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Research article

The organisation of larval feeding in bumblebees (Hymenoptera, Apidae) and its significance to caste differentiation

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Summary. In pollen-storing bumblebees, the rate at which workers nourish larvae has been proposed to be the main factor influencing caste differentiation since workers feed prospective queens more frequently and longer than worker larvae during the last instars. In order to determine how the frequency of feedings is established small groups of *Bombus terrestris* larvae were either subjected to starvation or nourished regularly by workers. Experimentally starved larvae were fed significantly earlier and more often than control larvae. Behavioural observations provide evidence for the existence of a stimulus of larval origin that releases feeding behaviour in workers. Intentional inspections in the sense of a recognisable and functional behaviour intended to organise the feeding process were never observed. We argue that worker inspections are not required for the adequate maintenance of larvae and that a simple stimulus-response chain appears to be sufficient to regulate feeding behaviour at the individual and the colony level in bumblebees. Furthermore, hand-rearing experiments with female larvae indicate that queen rearing is not dependent on a high frequency of feedings in itself, nor is worker development induced by larval deprivation. This makes it unlikely that workers impose a caste-specific frequency of feedings on larvae in order to actively control or manipulate caste development. Since caste fate seems to be predetermined early in larval life, we propose that early caste-specific differences in development are reflected in the physiology of the larvae and the associated nutritional needs to which workers respond accordingly. Consequently, caste-specific differences in feeding frequencies are a result, but not the cause of differences in development.

Key words: Bombus terrestris, larval feeding, caste differentiation.

Introduction

In the social Hymenoptera, differences in reproductive potential are related to dissimilarities in adult body size or morphology. Queens, the female reproductive caste, are generally larger than the non-reproducing workers. This is primarily an effect of trophogenic and social factors in the colony acting on the endocrine system during larval development (De Wilde and Beetsma, 1982; Wheeler, 1986).

In bumblebees, caste-specific size differentiation seems to be primarily affected by the quantity of food ingested during the larval stage (Cumber, 1949; Free, 1955; Röseler and Röseler, 1974; Plowright and Jay, 1977; Sutcliffe and Plowright, 1988; Pereboom, 1997). Qualitative differences in larval nourishment, like in honeybees (*Apis mellifera*) for example, do not seem to play a significant role, as the general food composition for all castes is the same (Pereboom, 2000).

Within the genus *Bombus*, two different ways of provisioning larvae have been reported, which are related to the way forage is stored in the colony (Sladen, 1912; Michener, 1974). Pocket-making bumblebees mass-provision larvae by stashing pollen in a pocket underneath a group of larvae. The larvae themselves feed from these stores directly, though prospective queen larvae are often nourished additionally by the workers. In such species, queens are usually larger than workers, though a considerable overlap in body sizes may occur within or between colonies. Queens usually emerge by the time the food supply to the colony is abundant and larvae can eat at will (Free and Butler, 1959).

The most conspicuous size differences between the female castes are found in pollen-storing species, like *B. terrestris*, who accumulate pollen and nectar in separate containers. In contrast to the pocket makers, all larvae are fed progressively by workers during their entire development (Michener, 1974). Queen production has been suggested to be related to high worker to larva ratios (Cumber, 1949;

Röseler, 1970; Duchateau and Velthuis, 1988), although there is no evidence of a direct causal relationship. Prospective queens, however, are fed considerably more often and have an extended development time which allows for a longer feeding period (Röseler, 1970; Plowright and Jay, 1968, 1977; Plowright and Pendrel, 1977; Katayama, 1973, 1975; Ribeiro et al., 1999). The prevailing view is that workers are able to control caste differentiation directly by increasing the duration and the frequency of feedings in order to rear queens, while deliberately undernourished larvae are destined to develop as workers (Röseler, 1970; Pendrel and Plowright, 1981). Recent studies, however, indicate that *B. terrestris* larvae can not be forced to become workers through malnutrition and that predetermined differences in larval development (e.g. physiology and development time) cause the observed differences in feeding rates (Pereboom, 1997, 2001; Bortolotti et al., 2001).

