Amino Acids (2003) 24: 205–212
DOI 10.1007/s00726-002-0311-y

Amino
Acids
Printed in Austria

Free amino acids in the haemolymph of honey bee queens *(Apis mellifera L.)*

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Received January 12, 2002
Accepted March 14, 2002
Published online September 4, 2002;

Summary. In queen honey bees the free amino acid content in the haemolymph clearly depends on the physiological function and social environment of the individual. While in drones and workers the content of free amino acids increases after emergence until it reaches a peak in 5-day-old animals and decreases afterwards, the amino acid content in queens reaches its highest level (>60 nmol/μl haemolymph) with the onset of egg laying (10 d of age). This level is about 2.5 times more than the highest level found in workers. Queens maintain this high level also when they are older (>30 d) and continue to lay eggs in average colonies. As in drones and workers, in queens the predominant amino acid is proline, which accounts for more than 50% of the total content of free amino acids in egg-laying individuals. When 10-day-old queens are prevented from mating and do not lay eggs, their amino acid content is significantly lower compared to laying queens of the same age. Also the social environment influences the contents of free amino acids in queens. When virgin queens were kept for 6 days with 20 worker bees and sufficient honey and pollen in an incubator, they had significantly lower concentrations of amino acids than virgin queens living for the same period with about 8000 workers in a colony. Most probably, the high amino acid concentration in the haemolymph is the basis for the high protein synthesis activity of laying queens.

Keywords: Amino acids – Queen – Apis mellifera – Proline – Egg-production – Social environment

Introduction

Generally, free amino acids are found in the haemolymph of insects in much higher concentrations than in the blood of vertebrates (Wyatt, 1961; Cheng, 1985). Concentrations in the honey bee have been well documented (Wang and Moeller, 1970; Crailsheim and Leonhard, 1997). Unlike most other insects, honey bees are highly eusocial. Each colony typically includes only one reproductive female – the queen – along with thousands of female workers, and, during summer, some hundreds of males, called drones.

The workers, which care for the nest, the brood and the other adults, are short-lived. Their age expectancy is from several weeks to several months. In contrast, queens can live from one up to 4 to 5 years or even longer (Winston, 1987; Page and Peng, 2001). In the normal life cycle of a queen, she emerges as an adult after a developmental time of 16 days. During the first days of adult life she fully develops her reproductive organs until, some days after emergence, she makes several mating flights to store sperm from about 6–24 drones in her spermatheca (Estoup et al., 1994; Neumann et al., 1999). After these flights she stays in the nest for the rest of her life (except when the colony splits, a process called swarming) and lays eggs for the rest of her life. In moderate climates a queen lays high numbers every day during the summer period. Up to 2000 eggs per day have been reported (c.f. Ribbands, 1953), amounting to about twice the queen's body weight. To enable this extraordinary effort, some of the workers, called nurse bees, feed the queen with high amounts of a proteinaceous jelly they produce in their hypopharyngeal glands (Crailsheim, 1991, 1998). Allen (1960) found that the laying of eggs is directly correlated to the frequency of feeding. The ability to process large amounts of protein for egg production implies special adaptations related to the queen's protein metabolism.

The way amino acid content in the haemolymph varies with age has been quantified for worker

Table 1. Conditions and ages of queens analysed for the content of free amino acids in the haemolymph (n = number of queens sampled)

Conditions	Age of queens when sampled	n
[1] freshly emerged queens (emerged in an incubator)	<12 h	11
[2] virgin queens, 5 d old, each kept with 20 nurse workers in a Liebefeld box in the incubator	6 d	10
[3] virgin queens, emerged in a 6-frame colony with a queen excluder at the entrance	6 d	6
[4] virgin queens, each kept in an Apidea mating box and prevented from mating by a queen excluder screen at the entrance	10 d	7
[5] freshly mated queens, each kept in an Apidea mating box; sampling occurred after the queen laid her first eggs	10 d	8
[6] normally laying queens from a 6-frame colony	>30 d	7

honey bees and drones. In both workers (Crailsheim and Leonhard, 1997) and drones (Leonhard and Crailsheim, 1999) the amino acid content depends significantly on age, and drones have higher absolute amounts than workers. In both drones and workers, the highest concentrations of free amino acids occur at 5 days of age, and after that levels decrease with age. The amino acid proline is present in relatively high concentrations in both workers and drones. This amino acid has been shown to be metabolised to a greater extent than leucine or phenylalanine in the drone's oxidative catabolism (Berger et al., 1997). For workers, Micheu et al. (2000) showed that proline is involved in flight metabolism.

