J Comp Physiol B (1992) 162:681–689

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

The flow of jelly within a honeybee colony

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Accepted September 10, 1992

Summary. The flow of jelly from 100 nurse bees to the members of two normal-sized colonies was measured during one night. To follow the flow, nurses were injected with ^{14}C-phenylalanine. They incorporated this label into the protein of their hypopharyngeal (brood food) glands and their own body protein. When they were allowed trophallactic contacts during the investigation period a loss of label and a shift away from the abdomen was observed, indicating protein synthesis in the hypopharyngeal glands from previously stored protein. Very young larvae were fed less frequently than older ones. Younger workers received larger amounts of jelly than older ones, but considerable amounts were given to foragers. Drones behaved similarly. Between one-third and one-half of the distributed jelly was given to imagines; 10% and 16% of all workers received radioactive jelly from 100 nurses in the two colonies during one night. Thus, jelly is a very important food for adult honey bees. There was a remarkable exchange of label within the class of nurses themselves that is interpreted as communication within the social system.

Key words: Food exchange – Protein metabolism – Drones – Division of labour – Honeybee, *Apis mellifera* L.

Introduction

In social insects individuals may obtain food in various ways. They may (1) ingest it in the environment and use it for metabolism; (2) bring it back to the nest where it is stored and consumed later, or (3) distribute it via trophallactic reactions in an either unaltered or a further processed form (e.g. brood food).

Trophallactic contacts play an important role in the flow of information within the honeybee community. In the complicated system of the bee's dance language (von Frisch 1965) the transfer of freshly foraged nectar informs hive mates about the availability and quality of outside food sources. Another important transfer involves foragers to food storers. The latter concentrate the nectar and store it as honey, thus saving valuable foraging time for those individuals which are best informed about the location of the source. The food transfer to brood and queen may exclusively serve nutritional requirements.

Honeybee food sources are nectar, honeydew (excrements from aphids) and pollen. The first two mainly contain carbohydrates, the last mainly protein and lipids. All these foods can be consumed fresh or as honey or beebread (processed pollen). Freshly foraged pollen and beebread are eaten mainly by bees that are some days old but not yet foraging (Crailsheim et al. 1992). Within this group most protein is consumed by the class of nurse bees. The other main classes within the system of division of labour (Rösch 1925, 1930; Lindauer 1952; Seeley 1985; Winston 1987), especially the foragers, consume proportionally less pollen.

Preliminary experiments in the laboratory and with small colonies (Crailsheim 1990a, 1991) have shown that the nurses feed the products of their hypopharyngeal glands, the protein-rich jelly also called brood food, not only to the larvae and the queen, but also to the other adults in the colony. This provides the other bees that are not eating much pollen with vital protein (Crailsheim 1990b). The present experiments were intended to quantify the flow of proteinaceous jelly from the nurse bees to adults of both sexes and different ages and to different stages of drone and worker brood in normal-sized colonies.

Material and methods

Bees. Two colonies of *Apis mellifera carnica* Pollm. on 9–10 combs (20 × 40 cm) each with a 1-year-old queen from the Styrian School for Beekeepers (breeder) were used. They were located each at the end of a row of four hives. The two queens were sisters. Adequate supplies of honey and beebread were available during the

Abbreviation: dpm, decays per minute

period of the experiment. During the period of maximum brood-rearing activity from the middle of June to the middle of July, brood combs were removed and put into an incubator at 34.5 °C and at 60% relative humidity. Only negligible amounts of open brood were lost by this procedure. On certain days 100–300 worker bees 0–12 h after emergence were colour coded and reintroduced together with all other emerged worker bees and the combs into their hives during the late evening. Thus, the natural structure of each colony was not disturbed. Young bees are readily accepted when added to a queenright colony (Kunert and Crailsheim 1988). When available, freshly emerged drones (but from another colony) were also labelled and introduced.

To test onset of foraging of the marked workers and to have a group of bees with a certain foraging status, a feeding station was established 28 days after the first marking of the bees; bees were trained to collect and were marked at the feeding station at a distance of about 8 m from the hives.

Labelling of the nurses, preparation of the control nurses and reintroduction into colonies with age-coded bees. In the evening of the day of the experiment, 9-day-old bees were removed from their colonies. They were fixed to a piece of wood with two crossed needles without being anaesthetized. In this position they were injected with 1 µl ^{14}C-phenylalanine (Amersham, 1.85 MBq·ml^{-1}, 18.98 GBq·mmol^{-1}). Phenylalanine was chosen as an essential amino acid for the bee (De Groot 1959) and is only slowly degraded to CO_2 (Crailsheim 1990a). Injection was dorsally with a Hamilton microsyringe between the 5th and 6th abdominal segments. If haemolymph was pressed out after the needle was removed, it was gently blotted away with a strip of filter paper. If the volume of haemolymph lost was estimated to be larger than 1 µl the animal was not used for the experiment. The wound was then sealed with a drop of warm beeswax and collophonium. The radioactivity of the collected drop of haemolymph was determined to calculate the exact amount of label received per bee. The amount primarily injected minus that found on the filter paper is referred to as the "injected amount". Bees were coded with numbered plates and stayed in the fixed position for 1–3 h at room temperature; 100 of them were then reintroduced into each of their hives. The entrance had been closed previously with a screen cage that did not inhibit air circulation, prevented bees from flying but allowed them to walk out from the hive for some centimetres. The volume of the cage was about 1 l. Timing was such that no natural flying activity was inhibited.

