Journal of Apicultural Research 45(4): 173–179 (2006)

ORIGINAL RESEARCH ARTICLE

Eggs from *Apis mellifera caucasica* laying workers are larger than from queens

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Received 11 April 2006, accepted subject to revision 23 July 2006, accepted for publication 6 September 2006.

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Summary

The size (e.g. length, width and volume) and the weight of eggs laid by *A. mellifera caucasica* queens (Qs) and laying workers (LWs) in both worker cells (WC) and in drone cells (DC) were compared. All measurements were taken at 3 different embryonic developmental periods (at 0–6 h, 18–24 h and 54–60 h after oviposition). The results showed that the size and weight of fertilized (in WC) and unfertilized (in DC) eggs laid by queens did not differ significantly. However, eggs from laying workers were 2.8% longer and 10.7% wider, they had as much as 22.2% larger volume and they were 22.3% heavier compared to eggs laid by queens. Thus, eggs from *A. m. caucasica* laying workers were larger and heavier than from queens. Significant decrease of size and weight occurred during embryonic development. Shortly before hatching, eggs from queens and workers lost 5.4% and 6.4% of their length, 8.9% and 9.1% of their width, 16.4% and 16.9% of their volume and 28.1% and 24.2% of their weight, respectively. Eggs laid by queens lost 3.9% more weight than eggs laid by workers, and shortly before hatching their density (ratio of weight/volume) was 86.3% of the initial in eggs from queens and 91.7% in eggs from laying workers. The decrease of the density indicates that the ratio of the lighter fat must have increased in relationship to the other egg components. The larger eggs from laying workers in comparison to eggs from queens may be the result of lower number of ovarioles in workers (2–12) than in queens (160–180).

Keywords: honey bee, *Apis mellifera caucasica,* laying workers, queen, egg weight, egg size, drone comb cells

Introduction

The variability in size of honey bee eggs has been investigated by several authors. The honey bee egg size and weight are highly variable under different conditions. The weight of queen-laid eggs varies depending on the race and age of queen, laying rate of queen and season (Bilash *et al.,* 1984). The length of eggs laid by different queens of the same race differs significantly (Woyke, 1998). Even the length and width of eggs produced by the same queen fluctuates throughout the season (Hejtmanek, 1961; Jordan, 1961; Henderson, 1992). Furthermore, size and weight of honey bee eggs change during embryonic developmental period due to metabolic process, and older eggs are smaller than younger ones in the European honey bees (Woyke, 1998) as well as in the Asian honey bees species (Woyke, 1993).

Also the size of queen-laid and worker-laid eggs of the same species differs. Winston (1987) writes that queen-laid eggs are larger than worker-laid eggs in *Apis mellifera* colonies, however, he does not present any data. Woyke (1994) found that eggs from queens are longer but narrower compared to eggs from laying workers in the European bee *A. mellifera,* as well as, in the Asian bee *Apis cerana* (Woyke *et al.,* 2003) while, Woyke and Wongsiri (1992) reported that eggs from *Apis florea* laying workers are longer and thicker compared to eggs from queens. According to Mackasmiel and Fell (2000) fertilized eggs from *Apis mellifera* queens are heavier than unfertilized eggs from laying workers.

In addition to differences in size and weight between queen-laid and worker-laid eggs, also physical and chemical differences of the surface structure have been reported between queen-laid and worker-laid eggs (Katzav-Gozansky *et al.,* 2003). Pheromonal differences have also been suggested (Ratnieks 1995), but nothing has been found till now.

According to Borodacheva (1973) queens reared from heavier eggs were heavier and had more ovarioles than those reared from lighter eggs. Increase of egg weight by 0.01 mg found to be in correlation with an increase by 2.6 ovarioles. It might be possible that drones from larger eggs laid by workers and reared under the same conditions as queen-derived drones will also show some beneficial traits. Therefore, it is important to know, whether eggs laid by laying workers are larger or not than those laid by queens in order, later on, to examine the significance of this difference.