Amino acid changes in honey bee queens have not been fully investigated. The honey bee queen, as a long-living potent reproductive female with the single and highly specialised task of egg-laying and with extraordinary feeding behavior, can be expected to differ from workers and drones in this respect. Wang and Moeller (1970) investigated the content of free amino acids in the haemolymph of 10- and 20-day-old queens, but did not give any information about the status of the queens, whether they were producing eggs or not. We investigated the influence of age and functional status on the concentration of free amino acids in the haemolymph of honey bee queens.

Material and methods

Haemolymph of honey bee queens (*Apis mellifera carnica* Pollmann) was sampled for determination of amino acid content in the summer of 1998. All the queens were bred in 1998, but different groups of the queens were kept under different conditions before samples were taken (see Table 1).

When comparing the data for queens to those for workers and drones, we used data from Crailsheim and Leonhard (1997) for workers, and data from Leonhard and Crailsheim (1999) for drones.

Conditions and ages of queens (see Table 1)

Queens were kept under 6 different sets of conditions, as follows:

[1] Queens emerged in an incubator at 34°C and 60% relative humidity (RH) in cages without attending workers and were provided with 30 μl of honey solution (honey/water = 1/1) in a small cup. They were 0–12 h old when haemolymph samples were taken.

[2] Queens emerged in an incubator at 34°C and 60% RH, and were transferred to Liebefelder cages with 20 potential nurse bees each (potential nurses were workers taken from a brood comb of a populous colony). The workers in each cage were fed with bee bread (pollen stores taken from a comb) and honey solution (honey/water = 1/1) ad lib. Haemolymph samples were taken 6 days after emergence of the queens.

[3] Queen cells were introduced into colonies with 6 combs (frame size: 22 × 42 cm) and approximately 8,000 workers each, from which the original queens had been removed, all the brood was sealed and all the emergency queen cells had been removed. Haemolymph samples were taken 6 days after emergence of the queens.

[4] Queens emerged in an incubator at 34°C and 60% RH; they were transferred with approximately 1,000 worker bees into Apidea mating boxes, each with a volume of 1,000 cm^3 and containing 3 frames (frame size: 10 × 10 cm) with 2 cm strips of wax foundation. The bees were fed with a mixture of powdered sugar (90%), brewers' yeast (5%) and water (5%). For 3 days the boxes were kept in a dark and cool room, then they were brought into the bee yard in the evening of the 3rd day. Each queen was confined to her Apidea mating box by a queen excluder screen at the entrance, so that the queens were not able to fly and mate, while the workers could pass through the screen and forage normally. Haemolymph samples were taken in parallel to [5].

[5] Queens were treated as in number [4], but there were no queen excluder screens mounted at the entrances of the Apidea mating boxes, so that the queens were able to perform mating flights. From the 5th day onwards the colonies were inspected for eggs. When there were eggs present on the 10th day, but still no larvae visible, haemolymph samples were taken.

[6] Laying queens, all more than 1 month old, were taken from hives that each had about 8,000 bees on 6 combs (frame size: 22 × 42 cm) and had brood in all stages.

Haemolymph samples were taken from 6–11 individuals in each of the conditions. Every queen was held separately from other queens, as queens that contact each other usually fight until death.

Analysis of haemolymph samples

To sample haemolymph, the queen was pierced with an insect pin dorsolaterally on the abdomen between the second and third segment. An amount of 2–5 μl of haemolymph was taken with a calibrated 5 μl glass capillary tube and mixed with 100 μl of acetonitril (1 : 1 diluted with H_2O). The samples were stored at −20°C until further analysis.

Aliquots of the diluted haemolymph representing 0.25 μl of haemolymph were lyophilized and analysed for free amino acids by reversed phase HPLC after derivatization with dabsylchloride. The method has been described in detail by Crailsheim and Leonhard (1997).

Data analysis

(I) To determine the content of free amino acids in the haemolymph of normally developed queens of different ages, queens from four different groups were sampled: freshly emerged queens (0–12 h old) [group 1], unmated queens (6 d old) from colonies [group 3], mated queens laying their first eggs in mating boxes (10 d old) [group 5], and queens laying normally in colonies (>30 d old) [group 6].

