

Bombus terrestris colonies were reared under standard laboratory conditions (28 °C, 60% rh) from artificially hibernated queens (Duchateau and Velthuis, 1988). The colonies were provided daily with a 50% sucrose solution and with pollen from a variety of plant species, collected by honeybee workers. These pollen pellets were blended in a coffee grinder to make an equal mix of the different pollen types. In several colonies, the development and the brood proliferation were monitored from the production of the first egg cell that was constructed by the queen until after the competition point, where workers start to lay eggs.

The processing of larval food

To assess the significance of exocrine secretions in larval food, samples of food provided to last instar second-brood worker larvae and last instar male and queen larvae from the third brood were collected with a micro-capillary pipette. The capillary was inserted into the drop of liquid food directly after a larva had received it. All samples were stored on ice immediately. The feeding worker was instantly removed from the colony after provisioning a larva. Its abdomen was dissected immediately and the remaining crop fillings were sampled for an estimation of pollen and protein contents. To determine whether or not workers had added exocrine products during the discharge of the mixture, the composition of each sample of regurgitated food was compared pairwise to that of the crop contents of the feeding worker. Crop fillings were not sampled in a regular fashion – by gently squeezing the worker's abdomen – because exocrine secretions might be discharged mechanically during the extrusion of the crop contents.

Glandular secretions might also be admixed during the intake of sucrose. So, the protein concentration in crop samples of workers that had been removed from the colony after drinking a 50% sucrose solution was compared to that of the sucrose that had been provided to the colonies.

The general composition of the larval food

Four well-developing colonies with sufficient workers, brood and egg cells were selected for observations from the beginning of the social phase onwards. The progression of the colonies and the position of the egg cells, groups of larvae, or single larvae were determined as exactly as possible, and registered by making drawings twice a day.

Food samples were collected from prospective queen, worker and male larvae of the second and third broods as described above. Each sample was tagged, documented, and instantly stored on ice. Shortly

afterward they were stored in a freezer until further analysis. The caste of each larva of which a food sample had been taken was determined by allowing them to complete their development. As a measure of the progression of larval development, the age of the larvae was determined using the day on which the egg had been laid.

Sample preparation and biochemical assays

Just before chemical analysis, the volume of each sample was determined by measuring the column of food in the capillary. All samples were diluted and briefly centrifuged to separate pollen grains from the liquid. The pellet was used to estimate the pollen contents and the supernatant of every single sample was used to measure the quantity of non-pollen proteins and carbohydrates by spectrophotometric assays.

Samples were not submitted to chemical treatment before the actual analysis to prevent the contents of pollen grains from leaking out and interfering with the determination of the protein concentration. Since osmotic shock might cause the pollen contents to come out (Kroon et al., 1974), dilutions prior to the chemical assays were performed with a saline solution, isotonic to a 50% sucrose solution. As a control for the effect of mixing pollen and sucrose on the protein concentration, an array of different mixtures of pollen and sucrose were analysed.

Protein concentration was determined by a dye-binding assay using folin-ciocalteu phenol-reagens and bovine serum albumin as a standard (Schacterle and Pollack, 1973). The amount of reducing sugars was determined using anthron-reagens with sucrose as a standard (Carrol et al., 1956).

Pollen counts

Pollen contents were estimated with a Burker haemocytometer. Mere pollen counts appeared to provide an inaccurate measure for a quantitative and comparative analysis of pollen samples (Biesmeijer and Sommeijer, 1992; Pereboom, 1997). To make an independent measure, the relative abundance and the pollen-grain volume of the 15 different pollen types or size categories which were present in the samples were estimated. With these estimates the relative pollen-volume was calculated. As a result, the quantity of pollen is expressed as a fraction (i.e., 0.0–1.0) of the total volume of the sample and not as the estimated number of pollen grains.