At present, we know practically nothing about how progressive provisioning of larvae is organised in bumblebee colonies. In honeybees (*Apis mellifera*), the ability of workers to recognise brood, its developmental progress, sex, and caste, is considered a prerequisite for adequate brood care and progressive feeding (Free, 1987). Larval provisioning involves a negative feedback mechanism, constituted by regular inspections intended to assess the nutritional status of the larvae (Free, 1987; Free et al., 1989; Huang and Otis, 1991). Since pollen storing bumblebee workers feed larvae progressively and the feeding rate is related to both caste and developmental stage, a similar mechanism was suggested for bumblebees. Although inspection behaviour was described for bumblebees (Röseler and Röseler, 1974; Katayama, 1973, 1975; Pendrel and Plowright, 1981; Ribeiro et al., 1999), the feeding of individual larvae appears to be highly variable and only weakly regulated (Stephen and Koontz, 1973; Pendrel and Plowright, 1981). Moreover, *B. terrestris* larvae seem to play a much more pronounced role in shaping the dynamics of the feeding process themselves (unpublished data, MJD, JJMP; Smeets and Duchateau, 2001) like in wasps (Ishay and Landau, 1972) and some ant species (De Wilde and Beetsma, 1982). This poses the question whether intentional inspections, aimed at providing workers with the necessary information to respond to nutritional needs of larvae regulate the feeding process in bumblebees, and what part larvae take in their own food intake.

If, indeed, larvae themselves were involved in regulating the feeding process they could in theory manipulate workers and 'self-determine' their own caste fate by demanding more food (Bourke and Ratnieks, 1999). In *B. terrestris*, however, queen-produced pheromones have been proposed to suppress queen development during the ergonomic stage of the colony development, supposedly by early predetermination of larval development (Röseler, 1970; Röseler and Röseler, 1974), and as such limit or remove the possibility of self-determination. It is not clear, however, whether queen pheromones inhibit the production of new queens by 'manipulating' worker behaviour as has been described in honeybees, stingless bees, wasps, and some ant species (De Wilde and Beetsma, 1982; Wheeler, 1986), or if they influence the development of larvae directly. Recent work on the physiological mechanisms underlying caste differentiation in *B. terrestris* suggests, however, that caste development is not under worker control (Cnaani et al., 1997, 2000; Cnaani and Hefetz, 2001; Hartfelder et al., 2000, Bortolotti et al., 2001). These studies provide evidence for a direct influence of the queen's presence on caste-specific endocrine events during the larval development, and that size dimorphism is primarily a result of different development duration.

The objective of our study is to investigate which factors regulate feeding behaviour in bumblebees, as well as its significance to the process of caste differentiation. First, in order to determine whether feeding rates are actively controlled by workers or induced by larval nutritional conditions, we designed a bioassay to observe feeding behaviour towards starved and control larvae. Second, diploid larvae taken from young queenright colonies should all develop as workers independent of the feeding regime. Larvae from queenless colonies should have retained the potential to develop as queens, provided the amount of nutrition received validates queen development (Pereboom, 1997). Alternatively, if caste development is induced by a change in worker behaviour towards the larvae, it should be possible to produce queens *in vitro* by (1) feeding *any* female larva excessively, by (2) lengthening the feeding period, or (3) by doing both, thereby assuming that workers do not add caste-determining compounds to the food. Likewise, workers should be obtained by selectively depriving female larvae of food.

Material and methods

(1) Regulation of the feeding process

Colonies of *B. terrestris* were kept in captivity in standard laboratory rearing conditions (28 °C, 60% rH; Duchateau and Velthuis, 1988). Larvae originating from the same egg cup were removed from the colony eight days after oviposition, matched according to size, and placed in two groups in artificial wax brood cells. Fresh pollen and a 50% (w/v) sucrose solution were provided ad libitum, and five workers were allowed to feed the larvae and reconstruct the brood cell. To obtain naïve workers that were as similar to one another as possible with regard to their age and motivation to feed larvae, callow workers were isolated in small groups until needed for feeding the larvae in the tests. All experiments and preparations were carried out in an air-conditioned room (28 °C and 60% rH) illuminated by red light.

The next day, experimental groups of larvae were placed in standard observation boxes without workers and starved, while control groups were fed by workers. After a period of 24 hours, one control and one experimental group were placed next to each other in a new observation box with ample supplies of pollen and 50% sugar solution. Five of the previously isolated naïve workers were then introduced and their behaviour recorded on video for four hours. The same treatment was repeated 14 times with new larvae and workers. The relative orientation of larval groups was alternated among trials to correct for possible worker preferences for position. In the analysis of the tapes, all feeding events were scored and we checked for the occurrence of inspection-like behaviour.