(II) To determine the influence of the social environment of young queens on amino acid content, two groups of 6-day-old queens were compared: queens kept with only 20 worker bees each in small boxes [group 2] vs. queens kept with about 8,000 workers in 6-frame-colonies [group 3].

(III) To investigate the influence of mating, mated and unmated queens of the same age, kept in mating boxes, were compared (group [4] vs. group [5]).

Statistics

A one-way analysis of variance was performed on the results (free amino acid content) at different ages.

To compare pairs of results, T-Tests were performed. To compare groups pairwise, an all pairwise multiple comparison procedure (Tukey Test) was applied when data were distributed normally. If the normality test failed, an analysis of variance on ranks (Dunn's method) was performed. Statistical significance was stated when $p < 0.05$.

Results

(I) Changes with age in the content of free amino acids in normally developed queens

Immediately after emergence the content of free amino acids in queens is relatively high (Fig. 1, Table 2). In the first phase of their lives, before mating it remains at a high level; no difference in the overall content of free amino acids was found between queens 0–12 hours old and those 6 days old (group [1] vs. group [3]). The amount of proline, the predominant free amino acid, increased from 12.28 ± 1.56 nmol/μl haemolymph in the youngest queens to 25.84 ± 1.51 nmol/μl in 6-day-old ones. This increase was counterbalanced by a decline in the amount of free essential amino acids: 0–12 h queens had 24.61 ±

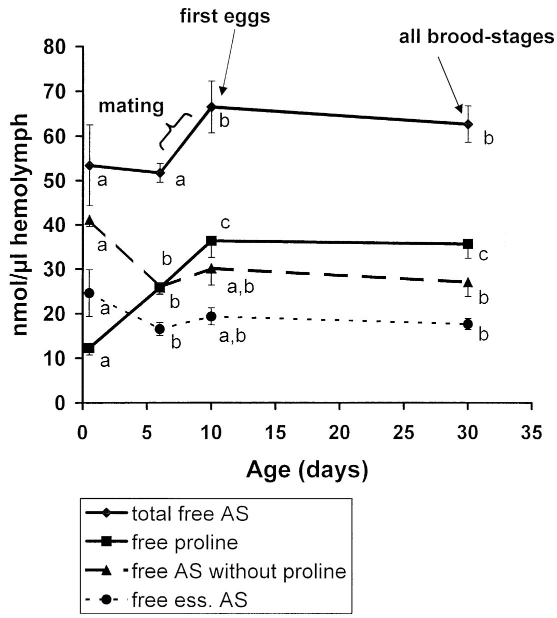

Fig. 1. Content of total free amino acids, of proline, of free essential amino acids, and of total free amino acids without proline in the haemolymph of honey bee queens aged <12 h, 6 days, 10 days and >30 days. The <12 h group had emerged in the incubator and was sampled from there. The 6-day-old queens were not mated yet, and were sampled from 6-comb colonies. The 10-day-old queens were mated and laying their first eggs in Apidea mating boxes, and the >30-day-old queens were laying eggs normally in 6-comb-colonies. Means and standard deviations are shown; standard deviations are occasionally given only in one direction to prevent overlapping; different letters indicate significant differences between age groups for a substance (all pairwise multiple comparison test, $p < 0.05$)

5.28 nmol/μl, and 6 d queens had 16.52 ± 1.47 nmol/μl, which was significantly less ($p < 0.05$) (Table 2).

After mating, with the onset of egg laying, a significant increase in the total content of free amino acids can be seen (Fig. 1). In these 10-day-old queens a maximum was reached with 66.52 ± 5.77 nmol/μl haemolymph, and that level was maintained in older individuals (>30 d) that were laying eggs in bigger colonies. The highest levels in queens (66.52 ± 5.77 nmol/μl) were lower than those in drones (90.5 ± 10.98 nmol/μl) (highest concentration is reached at the age of 5 days, from Leonhard and Crailsheim, 1999) and higher than those in workers (25.32 ± 7.42 nmol/μl) (highest concentration is reached at the age of 5 days, from Crailsheim and Leonhard, 1997).