The aim of this study was to compare the size and weight of eggs laid by queens or laying workers in both, worker (WC) and drone cells (DC) in *Apis mellifera caucasica* colonies. Changes were recorded during 3 embryonic developmental periods.

Materials and Methods

The experiment was conducted during May and June 2004 at Ankara University, Turkey, using six colonies of *Apis mellifera caucasica* under good foraging conditions. The experimental colonies were headed by naturally mated sister queens of the same age, they were full sized in one standard Langstroth hive body and they were provided with similar amounts of stores. In order to obtain worker-laid eggs of known age, three of the six colonies were induced to become laying worker colonies by removing their queens and combs with unsealed brood; the other three ones were maintained as queenright colonies (Qs). As soon as laying workers (LWs) appeared, a comb with worker cells (WC) and a comb with drone cells (DC) were introduced into the middle of the brood area of each colony for 6 hours at 1 or 2 day intervals. The queen in each of the three colonies was confined on a single DC and WC comb using a queen excluder push-in cage (25 × 15 cm) to ensure sufficient eggs of approximately the same age.

The queens remained confined on the combs for 6 hours. At the end of the six hours egg-laying period, the combs having sufficient eggs for egg weight and size measurements at 3 different embryonic developmental periods (at 0–6 h, 18–24 h and 54–60 h) were removed from the hives and the queens were released. After the 1st egg-sampling period, at 0-6 h of embryonic development, the combs were returned into the hives for subsequent sampling at 18–24 h and 54–60 h of embryonic development. When returning the combs, the areas containing the eggs were protected with wire mesh push-in cage (25 ×15 cm) in LWs colonies and with queen excluder push-in cage in Qs colonies, in order to prevent LWs and Qs from laying additional eggs on those areas.

The eggs were individually collected using a dental hook (Collins, 2002). Thirty eggs were collected from cells of each type (WC or DC) and from each Qs and LWs colony at every embryonic developmental period. In total, 1080 eggs (6 colonies × 2 cell types × 3 embryonic developmental periods × 30 eggs) were collected. The eggs were laid on a 1 cm piece of Scotch tape. A batch of thirty eggs fixed on the Scotch tape was weighed on an electronic balance (Sartorius BP-121S) to the nearest 0.1 mg, since it was not possible to determine individual weights of eggs due to the sensitivity range of the electronic balance. Then the results were divided by 30 to get the mean weight of 1 egg. Thus, the arithmetic mean and the s.e. were calculated based on the measurements of the 3 batches of eggs.

For measurements of length and width, the images of 25 eggs from each batch were taken with a digital camera (Leica DC 100) attached to a stereomicroscope. Egg length and width were measured from the image with a computer-based measuring software (Inspector-Matrox). Egg width was measured at the mid point of the egg length (Woyke, 1998), slenderness index of eggs was calculated as the ratio of egg length to width and density as the ratio of egg weight to the volume.

A three-way Analysis of Variance (ANOVA) was applied using cell type (WC and DC), colony type (Qs and LWs), and period (0–6 h, 18–24 h and 54–60 h) as fixed effect factors. Duncan's multiple comparisons test was also used to compare means and to detect significant differences between them ($P < 0.05$). Coefficients of variation (CV %) were also calculated. All statistical analyses were performed using SPSS v. 11.5 for Windows software package.

Results

Egg length

Eggs from workers were 1.530 mm long and 2.8% significantly longer than from queens (1.488 mm long) (main effect of colony type, $F = 83.837$, $df = 1$, $P = 0.000$). Furthermore, this difference was observed at every measurement period: worker-laid eggs were 2.9%, 3.8% and 1.8% significantly longer than queen-laid eggs at 0–6 h, 18-24 h and 54–60 h, respectively (Table 1).