Eight identically injected bees were killed 2 h after injection. At the same time another eight were isolated overnight (8 h) in small tubes (5 ml) in an incubator at 34 °C and 60% relative humidity in darkness, provided with honey and then killed 10 h after injection. Radioactivity was measured in the haemolymph (taken via an incision in the abdomen), head, thorax and abdomen in two steps. Samples were extracted after homogenization in 80% ethanol and centrifuged. Radioactivity in the supernatant was defined as soluble radioactivity and that of the pellet (after solution in sodium hydroxide) as protein-bound radioactivity. These two groups of nurses are the control nurses.

On the day of the experiment, i.e. 30 days after the first introduction of bees, colour codes were aged 30, 23, 16, 9, 4 and 1 day (with an accuracy of ±12 h with respect to ages up to 9 days and of ±1 day with respect to ages of more than 9 days). Thus, functional status could be defined by age (especially among the very young), developmental stage of the hypopharyngeal glands (nurses) and by behaviour (foragers). The size of the hypopharyngeal glands was measured with an ocular micrometer with a Reichert Diavar microscope (glands were embedded in 500 mmol·l^{-1} glucose solution). The largest hypopharyngeal glands were found in the investigated groups of nurse bees (9 days); foraging status was first demonstrated in the group of 16-day-old bees, detecting them at an artificial feeding place (Crailsheim et al. 1992).

Collecting of the samples and description of the colony. In the morning, 8 h after introduction of the injected bees and before the bees would have started flying, the whole hive was gassed with CO_2 for 10 min to anaesthetize the bees and placed in a refrigerated room at 3 °C. There the bees were removed; eight injected nurses per colony were fixed to a piece of wood with two needles and brought to room temperature where they recovered from narcosis. The remaining bees were frozen at −20 °C pending further preparation. Combs were removed and frozen.

The number of coded and non-coded bees in the hive was determined, brood area was measured according to Puchta (1949) and distribution of different larval stages estimated. Bees of each age-coded group which were not detected were assumed to have died. A survival rate for different ages was calculated based on the assumption that mortality was equal for coded and non-coded bees. An age distribution was modelled based on these calculations, the total number of bees, and the number of open and closed brood cells. Data are taken from Crailsheim et al. (1992). The same colonies were used for the experiment described there. Data are given in Table 1.

Preparation of the anaestized bees, larvae, honey and bee-bread. After narcosis and cooling, injected nurse bees were brought to room temperature to extract haemolymph and subsequently handled as the control nurses (see above).

All other injected and all colour-coded bees from the hives were powdered individually in toto in liquid N_2 and were dissolved in 1.5 mol NaOH·l^{-1} for 1 h at 60 °C. Samples were then neutralized with HCl, diluted and the radioactivity was determined in an aliquot of the supernatant. The radioactivity found was defined as having been received from the labelled nurses.

Samples of the non-coded bees were taken randomly (six times 400 bees of each colony), homogenized and dissolved in 2 mol NaOH·l^{-1}. Radioactivity was determined in aliquots after neutralization.

Worker larvae were categorized after Rembold (1974) and washed out of their cells together with the jelly with 300 µl Soluene-350 tissue solubilizer (Packard) after wetting the cells with 20 µl water. Drone larvae were similarly categorized. Weights are given in Fig. 1. As drone larvae were analyzed later than worker larvae, a weight loss had occurred during frozen storage and the weight for the small stages could not be determined.

100-µl samples of honey and 50 µg of pollen (wetted with 100 µl water) were mixed with 1 ml Soluene-350.

Measuring radioactivity and calculation of food flow. Aliquots of the dissolved total bees, ethanol extracts and NaOH-dissolved samples of injected bees were mixed with Hydroluma liquid scintillation cocktail (Baker). Solutions from pooled bees were mixed with isopropanol and bleached with H_2O_2; these, as well as samples dissolved in Soluene-350, were mixed with Hionic Fluor (Packard). All samples were counted with a Packard scintillation analyser (1900 CA Tri-carb). The technical threshold for taking an individual as a recipient was 30 dpm for adults and 10 dpm for larvae. As there is a continuous flow of proteinaceous and non-proteinaceous food among all colony members, not all individuals that were proven to be radioactive would have had to have received this radioactivity from the labelled (injected) nurses. An arbitrary second threshold was chosen to estimate primary recipients; this was 1% and 0.5% of the amount one labelled nurse had fed during one night (see below) per imago and larva, respectively.