Figure 2 shows how queens, drones and workers differ in the concentration of each amino acid. The predominant amino acid in queens, as in workers and drones, is proline, which accounts for 54.7% (36.36 ± 3.75 nmol/μl) of the total content of free amino acids

Table 2. Mean concentrations of free amino acids in the haemolymph of queens of different ages kept under similar conditions, or of queens of the same age kept under different conditions (n = 6–11). The ages are given in brackets. The sum of all amino acids (*sum*) and the sum of essential amino acids (*ess. AS*) are shown. Asterisks indicate amino acids reported essential for workers (De Groot, 1953). Data from Wang and Moeller (1970) were recalculated

Amino acids nmol/μL	Not mated								Mated				Wang & Moeller	
	[1] freshly emerged from incubator with no workers (<12 h) n = 11		[2] virgin from incubator with 20 workers (6 d) n = 10		[3] virgin from 6-frame-colony with 8,000 workers (6 d) n = 6		[4] virgin from mating box with 1,000 workers (10 d) n = 7		[5] freshly mated from mating box with 1,000 workers (10 d) n = 8		[6] normally laying from 6-frame-colony with 8,000 workers (>30 d) n = 7		(1970)	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	(10 d)	(20 d)
Asp	0,55	0,22	0,19	0,05	0,24	0,05	0,16	0,06	0,52	0,10	0,75	0,05	2,39	0,00
Glu	2,60	0,53	0,42	0,15	0,46	0,07	0,40	0,09	0,22	0,11	0,28	0,09	2,60	1,40
Ser	3,21	0,72	2,31	0,34	2,81	0,48	1,58	0,23	2,28	0,39	1,31	0,16	1,07	2,27
Thr*	0,66	0,28	0,42	0,06	0,54	0,09	0,51	0,18	1,28	0,17	1,19	0,12	0,67	0,96
Arg*	2,20	0,69	2,00	0,85	1,24	0,76	0,72	0,14	1,23	0,24	1,04	0,14	1,10	2,19
Gly	2,98	0,95	1,60	0,36	1,88	0,33	2,03	0,53	3,51	0,40	3,06	0,19	1,86	2,80
Ala	2,29	1,29	4,88	0,79	3,69	1,77	1,51	0,44	3,44	0,44	3,32	0,29	1,47	4,20
Pro	12,28	1,56	21,91	1,89	25,84	1,51	23,10	6,03	36,36	3,75	35,64	3,21	28,08	22,95
Val*	10,05	2,21	9,61	1,18	10,49	1,22	9,18	2,23	9,83	1,13	9,06	0,59	0,93	0,67
Met*	0,10	0,09	0,02	0,04	0,11	0,06	0,10	0,05	0,19	0,13	0,18	0,04	0,27	0,64
IIe*	0,96	0,19	0,56	0,05	0,58	0,05	0,54	0,03	0,99	0,16	0,84	0,14	0,62	1,04
Leu*	1,64	0,47	0,52	0,16	0,47	0,05	0,45	0,09	1,38	0,31	1,24	0,11	0,40	0,80
Phe*	0,84	0,38	0,04	0,04	0,00	0,00	0,03	0,05	0,10	0,02	0,11	0,01	0,54	1,00
Try*	3,18	0,70	0,62	0,16	0,83	0,06	0,53	0,07	1,14	0,15	0,82	0,10	0,00	0,00
Cys	0,17	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Lys*	3,30	1,34	0,87	0,12	1,72	0,40	1,38	0,59	2,08	0,30	2,23	0,44	2,12	0,50
His*	1,67	0,46	0,48	0,05	0,53	0,09	0,50	0,11	1,15	0,12	0,95	0,10	0,54	1,26
Tyr	4,74	1,83	0,16	0,14	0,31	0,27	0,06	0,06	0,83	0,35	0,70	0,28	0,54	1,24
ess AS	24,61	5,28	15,14	1,62	16,52	1,47	13,95	3,07	19,37	1,93	17,64	1,24	7,19	9,06
Sum	53,43	9,12	46,60	2,96	51,74	2,13	42,79	10,07	66,52	5,77	62,70	4,07	45,20	43,92