The mean length of fertilized and unfertilized eggs laid by queens or laid by workers into WC or DC did not differ significantly (Table 1) (main effect of cell type: $F = 0.338$, $df = 1$, $P = 0.561$). However, shortly after oviposition (0-6 h), the unfertilized eggs laid by queens into DC were significantly longer (1.541 mm) than fertilized eggs laid into WC (1.522 mm) as the two-way interaction showed (cell type × period: $F = 3.326$, $df = 2$, $P = 0.036$), while no such differences were detected between older fertilized and unfertilized eggs in WC or DC (Table 1). At the contrary, in LWs colonies, only the oldest eggs (54-60 h) were longer in WC (1.487 mm) than in DC (1.462 mm), (two-way interaction, colony type × period: $F = 3.836$, $df = 2$, $P = 0.022$).

The length of eggs deposited by both queens and workers decreased significantly during embryonic development (main effect of period: $F=134$, $df = 2$, $P = 0.000$). Shortly before hatching (at 54–60 h) the eggs from Qs and LWs were 5.4% and 6.4% shorter than at oviposition, respectively. Worker-laid eggs lost 1.0% more length than queen-laid eggs did. This indicates that length changes during embryonic development were different in eggs from queens compared to eggs from laying workers (two-way interaction, colony type × period: $F = 3.836$, $df = 2$, $P = 0.022$).

The variation coefficient of length was 1.64 times higher for eggs from workers (CV % = 6.23) than from queens (CV % = 3.81) (Table 1).

Egg width

Eggs from workers were 0.382 mm wide (Table 1) and 10.7% significantly wider compared to eggs from queens (0.345 mm wide) (main effect of colony type: $F = 617.402$, $df = 1$, $P = 0.000$). Furthermore, eggs from workers were significantly wider than eggs from queens at every measurement period: worker-laid eggs were 10.6%, 11.1% and 10.4% wider than queen-laid eggs at 0–6 h, 18–24 h and 54–60 h, respectively.

The mean width of fertilized and of unfertilized eggs laid by queens did not differ significantly. However eggs deposited by workers were wider in WC (0.386 mm) than in DC (0.378 mm) as the two-way interaction showed (cell type × colony type:

F = 12.600, df = 1, P = 0.000). Also, the oldest eggs (54–60 h) deposited by LWs in WC (0.369 mm) were significantly wider than those deposited in DC (0.352 mm) (as the three-way interaction showed, cell type × colony type × period: F = 4.2170, df = 2, P = 0.014).

The width of eggs laid by Qs and LWs decreased significantly during embryonic development (main effect of period: F = 192.021, df = 2, P = 0.000). The decrease was 8.9% in eggs laid by queens and 9.1% in eggs laid by workers, at the age of 54–60 h.

The variation coefficient of width was 1.38 times higher for eggs from workers (CV % = 8.35) than from queens (CV % = 6.05).

Slenderness of eggs

The slenderness index (ratio of egg length to width) of eggs laid by queens (mean 4.244) was higher as compared to that index of eggs from workers (mean 3.936) (table 1). Eggs from queens were more slender than eggs from workers at every measurement period (main effect of colony type: F = 292, df = 1, P = 0.000).

Fertilized eggs deposited by queens (4.268) were more slender than the unfertilized ones (4.221), however worker-laid eggs in WC or DC did not differ significantly (as the two-way interaction showed, colony type × cell type: F = 6.542, df = 1, P = 0.011).

Slenderness of eggs from Qs and LWs increased significantly during embryonic development (main effect of period: F = 158.999, df = 2, P = 0.000). This indicates that during the development period, the length of eggs decreased, proportionally less compared to the width. However, the oldest eggs laid by workers were significantly slenderer (higher SI) in DC (4.171) than in WC (4.046) (as the three-way interaction, cell type × colony type × period showed: F = 4.338, df = 2, P = 0.013), while queen-laid eggs were similar in both types of cells.

The variation of slenderness was 1.36 times higher for eggs from workers (CV % = 8.87) than from queens (CV % = 6.54).