The amount of radioactivity that was actually injected and had remained in all the 100 reintroduced nurses minus the amount that could be recovered in their bodies on the next morning, reduced by the percentage of loss seen in the control bees that had stayed in the incubator without the chance to donate jelly, was defined as having been spent. This count rate was taken as 100% of fed jelly for all food flow calculations.

Thus

$$\mathrm{dpm_{received}} - \frac{\mathrm{dpm_{recovered}}}{\%\ \text{recovered in controls} \cdot 100^{-1}} = \mathrm{dpm_{spent}}$$

where $\mathrm{dpm_{received}}$ is the dpm injected minus loss before sealing the wound in all reintroduced nurses; $\mathrm{dpm_{recovered}}$ is the dpm recov-

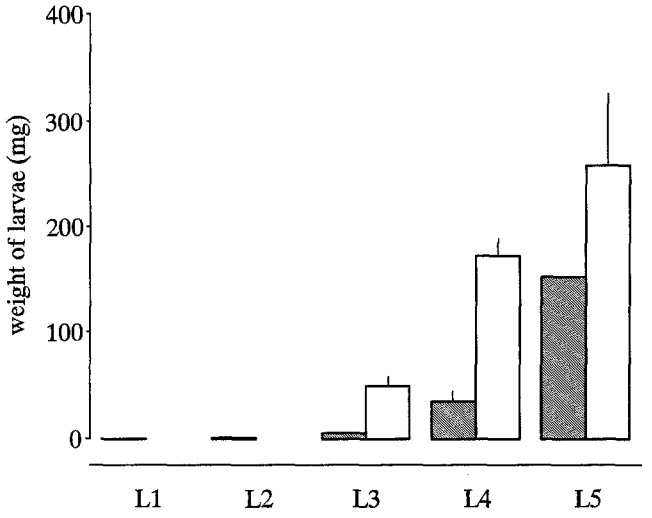

Fig. 1. The weight of worker and drone larvae in relation to their developmental stage. There are no results for the youngest drone larvae as they dried out during storage (see Materials and methods). *White*: drones, *grey*: workers

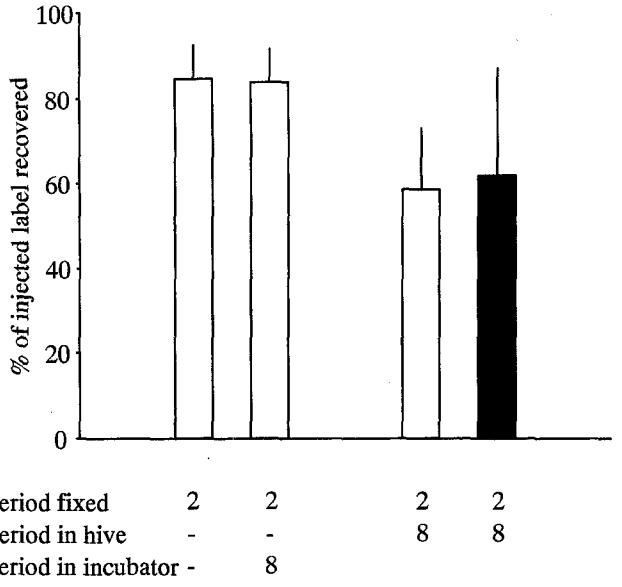

Fig. 2. The recovery of label in total bees at different periods after injection of ^{14}C-phenylalanine into the haemolymph, under different conditions for different periods (colonies 1 and 2). Means and standard deviations are given. *White:* sum of samples for different compartments; *black:* bees investigated in toto

ered in all reintroduced nurses in the morning after they had been in the hive all night; and % recovered in controls is the percentage of the label that could be recovered in control nurses that were handled as were the reintroduced nurses but had stayed separately in the incubator for the same period as the reintroduced bees had as a chance to donate jelly.

Means and standard deviations or box and whisker plots are given. Comparisons of means were done with the U-test, those of trends with the χ^2-test and significances of non-linear regressions were calculated with the *t*-test. The level of significance was chosen to be <0.01 if not indicated otherwise.

Results

Incorporation of the label and amount of spent jelly

When bees had no contact with others, the total label found within the animals did not differ whether the bees stayed in the laboratory for 2 or for 10 h (2 h at room temperature and 8 h at 34 °C). It was significantly lower when bees had been reintroduced into their hive for 8 h after the incorporation period of 1–3 h (Fig. 2). The eight narcotized injected nurses out of the group that had stayed in the hive overnight and were then sampled extensively (divided into body segments, haemolymph, extracted and dissolved fractions) showed the same total amount of label as the frozen bees sampled in toto (Fig. 2).

The longer period (2 h versus 2+8 h) for incorporation caused an increase in protein-bound activity in the head, but when the bees were not isolated and allowed to stay in the hive the protein-bound radioactivity was drastically lowered in the head. The content of the abdomen was also significantly lowered in these nurses. There were no differences in the protein-bound radioactivity in the haemolymph (Fig. 3).