Correction of protein and carbohydrate measurements

The quantity of pollen in the food appeared to be highly variable between samples. Some even contained no pollen grains at all. This made a comparison of samples difficult, as the size of the pollen fraction (i.e., the solid part of the food) may interfere with the measured concentration of protein and sugars in the liquid part of the sample. The higher the pollen fraction per µl sample, the smaller the liquid part. As a consequence, relatively lower protein or carbohydrate concentrations would be detected in samples with a high relative pollen volume. Therefore, in some cases, the concentration of these two constituents was also calculated in µg/µl in the *liquid fraction* (e.g., Table 1). This allows us to compare carbohydrate and protein concentrations in food samples with and without pollen, and with the original sucrose solution which was provided to the colonies. In all other results, concentrations are expressed as µg/µl of the *total sample.*

Table 1. Comparison of protein (n = 82) and pollen (n = 79) content in samples of worker crops and regurgitated food. Italics indicate protein concentration in the liquid fraction to facilitate a comparison with the results presented in Figure 1 and Table 2. No significant differences (p > 0.5; Wilcoxon signed-rank test)

	Crop	Larval food
Protein in total sample	38.08 ± 8.38	38.45 ± 8.43
(in liquid fraction)	*(64.47 ± 20.00)*	*(65.75 ± 23.87)*
Pollen volume	0.35 ± 0.11	0.35 ± 0.12

Feedings by the queen

Occasionally, queens were observed to feed a clear substance – containing no pollen – to the young larvae. Samples of this food were collected and analysed for protein, carbohydrate, and pollen content, and compared with samples which had been provided by workers. Since bumblebee larvae do not start defecation until their last instar, gut contents can be used as a measure of the cumulative amount of food ingested. In order to assess whether this transparent type of food was commonly provided by queens, the gut contents of larvae of the first brood (which are fed by the queen exclusively) was examined and compared to that of larvae which had been fed by workers only.

Results

The processing of larval food

Just before feeding a larva, workers drink sucrose prior to eating pollen. Crop contents of workers that had been feeding on sucrose alone, only occasionally contained traces of pollen. But, the crop contents of these workers contained on average 7.5 ± 3.4 (n = 72) µg protein per µl sample, while the sucrose solution itself contained only 0.3 ± 0.3 µg/µl (n = 16) (p = 0.0001, Student's t-test). Apparently, workers add protein containing secretions while imbibing sucrose.

Quantitative analyses of the control mixtures of pollen and sucrose revealed a strong positive correlation between the protein concentration and the amount of pollen (Fig. 1). The same correlation was found for samples of regurgitated food and worker crop contents, but they contained on average more protein than the control samples. Figure 1 shows that, regardless of the amount of pollen, a similar amount of protein had been admixed by the workers. This indicates that the protein in the crop samples must have originated from at least two sources: the pollen grains and possibly also from workers' head glands. Based on these results, it can be concluded that workers add proteinaceous secretions during the intake of sucrose.

The pairwise comparison of the samples of worker crop-contents and the food that had been fed to larvae revealed that neither protein concentration (n = 82; p = 0.96; Wilcoxon signed-rank test) nor pollen content (n = 79; p = 0.66; Wilcoxon signed-rank test) were significantly different before and after discharge of the food (Table 1). Similar results were found when the data were split up for queen larvae (protein, p = 0.61; pollen, p = 0.87), worker larvae (protein, p = 0.63; pollen, p = 0.78) and male larvae (protein, p = 0.84; pollen, p = 0.55). This clearly shows that workers do not change the composition of the food by adding proteinaceous glandular secretions while feeding a larva.