Volume of eggs

The volume of eggs laid by workers (0.077 mm^3) was 22.2% significantly larger compared to eggs laid by queens (0.063 mm^3) (main effect of colony type: F = 611.350, df = 1, P = 0.000). This difference was observed at every measurement period: worker-laid eggs were 22.1%, 23.1% and 23.2% significantly larger than queen-laid eggs at 0–6 h, 18–24 h and 54–60 h, respectively (Table 2).

No significant difference was found between volumes of fertilized (0.062 mm^3) and unfertilized eggs (0.063 mm^3) laid by queens, but eggs laid by workers were larger in WC (0.079 mm^3), than in DC (0.075), (two-way interaction, cell type × colony type: F = 12.196, df = 1, P = 0.01)

The volume of eggs decreased during embryonic development. Shortly before hatching (54–60 h), eggs laid by queens and workers were 17.6% and 16.9% respectively significantly smaller than after deposition (main effect of period: F = 178.252, df = 2, P = 0.000). Thus, in percentage, the decrease of the volume was similar in eggs laid by queens and workers. However, the older worker-laid eggs were larger in WC than in DC (three-way interaction, cell type × colony type × period: F = 3.702, df = 2, P = 0.025), while queen-laid eggs were similar in both types of cells. The variation of volume was 1.38 times higher for eggs from workers (CV % = 16.51) than from queens (CV % = 11.97). Figure 1 shows the volume range of eggs laid by queens and workers. The overall volume range of worker-laid eggs (0.107 – 0.054 = 0.053 mm^3) was 1.8 times higher than the overall volume range of queen-laid eggs (0.08 - 0.05 = 0.03 mm^3). The middle 25% to 75% interquartile volume span of eggs from all workers (0.087 – 0.072 = 0.015 mm^3) was 2.5 times higher than that interquartile span of eggs from all queens (0.006 mm^3). The volume of the smallest eggs from workers overlapped with the volume of the largest eggs from queens. However, the lower quartiles of the volume of eggs from workers in particular colonies were mostly larger than the upper quartiles of the volume of eggs from queens. Also, the overall shows that the lower quartile of the volume of eggs from workers (0.072 mm^3) was higher than the upper quartile of the volume of eggs from queens (0.069 mm^3). Thus, most of the eggs from workers were larger than eggs from queens.

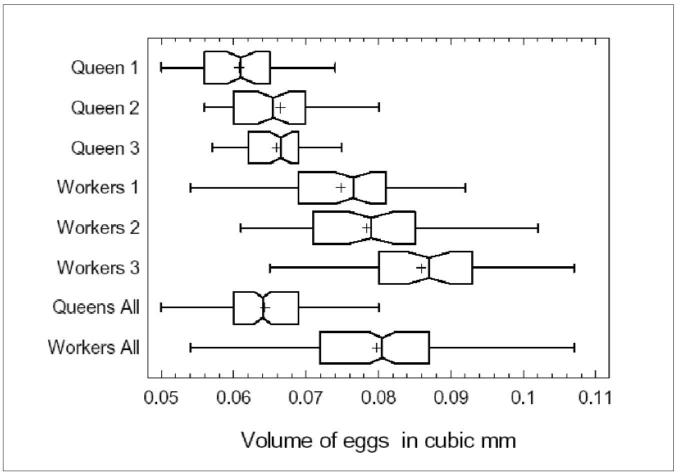

Fig. 1. Distribution of 0, 25, 50, 75 and 100 percentile of the volume of 18–24 h old eggs laid by laying workers in each of 3 colonies and by each of 3 queens (per 50 eggs); the overalls for all eggs from workers and from queens (per 150 eggs) are also illustrated. The cross in a notch indicates the location of the sample mean. The notch indicates 95% confidence level for the median, which is represented by the vertical line (a statistically significant difference between any pair of medians exists, when their notches do not overlap).