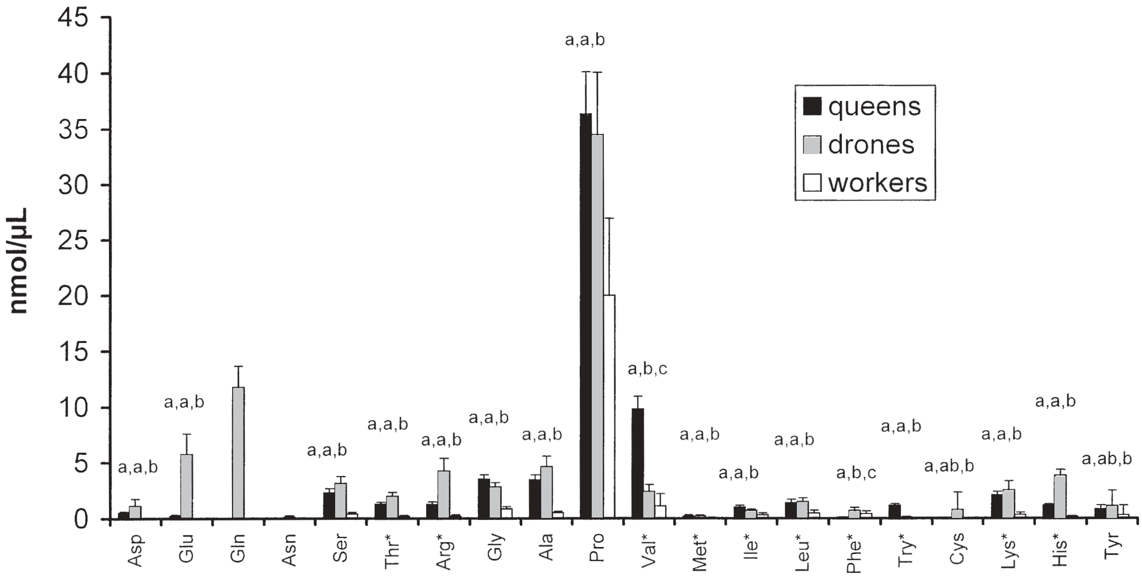

Fig. 2. Comparisons of the free amino acid content in the haemolymph of 10-day-old egg-laying queens (n = 8), 5-day-old drones (n = 23) and 5-day-old workers (n = 47). Means and standard deviations are shown; different letters above columns indicate significant differences for that amino acid (all pairwise multiple comparison test, $p < 0.05$). Glutamine and asparagin were present in drones only. The data given for drones and workers are cited from Leonhard and Crailsheim (1999) and Crailsheim and Leonhard (1997)

in 10-day-old egg-laying queens. Ten-day-old queens differ from drones and workers in having valine as the second most frequent amino acid, with a concentration of 9.83 ± 1.13 nmol/μl, amounting to 15% of the total content of amino acids. The amount of valine is about 4 times more than found in drones and 9 times more than in workers. While queens (10 d) and drones (5 d) do not differ in the haemolymph concentrations of most of the free amino acids, workers (5 d) have significantly lower concentrations of all free amino acids in the haemolymph.

The amount of essential amino acids slightly falls after emergence, then stabilises at this level in laying queens. When the amount of all free amino acids without proline is considered, we get the same picture as for the essential amino acids (Fig. 1). From this we conclude that the increase in the content of total amino acids in laying queens is driven primarily by proline and not by the other amino acids.

(II) The influence of the queen's social environment on the content of free amino acids

Six-day-old queens from the incubator, where they were kept with 20 companion workers (group [2]), were compared to 6-day-old normally emerged queens kept in 6-frame colonies with about 8,000 workers (group [3]). The data for individual amino acids are given in Table 2. The queens from the colonies had slightly higher amounts of total amino acids and of proline, but did not differ in the amount of essential amino acids (Fig. 3). Again we conclude that the difference in total amino acids is caused by the different amount of proline in the two groups and not by the other amino acids.

(III) The influence of egg production on the content of free amino acids

A more pronounced difference can be seen when 10-day-old mated, laying queens are compared with unmated queens of the same age kept under the same conditions (group [4] vs. group [5]). The laying queens had significantly higher levels in total amino acids, proline and essential amino acids (Fig. 4). Also the two groups differed in the total amount of free amino acids without proline ($p < 0.001$). Interestingly, there was no difference in the relative amount of proline: 54.0% of the total free amino acids in nonlaying queens and 54.7% in laying queens.

We found that the functional status of queens (i.e., laying eggs vs. nonlaying) had a greater impact on the free amino acid content than the social environment (compare Figs. 3 and 4).