(2) Hand-rearing experiments

a) We conducted two feeding trials to establish the effect of forced feeding on larval development. In the first trial, three-day old diploid larvae were isolated from three queenright laboratory-reared colonies (approximately two weeks after emergence of the first worker) and kept separately in small bee-wax containers placed in an air-conditioned room (32 °C, 60% rH). We used three-day old larvae because it was almost impossible to hand-feed younger larvae without mortality becoming unacceptably high. The larvae were divided into two groups of 20 individuals each and subsequently fed by hand until the end of the larval stage. One group (H = high freq.) was subjected to the feeding regime observed in laboratory colonies for queen larvae and the other group (L = low freq.) to that of worker larvae (Ribeiro et al., 1999). Feeding proceeded for 24 hours per day in accordance with the feeding scheme, and all larvae were fed for as long as they would continue to accept food and entered the prepupal stage. To ensure the accurate placement of food and to monitor the behaviour of the larvae, feedings were performed under a dissection microscope. The food consisted of a 50% sugar solution (glucose: fructose = 1 : 4 in water; Ono et al., 1994) mixed with fresh pollen (±35% v/v; Pereboom, 2000). In each feeding bout, small drops of food (1–2 µl/feeding) were applied ventrally to the larvae using a P10 Gilson pipette with 10-µl tips.

b) Before collecting larvae for the second trial, the queen had been removed from three colonies assuming that queen development is inhibited by the presence of queen pheromones. To eliminate the queen's influence as much as possible, we only collected diploid larvae originating from eggs laid on day the queen was removed or on the previous day. Like in the first trial, larvae were subjected to the two caste-specific feeding regimes.

In both trials, some larvae of the same batches as the experimental larvae remained in the colony as a control to check whether they had retained the potential to develop as queens. After the feeding trials, larval instar and caste were determined by measuring the width of the head-capsule and the body weight (Cnaani et al., 1997). The body size of an emerging adult was assessed by measuring the body weight and the length of the radial cell in the right wing (unpublished data MJD; Bortolotti et al., 2001).

Results

(1) Worker feedings and inspection behaviour

The feeding behaviour of workers in the experimental set-up was similar to the behaviour observed in laboratory colonies (Katayama, 1973, 1975; Ribeiro et al., 1999). Before food was being delivered, a worker inserted its antennae, mandibles and part of the head into the feeding hole and remained motionless for a moment, shortly later followed by a brief contraction of the abdomen. The abdominal contraction was taken as a characteristic of feeding behaviour, as it coincides with the regurgitation of the food from the crop.

During the 56 hours (14 four-hour recordings) of videotaped behaviour, inspections or inspection-like behaviour was never observed. We observed 307 feedings of which 193 (62.9%) were given to starved larvae and 114 (37.1%) to control larvae. In all 14 trials, the very first larva to be fed was a starved one (Table 1, χ^2-test, $p < 0.001$). This absolute preference for starved larvae diminished in subsequent feedings although in most trials the second to the fifth feeding was provided to a starved larva (Table 1). The differences in the number of feedings per hour to starved and control larvae were most obvious during the first ($p = 0.024$, Wilcoxon signed-rank test) and the second hour ($p = 0.022$) after the introduction of the workers (Fig. 1). Frequencies of feeding were not significantly different however for starved and control larvae during the third and the fourth hour ($p = 0.75$ and $p = 0.11$, respectively). Although the total number of feedings to control and starved larvae appears to be slightly lower during the first hour in comparison to the rest of the observation period, this difference was not statistically significant (Wilcoxon signed-rank test, $p = 0.12$).

(2) Hand-rearing experiments

In the first feeding trial, only worker-sized individuals emerged. Even high-frequency feeding did not result in the development of queens or queen-like individuals. Although larvae had the opportunity to continue feeding as long as they wanted, all larvae stopped accepting food and entered the prepupal stage after about two weeks. The total development duration of H and L-larvae was slightly, though significantly different (Table 2, $p = 0.03$, Student's *t*-test). The variable feeding regimes had a much greater effect on the ultimate body weight, however. Larvae in the H treatment were heavier than L larvae (Table 2, $p < 0.0001$, Student's *t*-test) and H adult individuals weighed significantly more (157.9 ± 27.4 mg, $p < 0.0001$, Student's *t*-test) than those of the L treatment (107.3 ± 24.9 mg). All experimentally nourished larvae and the control larvae in the colonies developed as workers (weight < 350 mg, radial cell < 3.375 mm, see Bortolotti et al., 2001) which suggests that all experimentally fed larvae must have been predetermined to develop as workers.