Weight of eggs

Worker-laid eggs (140.1 μg) were 22.3% significantly heavier than queen-laid eggs (114.6 μg) (main effect of colony type: F = 87.34, df = 1, P = 0.000). Similar difference was recorded at every measurement period: worker-laid eggs were 20.2%, 21.0% and 26.8% significantly heavier than queen-laid eggs at 0–6 h, 18–24 h and 54–60 h, respectively (table 2). No significant differences were found between eggs in WC or DC laid by either queens or workers in respect of egg weight (Table 2), (main effect of cell type: F = 0.000, df = 1, P = 0.998).

The weight of eggs from both Qs and LWs decreased significantly, during embryonic development (main effect of period, F = 64.01, df = 2, P = 0.000). Shortly before hatching

(54–60 h), queen-laid and worker-laid eggs had 28.1% and 24.2% less of their initial weight (0–6 h), respectively. Queen-laid eggs lost 3.9% more weight than worker-laid eggs. Thus, although eggs from laying workers were heavier, they lost less weight during embryonic development period than eggs from queens.

Variation of weight was similar for eggs laid by queens and workers (CV % = 14.42 and CV % = 13.10, respectively).

Density of eggs

The mean density (ratio of weight to volume) of all eggs laid by queens or workers was identical (1.83 mg/mm^3) (main effect of colony type, F = 0.001, df = 1, P = 0.972). The density of fertilized and unfertilized eggs laid by queens was also identical (1.83 mg/mm^3), and the density of eggs laid by workers in WC and DC did not differ significantly (Table 2) (main effect of cell type: F = 0.444, df = 1, P = 0.512).

The density of eggs from queens and workers decreased significantly during embryonic development (main effect of period, F = 10.680, df = 2, P = 0.000). However, shortly before hatching, fertilized and unfertilized eggs laid by queens had 11.3% and 15.1% less of their initial density respectively and the mean loss was 13.7% (Table 2, %60h/6h). At the same period, eggs laid by workers in worker and drone cells lost 9.3% and 6.8% of their density respectively, and the mean loss was 8.3%. Thus, eggs laid by queens lost 5.4% significantly more density than eggs laid by workers.

Discussion

After we investigated six traits of eggs laid by *A. m. caucasica* queens, we did not find significant differences between fertilized and unfertilized eggs. Till now only two traits, the length and the width have been described as not different between fertilized and unfertilized eggs laid by *A. m. ligustica* queens (Henderson, 1992).

The mean length (1.488 mm), width (0.345 mm) and volume (0.063 mm^3) of *A. m. caucasica* fertilized eggs found here were within the variations of mean length (1.492 mm), width (0.350 mm) and volume (0.096 mm^3) of eggs from queens of three other *A. mellifera* subspecies: *A. m. ligustica, A. m. carnica* and *A. m. caucasica* (Woyke,1998). The range of the weight of eggs laid by *A. m. caucasica* queens (94.4 to 133.3 μg) found was also within the range reported for four *A. mellifera* subspecies (*A. m. ligustica, A. m. mellifera, A. m. caucasica* and *A. m. carnica*) by Bilash *et al.,* (1984) and reported for *A. m. mellifera* (mean 133 μg) by Woyke (1998). Thus, both, the size and weight of eggs laid by *A. m. caucasica* queens presented here were within the ranges of both traits recorded for other *A. mellifera* subspecies. Concerning the differences between eggs laid by laying workers and queens, we found that *A. m caucasica* eggs from workers were 2.8% longer (1.530 versus 1.488 mm), 10.7% wider (0.382 versus 0.345 mm), and 22.2% larger (0.077 versus 0.063 mm^3) than eggs from queens. However, Woyke (1994) showed that eggs from *A. m. mellifera* laying workers were 2.5% shorter (1.579 versus 1.620 mm) but 3.3% wider (0.378 versus 0.366 mm) than eggs from queens. Similarly, eggs from the Asian *Apis cerana* laying workers were 5.5% shorter (1.688 versus 1.788 mm), but 13.7% wider (0.441 versus 0.388 mm) than eggs from queens (Woyke *et al.,* 2003). However, eggs from *Apis florea* laying workers were 8.3% longer (1.703 mm versus 1.572 mm) and 17.5% wider (0.436 mm versus 0.371 mm) than eggs from queens (Woyke and Wongsiri, 1992). Interestingly, the last relationship is similar to our results concerning *Apis mellifera caucasica* colonies.