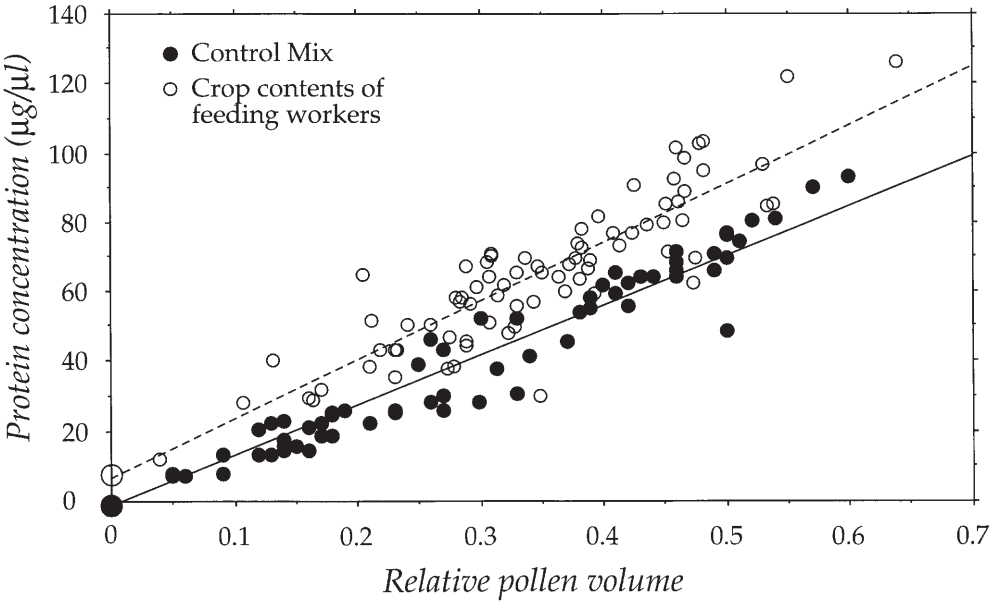

Figure 1. Protein concentration in the liquid fraction of the sample, plotted as a function of relative pollen-volume in samples of a standard control mixture (closed circles) and in crop samples (open circles) of workers feeding last instar queen, male, and worker larvae. The protein concentration in the food mixture is partly determined by the amount of pollen in the mixture and partly by an unknown protein source, which are probably exocrine secretions from the workers' head glands. Protein concentration is expressed as µg/µl in the *liquid part* of the sample to correct for the variable amounts of pollen in the samples

The general composition of larval food

The average amount of food retrieved from queen, worker and male larvae was 0.88 µl (± 0.47; n = 783). There was no difference for the three types of larvae. Only a slight correlation was found between the size of the food sample and the age of the larvae ($r^2 = 0.13$), which might have been caused by the fact that it is easier to collect food from bigger, and hence, older larvae. So, probably, all larvae received the same amount of food per feeding.

The average composition of the larval nutrition with respect to the amount of pollen, protein and sugars was exactly the same for queen, worker and male larvae (Fig. 2: ANOVA; pollen p = 0.11; protein p = 0.31; sugars p = 0.15). Pollen comprised on average 34% (n = 751) of the total sample, and the samples contained on average 35.12 ± 12.52 µg/µl protein (n = 751) and 530 ± 173 µg/µl carbohydrate (n = 596).

No correlation was detected for any of the three measured constituents in the larval diet with the age of the larvae ($r^2 < 0.01$). However, during the first days of their development larvae received food which contained less pollen and sugars, but slightly more protein than the average (Fig. 3). Although there are only few data, these young queen, worker and male larvae received the same food with respect to the three measured constituents (ANOVA; pollen p = 0.38; protein p = 0.29; sugars p = 0.16). All larvae received a "standard" food composition at a later age. This means that even during the first instars, when caste is supposed to be determined, all larvae received a similar diet. But, the presence of small amounts of caste determining factors could not be ruled out with the applied analyses.