Also in the second trial all larvae terminated the ingestion of food themselves by refusing any further feedings and entered the prepupal stage subsequently. The results (development duration, body weight, and the amount of food ingested) were not different from those of the first trial (all $p > 0.5$, Student's *t*-test) for most larvae. Three larvae in the

Table 1. The choice of workers for larvae of the experimental (starved) or the control group during the first five feedings in each of the 14 experimental trials. The number of feedings is expressed per group of five larvae, in stead of for individuals

	First feeding	Second feeding	Third feeding	Fourth feeding	Fifth feeding
Starved brood	14	9	10	10	9
Control brood	0	5	4	4	5
χ^2	14.000 $p < 0.001$	1.143 $p > 0.1$	2.575 $p > 0.1$	2.575 $p > 0.1$	1.143 $p > 0.1$

Table 2. Summary of the data on larval development in the hand-rearing treatments. Total development times and development times until the prepupal stage (larval development) are expressed in days (±s.d.) starting at the time of oviposition. Larval weight (± sd), is displayed in mg, the amount of food eaten (± sd) in µl. n.a. = no data available. In each row, different superscripts (a, b and c) indicate a significant difference, within and among trials

	Trial 1		Trial 2		
	HIGH freq (n = 13)	LOW freq (n = 19)	HIGH freq (n = 13)	LOW freq exc. L-3/L-4/L-10 (n = 6)	LOW freq L-3/L-4/L-10 (n = 3)
Larval development	n.a.	n.a.	15.5 ± 0.9^{a}	16.8 ± 1.2^{a}	21.3 ± 0.5^{b}
Total development	25.0 ± 0.5^{a}	26.2 ± 1.5^{b}	25.6 ± 0.7^{a}	26.2 ± 0.5^{b}	33.5 ± 0.5^{c}
Larval weight	239.2 ± 41.5^{a}	162.6 ± 37.7^{b}	231.3 ± 66.4^{a}	176.2 ± 36.2^{b}	652.0 ± 28.1^{c}
µl food eaten	548 ± 44^{a}	311 ±7 2^{b}	546 ± 85^{a}	369 ± 54^{b}	1275 ± 145^{c}

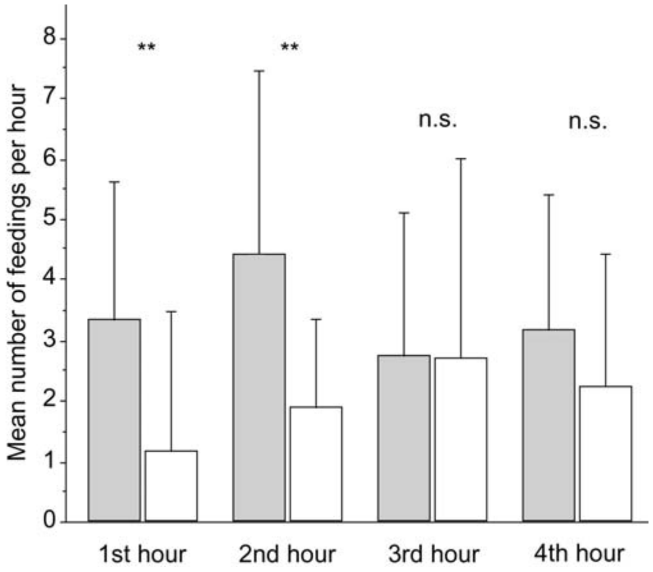

Figure 1. The average number (± sd) of feedings to starved and control larvae during the first to the fourth hour. The number of feedings is expressed per group of five larvae rather than for individuals. Shaded bars represent starved larvae and white bars control larvae. (n.s. = not significant; ** = significant $p < 0.05$, Wilcoxon signed-rank test; n = 14)

L-treatment (L-3, L-4 and L-10), however, had considerably longer development times, ingested more food and attained much higher body weights (Table 2). With the exception of these three, all L-treated larvae stopped eating slightly, though not significantly later than H larvae (Student's *t*-test p = 0.1). Nevertheless, the latter gained more weight, probably because they were fed more often ($p < 0.0001$, Student's *t*-test).