We found that eggs from laying workers of *A. m. caucasica* were 22.3% heavier (140.1 versus 114.6 μg) than eggs from queens. However, Mackasmiel and Fell (2000) reported that unfertilized *A. m. ligustica* eggs from laying workers were 18.0% lighter (108.0 μg) than fertilized eggs from queens (133.0 μg), a result which comes in contradiction with our findings. The size of eggs reported for other *A. mellifera* subspecies or other *Apis* species do not suggest that eggs from laying workers are lighter than from queens. Therefore, further investigations are necessary.

We found that the span of the middle 25% to 75% interquartiles of the sizes of *A. m. caucasica* eggs was higher in eggs from workers than from queens. The values of middle interquartiles were higher in eggs from workers than from queens and did not overlap. Woyke (1994) showed that in *A. m. mellifera,* the span of the middle 25% to 75% interquartile of the sizes was also higher in eggs from workers than from queens, similarly to our findings. However, the values of the middle interquartiles of the sizes of eggs from queens were within the span of those interquartiles of eggs from workers. Thus, the size difference between the eggs laid by workers and the ones laid by queens is larger in *A. m. caucasica* than in any *A. mellifera* subspecies examined till now.

Eggs of European and Asian honey bees decreased in respect of size and weight during embryonic development (Woyke, 1993, 1998). Eggs from *A. m. mellifera* queens lost 7%, 8%, and 35% of their initial length, width, and weight, respectively. Similar phenomenon was also observed in this study. However, this is the first time to report that changes during embryonic development occur differently in eggs from Qs and LWs. *A. m. caucasica* queen-laid and worker-laid eggs lost 5.4% and 6.4% of their initial length, 8.9% and 9.1% of their initial width and 28.1% and 24.2% of their initial weight, respectively. Thus, queen-laid eggs lost 9.0% more weight than worker-laid eggs and the loss of density (mg/mm^3) was higher in eggs from queens (13.7%) than from workers (8.3%) accordingly. The loss of weight indicates that some of the eggs' components are consumed to produce energy for metabolic process of embryo development. Furthermore, the decrease of the density indicates that the ratio of one or both of the lighter components, these are water and fat, must increase in relationship to the other components such as carbohydrates and proteins.

In this study, the variability for the length, width, and volume of eggs from workers was 1.6, 1.4 and 1.4 times respectively higher than for eggs from queens. This is similar to the higher variability of length and width of eggs from *A. mellifera* laying workers (3.5 and 2.9 times higher respectively), than from *A. mellifera* queens (Woyke 1994). Similarly, in the Asian honey bee *Apis cerana* the variability for the length and width was 2.0 and 1.3 times respectively higher for eggs from laying workers than from queens (Woyke *et al.,* 2003), and in *Apis florea* it was 2.4 and 1.7 times respectively higher for eggs from laying workers (Woyke and Wongsiri, 1992). We suggest that the higher variation in worker-laid eggs occurs because eggs collected from colony headed by laying workers originate from many workers, while eggs collected from queenright colony originate just from one queen.