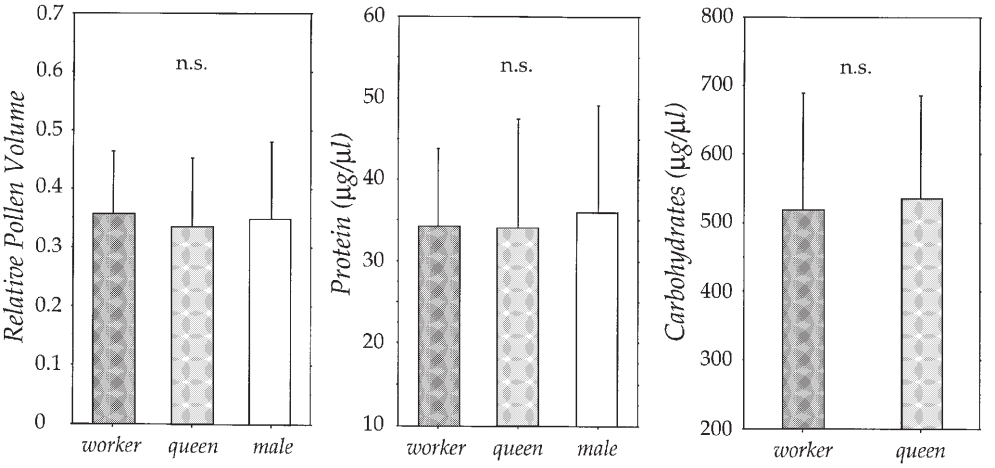

Figure 2. Average concentrations of pollen, protein and carbohydrates in food samples collected from worker larvae (n = 331), queen larvae (n = 332) and male larvae (n = 88), irrespective of their age. Protein and carbohydrate concentrations are expressed in µg per µl of the total sample. (ANOVA; n.s. = not significant; significance when p < 0.05)

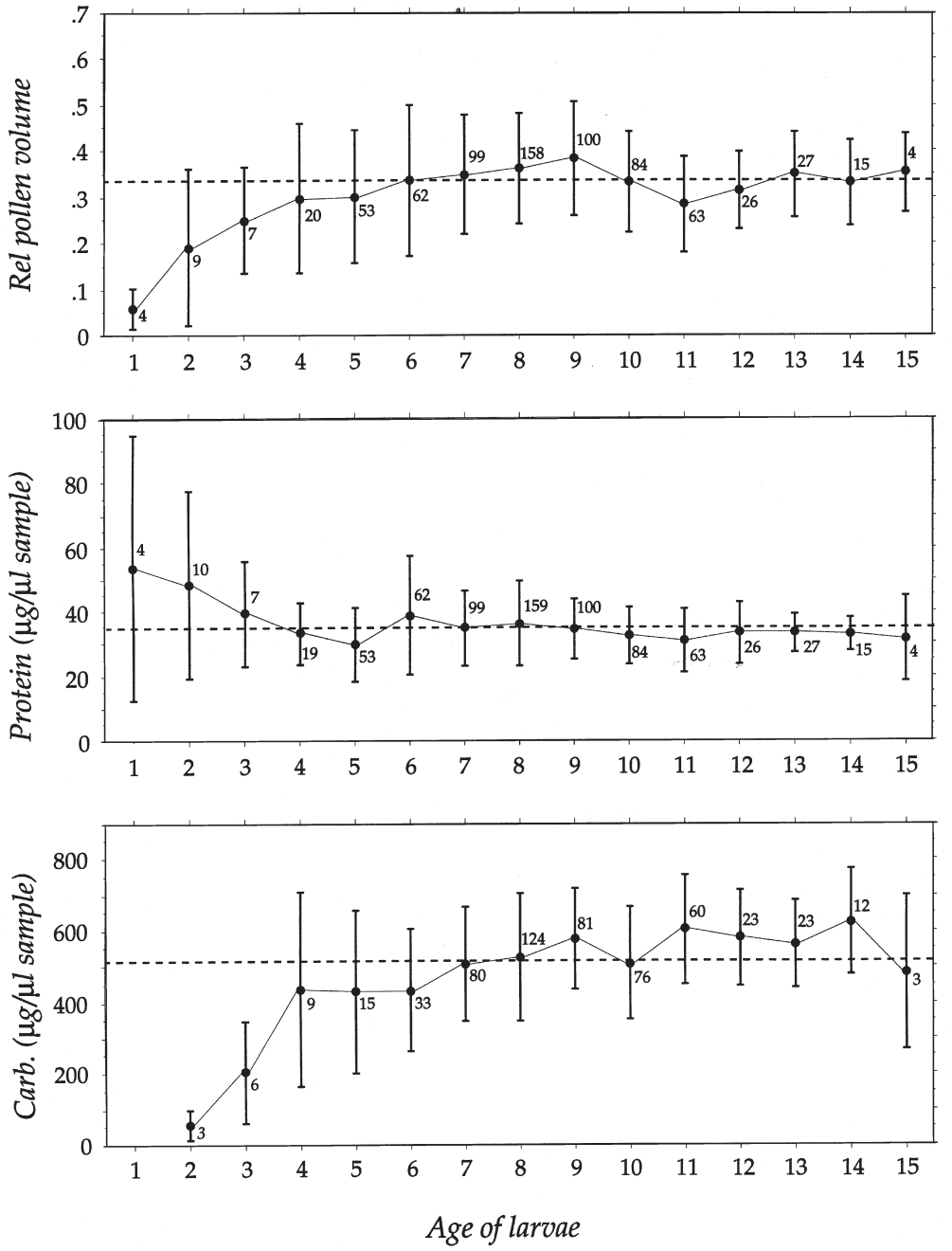

Figure 3. Averages of the different components of the larval food samples plotted as a function of larval age in days. In each plot all three types of larvae are taken together. The mean values measured for pollen, protein and sugar in the diet are indicated by the horizontal line. Small numbers in the graph indicate sample size