Although these particular three individuals were subjected to the low feeding regime, they continued to feed for about 4.5 days longer, and in the end weighed 3 to 4 times more than the other larvae (Table 2). In addition, their total development (egg – adult) took on average 8 days longer. The head-capsule width was 1.98 mm for L-3, 1.93 mm for L-4, and 1.86 mm for L-10, while that of all the other L larvae was 1.66 ± 0.06 mm (n = 9; p = 0.012, Mann-Whitney U test).

After emergence as adults, the length of the radial cell in the wings was determined as a measure of adult body size (see also Bortolotti et al., 2001). The radial cell was significantly larger for L-3, L-4 and L-10 (3.34, 3.40 and 3.20 mm respectively) than that of all the other individuals (p = 0.01, Mann-Whitney U test), independent of whether they were L or H larvae (L: 2.28 ± 0.08 mm and H: 2.30 ± 0.12 mm; $p > 0.1$, Student's *t*-test). Moreover, some of the larvae in the control larval batches in the colonies (Table 3) developed as queens (weight > 1000 mg, radial cell > 3.8 mm), which suggests that at least some of the larvae in the treatment were not fated to become workers.

The body weight at the end of the last instar clearly depended directly on the amount of food that was ingested (Fig. 2). Larvae in the H and L-treatment show a similar regression line; H larvae have the same slope as L larvae but a slightly lower intercept (L: $y = 2.4 + 0.53x$, p = 0.0001, $r^2 = 0.88$; H: $y = -42.42 + 0.52x$, p = 0.001, $r^2 = 0.46$).

Table 3. Queen production in 2nd trial of the in-vitro feeding experiments and in the colony controls. Only colonies II and III produced queens from the eggs in the control batches that had been laid just before queen removal. All batches in these colonies produced a mixed queen and worker brood. The batches that were used in the feeding experiment (exp.) are printed in bold type. In this table we included larvae or pupae that had died during the experiment in the case were it had been possible to determine the caste

	Eggcup number	Result
Colony I (0% queens)	**1 (exp.)**	**no queens**
	2 (control)	no queens
	3 (control)	no queens
Colony II (26% queens)	**1 (exp.)**	**2 queens and 10 workers (16.67%)**
	2 (control)	4 queens and 7 workers (36.40%)
Colony III (14% queens)	**1 (exp.)**	**1 queen and 8 workers (11.00%)**
	2 (control)	1 queen and 5 workers (16.67%)
	3 (control)	2 queens and 11 workers (15.40%)

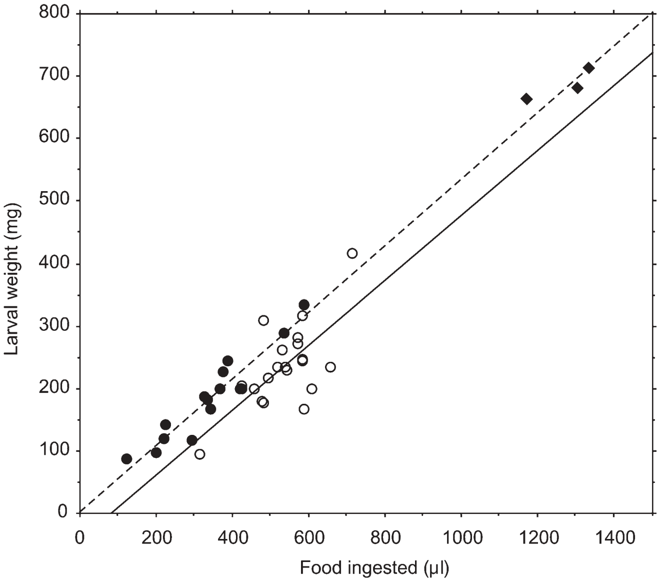

Figure 2. Larval weight at the end of the fourth larval instar as a function of the amount of food ingested. Closed circles denote larvae provisioned at L-frequencies and open circles those at H-frequencies. The top three closed squares represent the queen-like individuals fed at the L-regime. These were NOT included in the regression analysis but superimposed on the graph

Discussion

(1) Larval feeding rates and the significance of inspection behaviour

The 24-hour starvation experiments show that starved larvae were fed more often and sooner than control larvae. The differences disappeared as soon as the larvae of the experimentally starved groups had been fed, probably because they were nourished and the larvae of the control group had to be fed as well after some time. This indicates that the nutritional status of larvae influences the feeding behaviour of workers, and that larvae play an important role in the regulation of the feeding rate as suggested by Smeets and Duchateau (2001).