From the total amount of label in the injected nurses after their overnight stay in the colony and the known

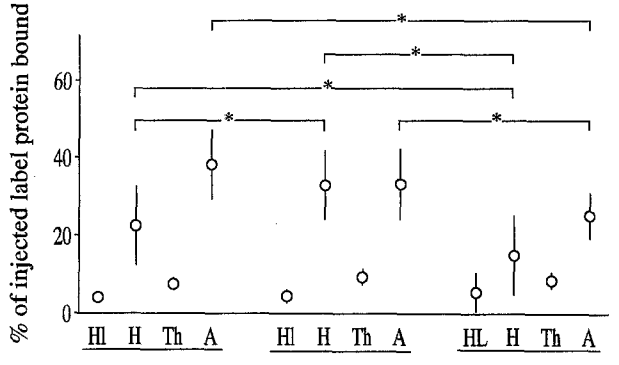

Fig. 3. Percent of protein-bound label found in different compartments of the body (*Hl:* haemolymph, *H:* head, *Th:* thorax, *A:* abdomen) at different periods after injection of ^{14}C-phenylalanine into the haemolymph and under different conditions. The results for the three body compartments include the haemolymph in this compartment. The content of the haemolymph sample was calculated from the measured content of 1 µl and the average haemolymph content of nurse bees [19 µl, Crailsheim (1985)]. Means and standard deviations are given; * indicates significance ($P<0.01$)

amount of originally applied label, the amount of label in the jelly fed to nestmates could be calculated; this was 2944751 and 2348107 dpm in colonies 1 and 2, respectively.

Recovery of the label in the recipients

All castes and within all castes all investigated age groups (except one drone group) could be demonstrated