Samples of clear food

In some occasions, a clear food-substance with little or without pollen was obtained while sampling larval food. In 26 occasions, at a total of 783, an entirely clear food was supplied to the larvae. Queens supplied these feedings to worker larvae younger than 5 days old, and to a group of mixed worker and queen brood (age = 1; i.e., the first day of larval development), and one group of male larvae (age 1). Workers fed this type of food to queen, worker and male larvae of all ages (Table 2).

Between the two types of food suppliers, however, there was a considerable difference in the composition of this clear liquid (Table 2). In all fifteen occasions where a queen had fed a larva, the food was either totally clear (n = 7) or it contained only a small quantity of pollen (n = 8). The carbohydrate concentration was on average as low as 77 ± 68 µg/µl, whereas that of the supplied sucrose (not in table) was 650 µg/µl ($p < 0.0001$; χ^2-test). In samples which contained some pollen, sucrose comprised only 194 µg/µl ($p = 0.0001$; χ^2-test). Worker-fed deposits, however, contained on average 730 µg carbohydrates per µl, while regular pollen-containing

Table 2. Number of feedings by queens and workers with either clear food or food which contained pollen, the age of the larvae, and the average composition of those food samples. The values for protein and carbohydrates are expressed as µg/µl *in the liquid part* of the sample since this table also compares the composition of clear food with normal pollen-containing food. (n.s. = not significant, * p < 0.05, ** p < 0.01)

Larvae fed by:	Number of samples	Age range[a] (days)	Pollen[b] (% sample)		Protein[c] (µg/µl)		Sugars[c] (µg/µl)	
Queens								
clear food	7	1–5	5		68	**	n.s. 77	**
+ pollen	*8*	*1–4*	*19*		*61*		*194*	
Workers			**					**
clear food	19	1–11	2		* 32		n.s. 730	
+ pollen	*752*	*1–15*	*34*		*58*		*830*	

[a] On day 1 the larvae hatched from the egg.
[b] Pollen quantity is expressed as a percentage of the total volume of the sample.
[c] The protein and sugar concentration were corrected for the size of the pollen fraction and are expressed in µg/µl of the liquid part of the sample.

food had a concentration of 830 µg per µl. The protein concentration in queen-fed samples was on average as high as 68 (± 31) µg/µl, even though they contained no pollen grains. The clear food regurgitated by workers measured on average only 32 (± 27) µg/µl (p < 0.01; Mann-Whitney U-test).

These results altogether suggest that workers regurgitated a plain sucrose solution to which they probably added glandular secretions during the uptake. But, the clear food that was fed by queens, appeared not to be simply regurgitated sucrose. It was either diluted by the addition of substantial amounts of protein-containing glandular secretions, or, queens are able to feed glandular secretions directly to the larvae, which is common practice in honeybee workers.

Although all samples that were provided by queens contained no or little pollen, queens seemed to feed this special brood-food only occasionally. Larvae of the first brood that had been fed by the queen exclusively and larvae which were fed by workers only, contained the same quantity of pollen in their midgut (p = 0.13; Student's t-test). The weight of the midgut in relation to the total body weight was on average 29% for queen-fed larvae (n = 37) and 31% for larvae fed by workers only (n = 47). Hence, in most cases queens must have fed first-brood larvae with a regular pollen-containing diet.