Workers seem able to detect differences between 'hungry' and nurtured larvae. First, workers were on average more attracted to, and spent more time on starved larvae, although this was not well quantified. Second, naïve workers provisioned starved larvae without performing any inspection-like behaviour towards either starved or control larvae. From this we infer that hungry larvae produce strong stimuli that elicit feeding behaviour (see also Smeets and Duchateau, 2001). It has been suggested that chemical cues are involved in the release of feeding behaviour because within a single feeding bout several larvae from the same broodclump are fed (Pereboom, 1997; Smeets and Duchateau, 2001). A single 'signal' from a single larva could elicit feeding of several larvae successively.

In contrast to earlier studies on bumblebees we never observed workers inspecting the brood. Such worker inspections were defined as the same sequence of behavioural elements as in a regular feeding without the characteristic food discharge behaviour: a short contraction of the abdomen (Pendrel and Plowright, 1981; Ribeiro et al., 1999). Nevertheless, we noticed that larvae regularly received food without the worker displaying the typical abdominal contraction, which suggests that the absence of a contraction does not imply a worker has performed an inspection (Pereboom, 1997). What does this behaviour represent then? First, wax manipulation can easily be mistaken for an inspection since it looks very similar. Second, the use of the words 'feedback mechanism' and 'inspection' suggests an intentional and causal relationship between inspections and the frequency of feedings. Inspection-like behaviour, however, might not be intended to inspect larvae, and could be a by-product of a stochastic feeding process at the individual level. The larvae in our hand-rearing trials clearly responded when a drop of food was deposited on the ventral surface of their body. They adopted a characteristic curled posture, preventing the liquid food leaking away, and moved their mandibles towards the liquid making chewing movements. Larvae reacted in exactly the same way when being slightly touched with a pipette or forceps. Apparently, the combination of touching and moistening stimuli seems to elicit food intake by the larvae, but only if they are motivated to eat since larvae remained immobile and did not react to such stimuli at all during ecdysis or when they had been fed recently. The retention time for not showing a reaction was longer for younger larvae, and decreased rapidly with larval age, which suggests that feeding is regulated by the rate at which larvae react to the workers' presence. A larval response, in turn, could be the stimulus for workers to actually regurgitate some food. From this perspective, inspections can be viewed as aborted feeding attempts instead of functional elements of a negative feedback mechanism regulating the feeding rate of larvae.

In sum, we hypothesise that the feeding process of larvae is organised by a simple stimulus-response chain, initially released by chemical signals originating from a 'hungry' larva. Since workers were observed to perform wax manipulation and brooding behaviour on the larval clumps almost continuously during inter-feeding intervals, their mere presence on the brood might be sufficient to perceive such chemical stimuli from larvae. Next, the absence of a larval response could be a reason for a worker not to finish a feeding sequence, omitting the abdominal contraction. Although this behaviour complies fully with the definition of an inspection, we certainly would not qualify it as one. Such a stimulus-response chain may just as well explain why larvae are fed at more regular intervals than at random.

(2) Frequencies of feeding, size dimorphism and caste differentiation

Queen development was not triggered by a high frequency of feeding, nor was it inhibited by a low frequency. Furthermore, the hand-rearing trials clearly showed that neither high rates of feeding nor an extended feeding period could be imposed upon larvae in order to force larvae to develop as queen. Larvae refused to consume offered food at what was apparently a pre-programmed completion of the larval development. In accordance with the caste-specific endocrine regulation of instar duration in *B. terrestris* (Cnaani et al., 1997, 2000; Hartfelder et al., 2000; Bortolotti et al., 2001), the rate at which larvae ingest food seems to be under endogenous (i.e. developmental) control. Differences in feeding frequency are likely the outcome of variation in body size, which result from differences in hormone-mediated larval development.