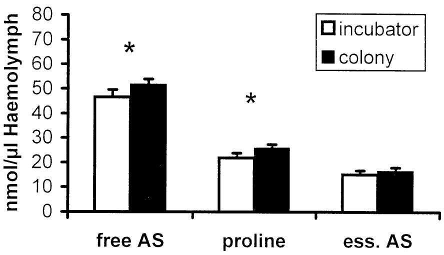

Fig. 3. Influence of social environment on the amount of free amino acids in the haemolymph of 6-day-old honey bee queens. Queens kept in an incubator with 20 companion workers were compared to queens who emerged normally in big 6-comb colonies with about 8,000 workers each. Means and standard deviations are shown. Stars between columns indicate a significant difference (T-Test, $p < 0.05$, $n = 6–7$)

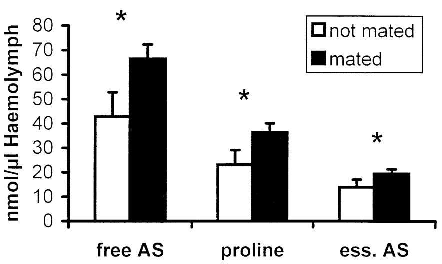

Fig. 4. Influence of the functional status of 10-day-old queens on the contents of free amino acids. Mated, egg-laying queens are compared to unmated, nonlaying queens. All queens were kept in Apidea mating boxes. Means and standard deviations are shown. Stars between columns indicate a significant difference (T-Test, $p < 0.05$, $n = 7–8$)

Discussion

Honey bee queens, like the queens of other eusocial insects, perform their main task, i.e. the production of eggs, for long periods during their lives. The amount of free amino acids in the queen's haemolymph increases to a maximum level with the beginning of egg laying and this high level is apparently maintained in laying queens. Whether honey bee queens maintain this level during wintertime, when egg production ceases, has not been investigated. Interestingly, a similar trend has been described for the amounts of queen pheromones in the mandibular glands: low in virgin queens and high in mated egg-laying queens (Pankiw et al., 1996; Engels et al., 1997).

Our data show that queens reach a level of total free amino acids more than twice that of workers (compare Fig. 1 with data from Crailsheim and Leonhard, 1997). This might reflect a higher synthesis activity – for instance for egg proteins – in queens than in workers. In relation to drones, which reach a maximum amount of free amino acids on the 5th day of their life (Leonhard and Crailsheim, 1999), the maximum amount found in laying queens is smaller, about two-thirds that of drones. This difference is caused primarily by the amino acids glutamine and asparagine, which were present in drones but not found in queens (see Fig. 2, Table 2). For most of the other amino acids no differences between the highest level in queens and drones were found.

In various other insects quantitiative differences in the free amino acid content between females and males have been reported, as for instance in mosquitoes *Culex pipiens* (Chen, 1958) or in the stable fly *Stomoxys calcitrans* (Chen and Wagner, 1992). In *Drosophila melanogaster* the concentration of amino acids is consistently higher in females than in males. Chen (1972, 1985) attributes this to the production of eggs, as the increase of amino acids in virgin females is clearly smaller than in mated ones of the same age.

In workers and drones the amount of free amino acids clearly depends on age, and the progression with age is quite similar in both groups, with an increase after emergence and a maximum at about the fifth day of life, followed by decreasing levels in older animals. In queens the total amount of free amino acids does not primarily increase with age (investigated in 0–12 h, 6-day and 10-day-old virgin queens) but with functional status (egg-laying). Even in older queens (>30 d) the amount remains on a high level (Fig. 1), which is strikingly different to what was found for workers and drones. Although queens and workers are both female, their task performance and physiology differ fundamentally. While workers perform various tasks to maintain the nest and care for the brood, the queens only produce eggs. And while workers, as they age, proceed from a sequence of within-hive duties to foraging flights, thereby changing their occupation dramatically, the queens stay in the nest and do not change to other tasks.

The high level of free amino acids in laying queens is due to the relative high content of proline, which is the predominant amino acid in workers and drones as well (Crailsheim and Leonhard, 1997; Leonhard and Crailsheim, 1999). Our results agree with those of Wang and Moeller (1970), who investigated 10- and 20-day-old queens from colonies (see Table 1). They also found a high amount of free proline in the haemolymph of queen bees. Generally the amounts they report are more similar to those we found in virgin queens than to those we found in queens laying eggs. Unfortunately Wang and Moeller did not state whether their queens were laying eggs or not. Their results differed from ours in the relatively small amounts of valine they found. In our study valine was the second most frequent amino acid after proline. Interestingly, the concentration of this amino acid was very constant at about 10 mM independent of the queen's age or the conditions in which she was kept. One might attribute the concentration of valine to the composition of the proteinaceous food (jelly) fed to queens by workers. In the locust *Locusta migratoria*, the concentration of various amino acids depends partly on the quality of food the insect feeds on (Zanotto et al., 1996). However, valine is not a prominent constituent of royal jelly (Howe et al., 1985).