One would expect that eggs from LWs are smaller than from Qs, because of anatomical difference between worker and queen. However, there is not any relationship between size of eggs and body size of queens and laying workers. Although the queens of *Apis cerana* and *Apis andreniformis* are smaller than those of *Apis dorsata,* the volume of eggs from *Apis cerana* and *Apis andreniformis* queens are larger than that of eggs from *Apis dorsata* queens (Woyke *et al.,* 2003). We suggest that the dimensions and the weight of eggs from laying workers are larger than from queens because the queen has 160–180 ovarioles in one ovary (Snodgrass, 1956) and lays many eggs per day, while a laying worker has only 2–12 ovarioles and lays few eggs per day. Hejtmanek (1961) already pointed out that eggs laid in period when the queen lays many eggs are smaller than at the time when few eggs are deposited. The nutrition mechanism of eggs is probably less efficient in a laying worker than in a queen. Nevertheless, the effect per ovary unit could be more efficient for eggs of a worker bee than of a queen.

Table 1. Comparison of mean ± s.e. length, width and slenderness index of eggs deposited by queens (Qs) and laying workers (LWs). Different capital letters denote significant differences (P < 0.05) between means in a row (overalls are compared with overalls). Different small letters denote significant differences between means in a column.

	Queen-deposited eggs			**Laying worker-deposited eggs**		
	in worker cells (*n* = 75)[*]	**in drone cells** (*n* = 75)	**Overall** (*n* = 150)	**in worker cells** (*n* = 75)	**in drone cells** (*n* = 75)	**Overall** (*n* = 150)
			Length (mm) of eggs			
0–6 h	1.522 ± 0.0045 Ac	1.541 ± 0.0041 Bc	1.531 ± 0.0031 Ac	1.570 ± 0.0111 Cc	1.579 ± 0.0091 Cc	1.575 ± 0.0072 Bc
18–24 h	1.491 ± 0.0067 Ab	1.477 ± 0.0046 Ab	1.484 ± 0.0041 Ab	1.542 ± 0.0122 Bb	1.540 ± 0.0097 Bb	1.541 ± 0.0078 Bb
54–60 h	1.450 ± 0.0065 Aa	1.447 ± 0.0042 Aa	1.448 ± 0.0039 Aa	1.487 ± 0.0075 Ca	1.462 ± 0.0092 Ba	1.474 ± 0.0060 Ba
Mean	1.488 ± 0.0040 A	1.488 ± 0.0036 A		1.533 ± 0.0064 B	1.527 ± 0.0063 B	
Overall Qs – LWs	1.488 ± 0.0027 A [**]CV % = 3.81			1.530 ± 0.0045 B CV % = 6.23		
			Width (mm) of eggs			
0–6 h	0.360 ± 0.0014 Ac	0.358 ± 0.0015 Ab	0.359 ± 0.0010 Ac	0.398 ± 0.0032 Bb	0.396 ± 0.0033 Bc	0.397 ± 0.0023 Bc
18–24 h	0.348 ± 0.0014 Ab	0.353 ± 0.0025 Ab	0.350 ± 0.0014 Ab	0.392 ± 0.0031 Bb	0.386 ± 0.0029 Bb	0.389 ± 0.0021 Bb
54–60 h	0.324 ± 0.0017 Aa	0.329 ± 0.0021 Aa	0.327 ± 0.0014 Aa	0.369 ± 0.0031 Ca	0.352 ± 0.0034 Ba	0.361 ± 0.0024 Ba
Mean	0.344 ± 0.0013 A	0.346 ± 0.0015 A		0.386 ± 0.0020 C	0.378 ± 0.0022 B	
Overall Qs – LWs	0.345 ± 0.0010 A CV % = 6.05			0.382 ± 0.0015 B CV % = 8.35		
			Slenderness index (ratio of length to width) of eggs			
0–6 h	4.038 ± 0.0271 Ba	4.050 ± 0.0199 Ba	4.044 ± 0.0168 Ba	3.746 ± 0.0307 Aa	3.705 ± 0.0312 Aa	3.725 ± 0.0219 Aa
18–24 h	4.285 ± 0.0245 Bb	4.202 ± 0.0258 Bb	4.244 ± 0.0180 Bb	3.950 ± 0.0406 Ab	4.001 ± 0.0383 Ab	3.975 ± 0.0279 Ab
54–60 h	4.481 ± 0.0281 Cc	4.409 ± 0.0284 Cc	4.445 ± 0.0201 Bc	4.046 ± 0.0353 Ac	4.171 ± 0.0376 Bc	4.108 ± 0.0262 Ac
Mean	4.268 ± 0.0195 C	4.221 ± 0.0174 B		3.914 ± 0.0222 A	3.959 ± 0.0243 A	
Overall Qs – LWs	4.244 ± 0.0131 B CV % = 6.54			3.936 ± 0.0165 A CV % = 8.87		