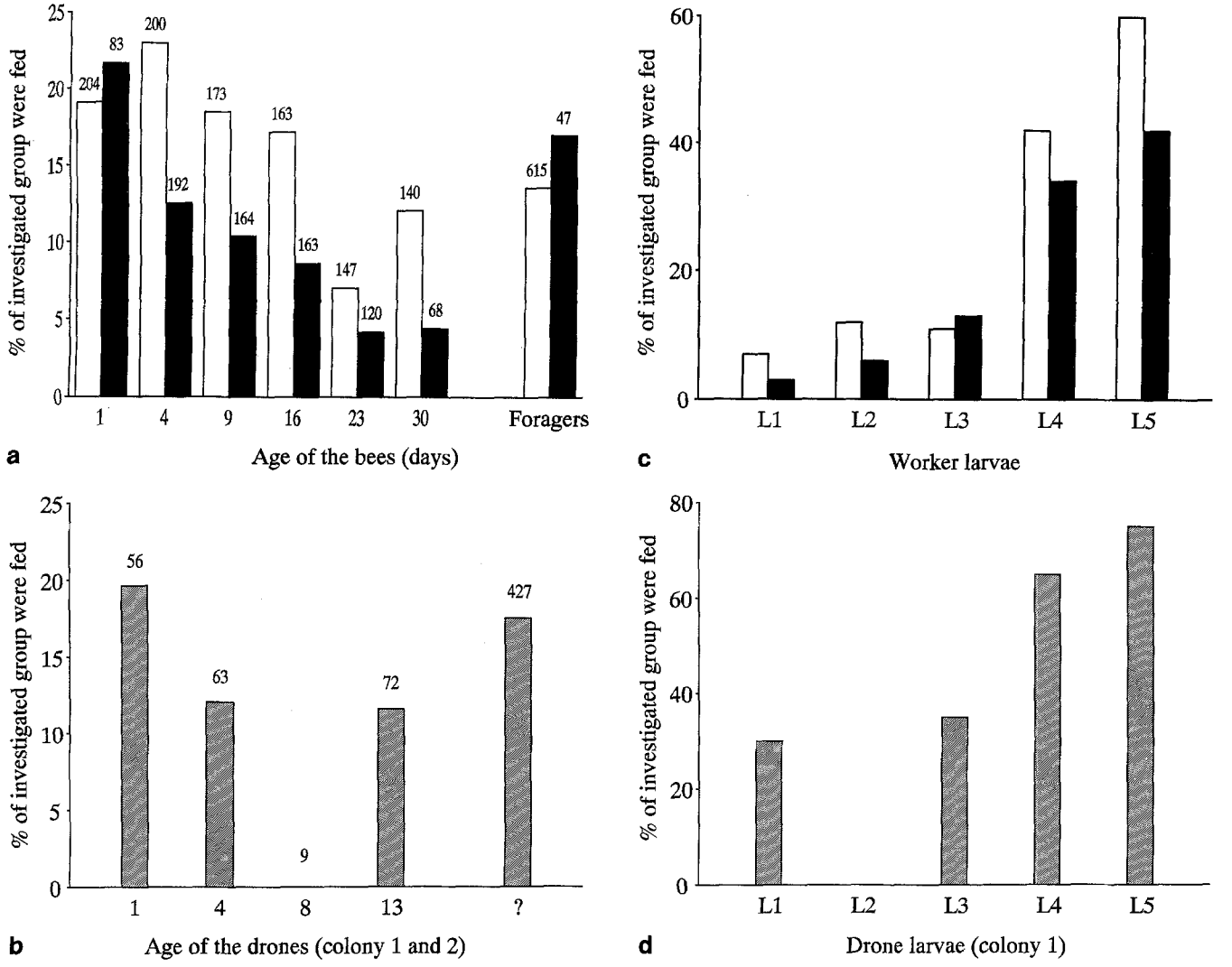

Fig. 4. Frequency of protein-transfer transactions among different-aged adult workers (**a**), adult drones (**b**) and larvae of both sexes [workers (**c**) and drones (**d**)]. The correlation between age (stage) and percentage of fed members of the group was significant for worker larvae and adult workers ($P<0.01$). Significance was not tested for drones. Sample sizes for adults are given above the bars. *White:* colony 1; *black:* colony 2

to have received radioactive label. Within the group of workers defined by age there is a significant tendency for more members of the young groups to be recipients (Fig. 4a); the same trend could be shown for drones (Fig. 4b) but was not tested for significance (small sample size). A higher proportion seems to have been fed to the group of proven foragers in colony 2 but the sample size was small.

With increasing age, significantly more worker larvae were fed (Fig. 4c). Although there was a larval drone stage (L2) that was not fed at all, the same trend was seen for drone larvae (Fig. 4d, significance not tested, $n=20$). A correlation of larval weight and frequency of feeding produced good significance (Fig. 5a), depending on weight. When the threshold for classification as a recipient was not 10 dpm (see Materials and methods) but 0.5% of the amount of jelly one labelled nurse had donated – which is much higher – the curves showed a shape similar to that obtained with the lower threshold (Fig. 5a, b). This second type of calculation (higher threshold) was done to have a group of fed larvae that were probably fed by an injected nurse as opposed to a nurse that had received labelled jelly from an injected nurse (see Discussion). The amount given to a single larva varies and is documented in Fig. 6a.

There is a tendency for individual younger workers to receive more jelly than older ones (Fig. 6b), but the significance, due to the large variance, is low ($P<0.05$). No clear trend could be seen for drones, probably due to the smaller sample size (data not shown).

When the threshold for a successful jelly transfer to an imago was increased from 30 dpm to 1% of the amount one labelled nurse had distributed, a significant negative correlation could be seen between age of the recipients and number of high-level protein transfers (supposed to come from one labelled nurse; see Materials and methods and Fig. 7).

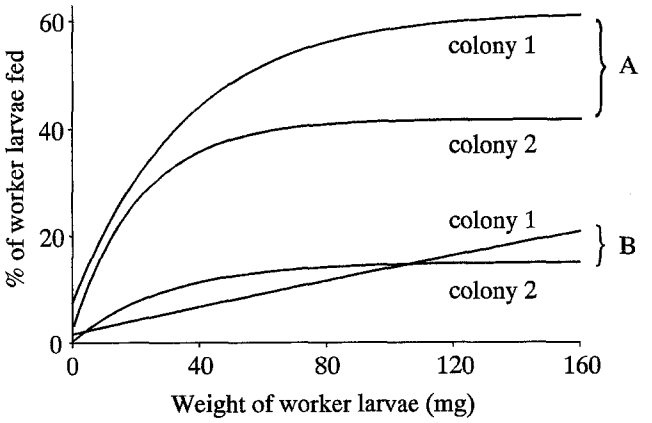

Fig. 5. Portion of worker larvae receiving jelly in relation to their weight (stage). *A* threshold given by exactness of the method; *B* threshold was 0.5% of the average total amount given by one nurse. All curves show a similar shape, dependence was significant for all curves ($P<0.01$)