Proline is considered to play a role as fuel for flight metabolism of various insects, like the African fruit beetle *Pachnoda sinuata* (Zebe and Gäde, 1993; Auerswald et al., 1998), the tsetse fly *Glossina pallidipes* (Hargrove, 1976; Bursell, 1963), the blow fly *Phormia regina* (Sacktor and Childress, 1967), the stable fly *Stomoxys calcitrans* (Bursell, 1975), the cockroach *Periplaneta americana* (Crabtree and Newsholme, 1970) and many more. This amino acid has also been discussed as involved in flight metabolism in honey bee workers (Micheu et al., 2000). Although its contribution to the overall energy turnover is, in relation to glucose, quite small, proline might have a starter function to provide some intermediates of the tricarboxylic acid cycle in the first period of flight (Crabtree and Newsholme, 1970). Although queens do not perform flights when they are inside the colony, being capable of flight enables a queen to abscond with the rest of the colony when their nest is destroyed, which is crucial for the survival of the colony. On the other hand, our results show that queens at the age of main flight activity (6 days old) have significantly lower proline concentrations in their haemolymph than queens staying exclusively in the nest (10-day- and >30-day-old egg-laying queens), which means that high proline levels in queens are not necessarily associated with flight performance.

As mentioned above, the total concentration of amino acids increases with the queen's age in a similar way to the amount of queen pheromones, extracted from the mandibular glands. Since proline accounts for the elevated total amino acid concentration in the haemolymph we might speculate that proline is involved in the synthesis of queen pheromones. But, although in various insects pheromones are synthesised by simple transformation of amino acids (Tillman et al., 1999), this does not seem to be the case in honey bee queens. Here the main compounds of the pheromones, the 9-hydroxy-(E)2-decenoic acid and 9-keto-(E)2-decenoic acid, are synthesised from stearic acid (Plettner et al., 1996). Nevertheless proline could act as a precursor for this fatty acid, but as in honey bee nutrition carbohydrates are present in high quantities the pathway via proline is questionable.

Proline also seems to work, together with other substances, as a cryoprotectant (Lee, 1991). In cold-acclimatised granary weevils, *Sitophilus granarius* (L.), and rusty grain beetles, *Cryptolestes ferrugineus* (Stephens), elevated levels of proline, but also some other free amino acids, were shown for whole body extracts (Fields et al., 1998). But as honey bees heat themselves actively when temperatures are low (Stabentheiner et al., 1995; Kovac and Schmaranzer, 1996), this function is not very likely to play a significant role in honey bees.

Generally, in honey bees the social environment a worker or queen lives in has a significant impact on the development of the individual. A queen usually does not provide herself with food, but is fed by workers, which form a court around her. The court of a stationary queen consists, on average, of about 10 workers (Free et al., 1992; Brodschneider and Crailsheim, 2001), which are exchanged quickly (Allen 1960). It seems clear that low numbers of workers (20), in the artificial environment of a cage, are not able to provide food for the queen as well as high numbers of workers (8,000) in a hive can. The consequence of this malnutrition is reflected in a lower proline concentration in the haemolymph, as shown in the present investigation for 6-day-old individuals. Nevertheless, for this age group no impact on the amount of essential amino acids was found. This is quite astonishing, because the queens kept with just 20 nurses were not only subjected to a reduced level of maintenance, but were also kept in small cages in an incubator far from their natural environment of a normal hive. We suppose that, if the queens were kept for a longer period under these restricted conditions, the differences in amino acid content between normally kept animals and the caged ones would increase.

In the present investigation we showed that in queens the relatively high concentration of free amino acids in the haemolymph depends on age, on egg-laying status and on social environment. It seems that, in contrast to drones and workers and also other insects, there are no big alterations with age after egg-laying begins normally. Whether this is also the case during times of the year with reduced egg production, or in very old queens still has to be investigated.

Acknowledgements

We are grateful to Richard Nowogrodzki for improving the English of the manuscript. This project was supported by the Austrian Science Foundation.

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