Food composition and colony development

In *B. terrestris*, the timing of the production of sexuals and the behaviour of the workers are highly correlated with the social development of the colony (Duchateau and Velthuis, 1988). Therefore, the concentrations of the constituents of larval food were plotted as a function of social development (Fig. 4). All colonies were synchronised with reference to the

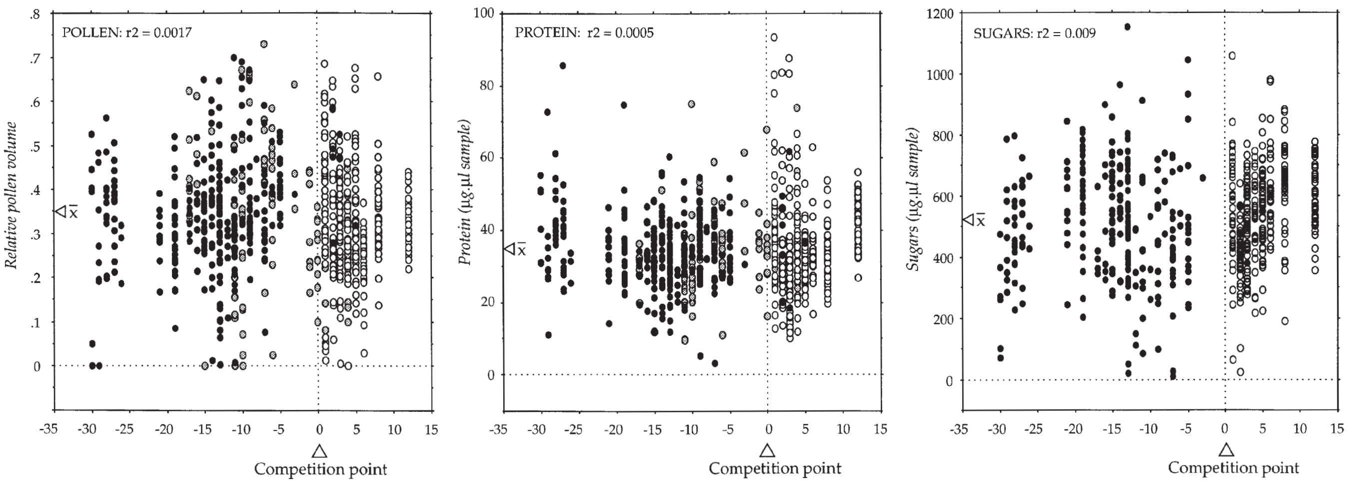

Figure 4. Composition of food samples plotted as a function of the progression of colony development. All colonies were taken together in this figure and synchronised to the competition point, here indicated as day 0. Closed black dots indicate samples obtained from worker larvae, open dots samples from queen larvae, and grey dots samples from male larvae

Table 3. Spearman rank correlation coefficients of the different constituents of the larval food with the age of the feeding workers. The upper two rows indicate the r^2-values for workers which originated from the first or the second brood, the lower to rows display the r^2-values for two individual workers

Workers	r^2 values		
	Pollen	Protein	Sugars
First brood	0.004	0.030	0.005
Second brood	0.001	0.0005	0.039
Individuals			
worker 1	0.106	0.061	0.003
worker 2	0.015	0.009	0.007

competition point, an important parameter which characterises colony development (Duchateau and Velthuis, 1988).

There was no correlation for either of the three components with the developmental progress of the colony (pollen: $r^2 = 0.0017$; protein: $r^2 = 0.0005$; sugars: $r^2 = 0.009$; Spearman Rank Correlation). Neither were there any significant differences in the composition of the samples that had been supplied to larvae of the second brood (always workers) or the third brood (in which all three castes are produced) (Student's t-test: pollen $p = 0.68$; protein $p = 0.27$; sugars $p = 0.49$).

Furthermore, the results show that when workers get older they do not change the composition of the food they provide to the larvae; no significant correlations were found between worker age and food composition for any of the measured constituents (Table 3). First brood workers were primarily involved in the feeding of worker larvae, while workers originating from the second brood were involved in feeding all three types of larvae during the later stages of colony development. Individual workers that had been feeding larvae for several weeks (i.e., > 30 days) also showed no significant changes with age for any of the three food components (Table 3 lower rows).

Discussion

The significance of exocrine secretions in larval feeding

Protein concentration in the samples appeared to be strongly correlated with the amount of pollen in the food mixture, in spite of the precautions that were taken to prevent the pollen contents from leaking out. The pollen had been collected by honeybees, which usually moisten pollen with crop contents and gland secretions before they return to the colony. The major part of the measured "free proteins" probably originated from such secretions and broken or leaking pollen grains.