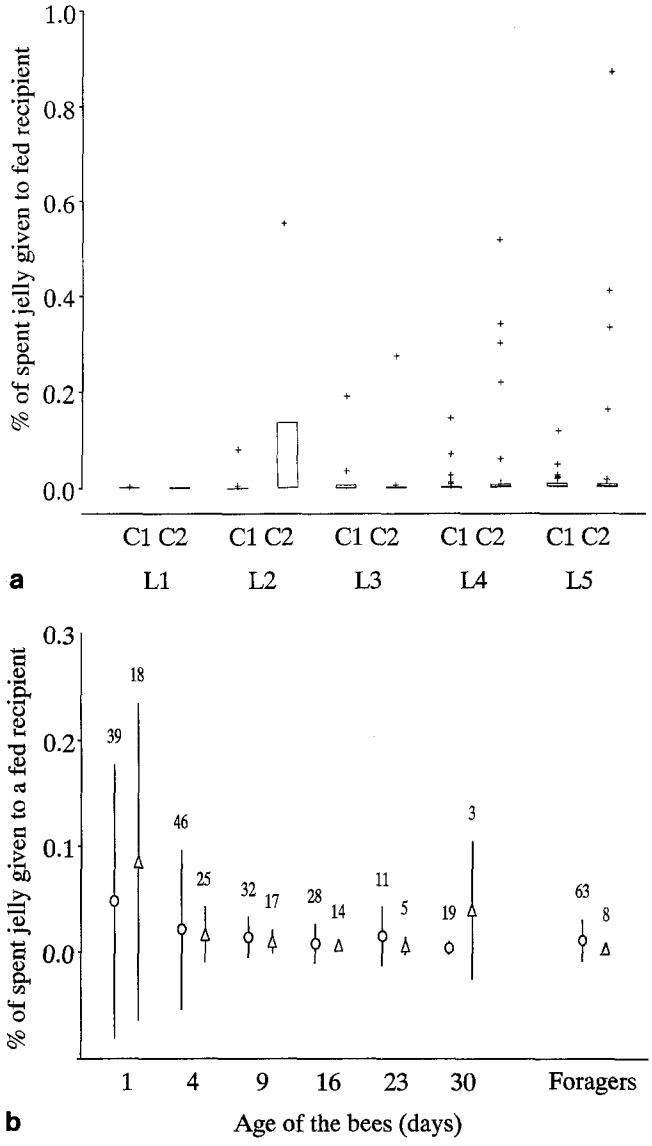

Fig. 6. Jelly given (**a**) to a fed worker larva (box and whisker plot) and (**b**) to adult workers (means and standard deviations, numbers indicate sample size). The decrease with age was significant for the latter ($P<0.01$). ○, *C1:* colony 1; △, *C2:* colony 2

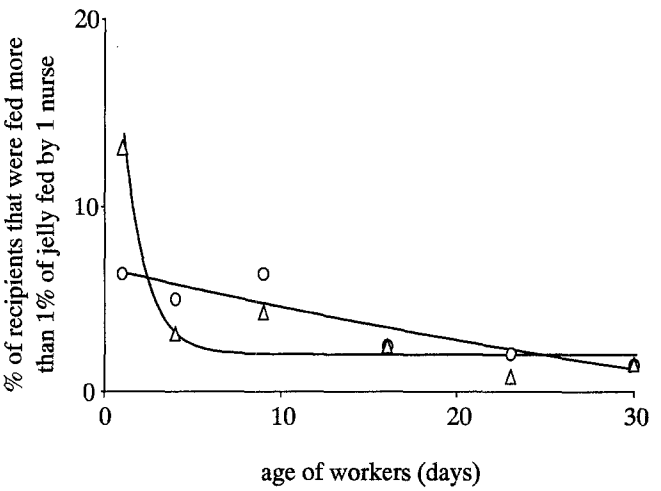

Fig. 7. Portion of workers that received more than 1% of the jelly that was distributed on average by one nurse in relation to their age. Bees receiving this amount of jelly were probably primary recipients (fed directly by an injected nurse). Means and standard deviations are given. ○ colony 1; △ colony 2

Total recovery of the label in the colonies

The total amount of label in the colony could be calculated from the estimated age distribution of the adult members of the colony, the measured numbers of brood cells and the average amount of label in the analysed individuals (adults and larvae); this was 69% and 106% in colony 1 and 2, respectively (Table 1). The amount of jelly that was fed to all workers was calculated in two ways: (1) by investigating the coded bees and assuming that all similar-aged bees were fed equally in amount and frequency, the total of fed jelly was estimated from the age distribution model. (2) Six samples, consisting of 400 bees each, were analysed and the results were extrapolated to the total number of bees. The results obtained with these two methods (Table 1) differed for the two colonies by 8% and 22%.

Calculated from the model data of age distribution and the number of bees fed in each coded group, 15.6% and 9.6% of all workers (3756 and 2409 individuals) had received jelly produced by the 100 injected nurses. In a large sample of non-coded drones 17.5% had been fed. There was no label in honey and beebread samples.

Comparison of the colonies

The two colonies with sister queens had about the same number of members but differed somewhat in the composition of age classes (Table 1). The number of foragers that could be trained to the feeding place was much larger in colony 1 (Fig. 4a). The youngest group found at the feeding station was 14 days old. 8.6% of this living, marked group of colony 1 was observed collecting there, indicating an early start of foraging [detailed data in Crailsheim et al. (1992)]. The portion of coded bees (taken to be representative of all members) that had drifted from colony 1 into colony 2 was 3.5%, and 5.1% for the reverse drift.