In theory, workers could still force female larvae to become workers by restricting their feedings, although Bortolotti et al. (2001) mention that workers never do this. Moreover, our results demonstrate that even with low frequencies of feeding queen-like larvae can be reared. In addition, selective starvation of female larvae from colonies in the ergonomic (i.e. worker-producing) phase leads to the emergence of small workers, while female larvae from colonies in the reproductive (i.e. queen-producing) phase become intermediate in size (Pereboom, 1997). Behavioural data on larval feeding rates in unmanipulated colonies give further evidence that queen development is causally independent of the frequency at which larvae are fed (Ribeiro et al., 1999). In some of the colonies in that study, queens were reared on a lower feeding regime than observed for worker larvae, and, most importantly, the lower frequency of feedings coincided with an extended larval development time, which is considered the main cause of queens becoming larger in size than workers (Cnaani and Hefetz, 2001; Pereboom, 2001). Considering these results makes it very implausible that restricted feeding yields worker larvae and increased feeding leads to queen development.

The weight and development times of all larvae in the first trial of the hand-rearing experiments, were well within the ranges measured for workers in laboratory and field colonies (70–350 mg and 19–34 days; Duchateau and Velthuis, 1988; Sutcliffe and Plowright, 1990; Cnaani et al., 1997; Bortolotti et al., 2001). All larvae that had remained in the colonies as a control for queen-rearing conditions developed as workers, suggesting that all experimentally fed larvae must have been fated irreversibly to worker development, probably because of the presence of the queen.

All but the three larvae in the second trial (table 2, L-3, L-4 and L-10) were found to be within the normal weight and time range for workers. Although no queen-sized individuals emerged from these three larvae, they weighed approximately twice the maximum of workers, but only half that of normal queen larvae. The width of their head capsule, was similar to the average of 1.93 mm for last-instar queen larvae (Cnaani et al., 1997). The head width of the worker-sized individuals, however, equals that of worker larvae in the ultimate instar (1.67 mm; Van den Toorn and Pereboom, 1996; Cnaani et al., 1997; Bortolotti et al., 2001). In addition, two of the three control colonies produced queens, but always in broods bringing forth both queens and workers (Table 3). The fact that L-3, L-4 and L-10 had been collected from those queenless colonies indicates they remained uncommitted, which allowed them to continue their development beyond that of worker larvae, eat more, and thus become larger.

Plowright and Jay (1977) proposed a size-determination model for *B. rufocinctus* where adult size is the result of differential rates of spinning and growth brought about by different feeding regimes. This size-determination model does not seem to be valid for *B. terrestris*. To ensure that larvae would not enter the prepupal stage because of insufficient space or starvation, spinnings were continuously removed and larvae were transferred to bigger containers if they grew too large.

For all hand-reared larvae, the body weight at the end of the last instar clearly related to the amount of food that was ingested which agrees with previous findings (Pereboom, 2001). Because larvae in the H and L treatment show the same relation between body size and food ingested, we conclude that body size is independent of the frequency of feedings in itself, but depends solely on the amount of food received. The three queen-like larvae fit the same correlation exactly, which suggests that they are intermediate in size because, being in the L treatment they received an insufficient amount of food. Considering that none of the worker-sized individuals came near to the size of colony-reared workers, it seems plausible to assume that the in-vitro rearing conditions (including the food quality) were inadequate. Although our results render it unlikely that the smaller body sizes were a result of the absence of essential components or a 'growth-factor' in the food mixture (cf. Ribeiro, 1999 and Cnaani et al., 2001), we can not exclude this possibility. Nevertheless, the extended development time, the wider head capsule, and the fact that control larvae in the colonies developed as queens, suggests they were queens – or at least queen-like individuals – be it of intermediate size.

In conclusion, the concept of a queen controlling caste development through the feeding behaviour of her workers does not seem to apply to *B. terrestris*. Caste-related variation in the rate of feeding is likely to be associated to differences in body weight which result from a pre-programmed caste-specific physiology (and a longer development time), and the associated nutritional needs to which workers respond accordingly. This strengthens the view that queen signals (e.g. pheromones) have a direct influence on larval development, either blocking a larva's potential to become a queen or signalling female larvae not to develop as queens as long as it not in their kin-selected interest.

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