Apart from this, on average 12% of the total amount of these proteins was proven to originate from others sources. The results demonstrate that bumblebee workers add proteinaceous glandular secretions during the intake of sucrose and pollen, but not during the regurgitation of the larval food. Furthermore, no differences were found in the amount of pollen and protein in the nutrition, nor in the amount of the non-pollen protein in food that was offered to either of the three types of larvae. This supports the hypothesis (Katayama, 1975) that the quality of the food is equal for the three castes since queen, worker and male larvae can be fed with the same crop content in rapid succession.

Ribeiro (1999) hypothesised that such protein containing secretions are a major nutritional component of the larval diet and that they might be involved in caste-specific larval development. But, based on the observations on the processing and discharge of the food it can be expected that these extra proteins are most likely digestive enzymes, which is in contrast to what has been suggested by Ribeiro (1999). The exocrine secretions presumably originate from the hypopharyngeal glands (HPG). Simpson (1960) made clear that in honeybee workers, secretions from the mandibular glands (MG) cannot be released during the intake of liquid food. The orifices of the HPG however, do lie within the in-going stream during feeding which might be sufficient for secretions to pass into the pharynx. These glands have no reservoir from which secretions might be discharged actively. But, according to Kratky (1931), their products may be released by an underpressure created by the pharynx pump during feeding. For bumblebees, Palm (1949) suspects a continuous flow of secretions from the HPG, which is to some extent stimulated by the intake of food. Moreover, like in honeybees, the efferent ducts of the HPG are thick chitinised tubes (Palm, 1949), which makes it altogether not unreasonable to expect similar discharge mechanisms in bumblebees.

As for the function of these secretions, Simpson (1960) and Simpson et al. (1968) found substantial amounts of invertase in the HPG, but none in the MG. This indicates that HPGs play an important role in inverting carbohydrates. In bumblebees also, the secretions of the HPG seem to be primarily involved in the salivary breakdown of carbohydrates. According to Palm (1949), the product of the HPG in *Bombus* and *Psithyrus* is a protein-rich fluid which contains invertase and amylase to break down carbohydrates. Its presence was not only demonstrated in the glands of workers, but also in those of queens and males. In-vitro tests by Ono et al. (1994) showed that sucrose was inverted into fructose and glucose within a few minutes by adding macerated HPG from *B. terrestris* workers. Hence, the proteins that were detected in the crop contents of the workers and in samples of larval food are very likely enzymes involved in inversion of polysaccharides. It makes sense, therefore, that such enzymes should be added during the intake of carbohydrates.

In honeybee nurses, the HPG and MG produce a nutritious brood-food to nourish the larvae and the queen. Only young workers that are specialised in feeding the brood have highly developed food glands. Röseler (1967) mentioned the existence of food glands (Futtersaftdrüsen) in *B. terrestris* and *B. hypnorum*, and observed that the HPG of bumblebee workers between the age of 2 to 10 days are at their maximum size, which could be related to larval feeding activity. Although there is no strict division of labour,

bumblebee workers do seem to be primarily involved in feeding when they are young (Duchateau, 1989; Ribeiro and Velthuis, 1997). But, Free (1955) and Free and Butler (1959) described that foraging workers and workers that are involved in the feeding of larvae have equally well developed HPG. Moreover, my results showed that when workers continue feeding when they grow older, the composition of the food they provide to the larvae does not change. Furthermore, Bailey (1954) demonstrated that the proventriculus of bumblebees does not transport pollen as quickly as in honeybees, suggesting that bumblebees are unable to digest the huge amounts of pollen that are needed to supply food-producing glands with proteins at a sufficient rate to serve as a supplement to the larval diet.

In conclusion, all these features suggest that in bumblebees exocrine secretions from the workers' HPG are part of the larval nutrition but that they are probably exclusively salivary in function. Bumblebee workers do not feed glandular secretions as a functional brood-food like in honeybees; their larval food is primarily composed of honey and pollen. Such a functional difference between the HPG of bumblebees and honeybees is also reflected in the observation that the food supplied to bumblebee larvae contains substantially more sugars than that of honeybees. This is in concurrence with Velthuis (1992) who suggested that the original function of the HPG secretions in bees in general was a digestive one, and that this feature is still prevalent in bumblebees and stingless bees.