Table 1. Distribution of jelly given by 100 9-day-old nurse bees to different age classes of imagines and larvae in colony 1 and 2

Colony 1 Investigated age group (days)	Artificial age class (days)	No. of investigated individuals (samples)	Average % of totally spent jelly per individual (sample)	Estimated no. of individuals	Estimated % of totally spent jelly per class or total sample	% total
1	1	204	0.00920	679	6.25	
4	2–5	200	0.00491	3409	16.72	
9	6–12	173	0.00256	4714	12.09	
16	13–20	163	0.00135	5073	6.85	
23+30	>20	902	0.00102	10201	10.37	
Sum worker		1642		24076	52.28	52.28 (48.04)
Sum drones	all	347	0.00503	counted: 1065		5.36
Queen					calculated: 0.15	0.15
Sum drifted bees		45				0.43
Workers L1		100	0.000055	600	0.03	
Workers L2		100	0.000968	600	0.58	
Workers L3		100	0.002430	1013	2.46	
Workers L4		100	0.003249	738	2.40	
Workers L5		100	0.004334	1088	4.72	
Sum worker larvae				4039	10.19	10.19
Drone L1–L5		100		385	0.31	0.31
Honey		100	0		0	0.00
Beebread		50	0		0	0.00
Total						68.72

Colony 2 Investigated age group (days)	Artificial age class (days)	No. of investigated individuals (samples)	Average % of totally spent jelly per individual (sample)	Estimated no. of individuals	Estimated % of totally spent jelly per class or total sample	% total
1	1	83	0.01844	822	15.16	
4	2–5	192	0.00210	4207	8.82	
9	6–12	164	0.00100	5936	5.92	
16	13–20	163	0.00053	6285	3.30	
23+30	>20	235	0.00071	7846	5.54	
Sum worker		837		25096	38.73	38.73 (47.21)
Sum drones	all	280	0.00170	counted: 280		0.48
Queen					calculated: 0.00166	0.00
Sum drifted bees		60				0.07
Workers L1		100	0.000044	913	0.04	
Workers L2		100	0.006914	1750	12.10	
Workers L3		100	0.002956	2550	7.54	
Workers L4		100	0.015325	1663	25.49	
Workers L5		100	0.019150	1100	21.07	
Sum worker larvae				7976	66.23	66.23
Drone L1–L5		0			0	0.00
Honey		100	0		0	0.00
Beebread		50	0		0	0.00
Total						105.51

The amount of injected label that was no longer found in the nurses was taken as 100% (see Materials and methods). Numbers in brackets beside the result of total gain in the worker group (calculated from the model) represent the results calculated from the analysis of six pools consisting of 400 bees each. The estimated numbers of members of each artificial age class (model) are from Crailsheim et al. (1992)

Discussion

Nurse bees show a high synthesis of protein in their hypopharyngeal glands (Brouwers 1982; Takenaka and Kaats 1987; Huang and Otis 1989; Huang et al. 1989; Knecht and Kaatz 1990; Takenaka et al. 1990). This is reflected in the high incorporation rate of the injected ^{14}C-phenylalanine in the head compartment seen 2 h and, to a greater extent, 10 h after the injection. A comparison of the level of protein incorporation in the head and the abdomen of bees that had only stayed in the laboratory for 2 h with those that had spent an additional 8 h in their colony shows a significant reduction in both compartments. The reduction in the head compartment indicates a donation of synthesized jelly as previously shown in laboratory experiments (Crailsheim 1990a); the reduction in the abdomen shows that amino acids or protein were probably already shifted from the fat body elsewhere. As the hypopharyngeal glands are the most active protein-synthesizing organs, they are probably the place to which the label was shifted. After a 2-h incorporation period, 85% of the injected label was recovered. Some of the lost label might be exhaled as $^{14}CO_2$, although the conversion of phenylalanine to CO_2 has been shown to be small (Crailsheim 1990a), and some might be lost during preparation.

More bees out of the younger groups were proven recipients than older bees. This could be caused by the age-dependent distribution of the bees within the colony. Young bees tend to stay near the brood nest (Free 1960; Furgala and Boch 1961) where there are also larger numbers of nurse bees. A second reason might be a more intense begging behaviour by very young bees that need large amounts of protein to achieve their final body composition (Haydak 1934). This hypothesis is strengthened by the finding that the 1-day-old bees are not only fed most frequently but also receive the largest amounts of food; thus, as a group they receive a high proportion of jelly in spite of a relatively small group size (Table 1).

Considerable amounts of jelly are fed to older bees and exchanged within the nurse age class. Within the latter the exchange might have mainly informational character, as they are the class that consumes the largest amounts of pollen (Crailsheim et al. 1992) and is also best equipped to digest it (Grogan and Hunt 1980, 1984; Moritz and Crailsheim 1987; Jimenez and Gilliam 1989). Since they are the protein-digesting temporal caste within the colony they should not have to depend on jelly from another nurse. However, if poor pollen supply causes a reduced willingness to exchange or to spend jelly, then these trophallactic contacts might provide them with information about availability of protein in the hive. Furthermore, there may be a pooling of the brood food if the jelly received is distributed together with the bee's own jelly.

Older workers and especially foragers consume negligible amounts of pollen (Crailsheim et al. 1992) and have very low levels of proteolytic enzymes (Moritz and Crailsheim 1987; Crailsheim and Stolberg 1989) but have a high protein turnover (Crailsheim 1986); this means that they depend on an additional source of protein. More bees of the group of foragers seem to have received jelly than other bees of the same age (16 days and older, Fig. 4a), but no significance was tested as the age data for the foragers was incomplete.

The tendency of young bees to receive more frequent and larger amounts of jelly can be seen when the lowest level of detectable radioactivity was chosen as threshold, but also when 1% of the amount that was distributed by one labelled nurse was chosen. If the second means of transfer is accepted as having taken place between an injected nurse and a recipient, then there are more such primary recipients within the younger groups. Nothing can be said about the amount of jelly given during one trophallactic reaction as it is not known whether the amount fed to one bee was transferred in one or in more transactions.

Early studies suggested that adult drones receive jelly (Nixon and Ribbands 1952; Free 1957a; Mindt 1962). Free (1957a) reported that the drones are mainly nourished by young workers, and further observed that young drones are more likely to be fed than older ones and that the young drones were fed at the same time as the older drones were being attacked by workers. The present study did not reveal any tendency for the older drones to be more poorly provided with food (4 days versus 13 days). It is possible that since the investigated colony accepted drones very well, even though they originated from another colony, older drones were also treated well. Furthermore, the sample size of the age-coded drone groups was not very large and there was considerable variability. The small sample size ($n=9$) of the 8-day group might be the reason for the lack of a significant difference. Nevertheless, the number of non-coded drones was large and showed that 17.5% of this total group had received considerable amounts of food from the 100 labelled nurses.

More larvae were fed with increasing age. This was calculated to be significant for the worker larvae and was shown for feeding in general and for primary feedings (under the assumption that a larva that has received more than 0.5% of the jelly produced by one injected nurse is a primary recipient). Assuming that all nurses, independent of their status (early or late nurse), are feeding larvae in a similar manner, the curves describing the dependence of receiving food from nurses are representative in their course for jelly receiving in general. Owing to the small sample of drone larvae, this calculation was not performed; the small number of available drone larvae might have meant that none of the L2 stage could be proven to have been fed, as it is not likely that there will be a young stage that is not fed at all during a given night. In both sexes the amount given to each fed larva showed a huge variability and was only documented for workers (Fig. 6a).

As the nurses have been shown to feed each other, and as all other age classes show trophallactic reactions with all other groups (Free 1957b), a bee that is found to have received radioactive jelly has not necessarily been fed by one of the injected nurses. It is important to note that this distribution causes a considerable dilution of the label. This dilution, also indicated by the fact

that the jelly of the 100 nurses was found in 3756 and 2409 individuals, means that many bees will have a label quantum below the chosen limit of 30 dpm (10 dpm per larva). Such an underestimation might influence the gain of recovered label in dependence on number of workers fed. This will be important in the discussion which follows.

There are differences between the two colonies. In colony 1 the recovery is 69% and much more is fed to the adults than to the larvae, whereas the recovery is much better in colony 2 (106%). One reason is certainly that there was much less brood in colony 1 than in colony 2, but this is not a sufficient explanation. If nurses at different stages have different donating behaviours and if the nurses of colony 1 and 2 happen to be at different stages, then this might account for finding more in adults than in larvae, or vice versa, in colony 2. The results of pollen analysis with bees in these colonies have shown differences in pollen consumption of bees aged 4 and 9 days, indicating that there could be another stage; the bees with maximum pollen consumption were 4 days old in colony 2 and 9 days old in colony 1. Furthermore, the diameters of the hypopharyngeal glands were generally smaller in all age classes of bees in colony 2 compared to colony 1 [data generated with bees of the identical groups given in Crailsheim et al. (1992)]. Although the queens were sisters, genetic differences can cause both morphological and behavioural differences (Robinson et al. 1989; Calderone and Page 1991). If such a difference in nourishing behaviour causes differences in the kind of jelly recipients, such behavioural variances might also occur in the begging behaviour of the recipients themselves.

The larger amount given to the adults in colony 1 might have also caused a greater loss to analysis, as the threshold (30 dpm) for rejection was higher for adults than for larvae (10 dpm). Furthermore, jelly that was fed to adults was certainly diluted by transfer to secondary recipients, whereas jelly fed to a larva stayed in that animal.

Thus, methodological and biological reasons might be responsible for the differences in the results of the two colonies. Major biological differences were previously shown in experiments with extremely small colonies (Crailsheim 1991), e.g. that the amount of jelly fed to adults was between 11% and 37%, indicating that honeybee colonies have their own very distinctive traits.

The similar amounts of jelly donated to the workers, as calculated with the estimation model and with the pooled bees (Table 1), confirms the method. Together with previous papers (Crailsheim 1990, 1991), the results show that nurse bees transfer food not only to the brood but also give up to half of their jelly to adult members of their colony and thus have the function of general protein supplementors within their community.

Acknowledgements. I gratefully acknowledge technical and mathematical assistance from Mag. Rudolf Gmeinbauer, Mag. Norbert Hrassnigg and Herbert Marchl and the critical reading of the manuscript by Ms. Eugenia Lamont. I also want to thank the two anonymous reviewers who helped to improve the manuscript. This project was supported by the Fonds zur Förderung der wissenschaftlichen Forschung, Austria.

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