Food composition and caste differentiation

It is important to keep in mind that the food samples were not analysed in detail to determine the qualitative composition of the food components themselves. This study primarily concentrated on the question whether the differences in body size between the castes could be explained by pronounced differences in the general composition of the larval nourishment during their development.

The results make clear that in *B. terrestris* the composition of the supplied food with respect to the amount of pollen, protein and sugars, is the same for queen, worker and male larvae. In the last larval instars, when major differences in body size between queen and worker larvae become apparent, there were no differences in the proportion of the three components of the diet, which makes it unlikely that this plays any role at all in the pronounced caste-specific differences in size. This is supported by observations which suggest that size differences occur primarily as a result of the longer duration of the queens' larval development (Cnaani et al., 1997; Pereboom, 1997).

Although the youngest larvae received food with a composition that differed slightly from that for older larvae, there were no caste-related differences which might be responsible for the induction of a caste specific development, similar to what was found in honeybees (Shuel and Dixon, 1959; Asencot and Lensky, 1976; Goewie, 1978). However, the existence of a specific caste-determining factor in the food during this period has not been tested here.

Clear food supplied by queens and workers

The observed differences in the composition of clear food produced by queens and workers (Table 2) suggest that queens provide a special brood-food to the youngest larvae. The extremely low values of sucrose and pollen in the food in combination with the high protein contents indicate that the mixture must have been diluted by the addition of relatively large amounts of proteinacious exocrine secretions by the queen. Such secretions could serve another function as in workers which add secretions during the intake of sucrose, suggesting an enzymatic function. Queens might secrete glandular products in the same way as honeybee workers do – just prior to feeding a larva – by creating an underpressure in the pharynx. And, while regurgitating a small amount of honey from the crop they may feed these secretions directly to the larvae. In this case, the queens' glandular products could act as a nutritional enrichment of the larval food, in contrast to the mere enzymatic function of it in workers. This is an interesting thought, since it could be a first step towards the evolution of a functional food-producing gland.

In workers, however, the feeding of clear food could have another origin. Katayama (1975) observed that bumblebee workers that were regularly involved in feeding activities, sometimes refilled their crop in between feedings with honey, without taking in pollen. Such behaviour may not only clarify the seemingly random supply of plain regurgitated sucrose, but also the high variability of the food composition per feeding. Ribeiro (1999) found that as much as 28% of the samples provided by workers contained no pollen. The experimental situation, and the seemingly relatively random processing of larval food might explain such a high presence of clear food in that study.

General conclusion

As explained before, it seems obvious that queens grow larger since they obtain more food than worker larvae. This can partly be explained by the longer duration of their development and, directly related to that, the higher frequency of feedings during the last instar (Röseler and Röseler, 1974; Cnaani et al., 1997; Pereboom 1997; Ribeiro et al., 1999). But, the volume of the drop of food that workers regurgitate appears to be about the same for all larvae. And, although the intensity of the workers' abdominal contraction – which coincides with the discharge of food – can be highly variable, Ribeiro (1999) found no relationship between the extent of the contraction, the size or age of the larva which was fed, and the amount of food discharged.

These results, together with the present results, suggest that the exact amount of food and the proportions of the three food components are not essential for the development of the larvae, let alone for caste differentiation. The composition and amount of the food may be highly variable among separate feedings. On average, however, all larvae receive the same food, and, within each caste, approximately the same amount of food. Hence, it seems unlikely that larval devel-

opment or caste development is strongly dependent on a poised composition or a fixed amount of food per feeding. Moreover, recent work by Ribeiro et al. (1999) and Pereboom (1997) suggests that even the frequency of feedings does not matter all that much. Therefore, larval development and caste differentiation can not be a result of differences or gradual changes in the composition or the amount of larval food provided. A qualitative analysis of the different components within the food might be a necessity to find possible caste-determining factors in the food, although the quest for such "determinators" in honeybees has been quite unsuccessful so far.

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