[*]*n* The numbers of eggs measured at each measurement period.

[**]Coefficient of Variation %

Table 2. Comparison of mean ± s.e. of volume, weight and density of eggs deposited by queens (Qs) and laying workers (LWs). Different capital letters denote significant differences ($P < 0.05$) between means in a row (overalls are compared with overalls). Different small letters denote significant differences between means in a column.

	Queen-deposited eggs			Laying worker-deposited eggs		
	in worker cells ($n1$ = 75, $n2$ =3)*	**in drone cells** ($n1$ = 75, $n2$ =3)	**Overall** ($n1$ = 150)	**in worker cells** ($n1$ = 75, $n2$ =3)	**in drone cells** ($n1$ = 75, $n2$ =3)	**Overall** ($n1$ = 150)
			Volume (mm³) of eggs			
0–6 h	0.068 ± 0.0005 Ac	0.068 ± 0.0006 Ab	0.068 ± 0.0004 Ab	0.083 ± 0.0014 Bb	0.083 ± 0.0014 Bc	0.083 ± 0.0010 Bb
18–24 h	0.064 ± 0.0005 Ab	0.065 ± 0.0009 Ab	0.065 ± 0.0005 Ab	0.081 ± 0.001 Cb	0.078 ± 0.0011 Bb	0.080 ± 0.0009 Bb
54–60 h	0.055 ± 0.0006 Aa	0.057 ± 0.0007 Aa	0.056 ± 0.0005 Aa	0.072 ±0.0012 Ca	0.065 ± 0.0012 Ba	0.069 ± 0.0009 Ba
Mean	0.062 ± 0.0005 A	0.063 ± 0.0005 A		0.079 ±0.0008 C	0.075 ± 0.0009 B	
Overall Qs – LWs	0.063 ± 0.0004 A **CV % = 11.97			0.077 ± 0.0006 B CV % = 16.51		
			Weight (μg) of eggs			
0–6 h	131.1 ± 2.23 Ac	133.3 ± 3.33 Ac	132.2 ± 1.86 Ac	159.9 ± 3.33 Bc	157.7 ± 4.43 Bc	158.9 ± 2.53 Bc
18–24 h	115.5 ± 4.00 Ab	117.7 ± 4.83 Ab	116.6 ± 2.85 Ab	138.9 ± 6.78 Bb	143.3 ± 6.93 Bb	141.1 ± 4.45 Bb
54–60 h	94.4 ± 2.94 Aa	95.5 ± 2.94 Aa	95.0 ± 1.87 Aa	124.4 ± 6.18 Ba	116.6 ± 5.77 Ba	120.5 ± 4.16 Ba
Mean	113.7 ± 5.54 A	115.5 ± 5.80 A		141.1 ± 5.88 B	139.2 ± 6.69 B	
Overall Qs – LWs	114.6 ± 3.90 A CV % = 14.42			140.1 ± 4.33 B CV = % 13.10		
			Density (mg/mm³) of eggs			
0–6 h	1.94 ± 0.064 Ac	1.99 ± 0.036 Ac	1.97 ± 0.035 Ac	1.93 ± 0.100 Ab	1.92 ± 0.070 Ab	1.93 ± 0.055 Ab
18–24 h	1.82 ± 0.076 Ab	1.81 ± 0.067 Ab	1.82 ± 0.045 Ab	1.73 ± 0.079 Aa	1.83 ± 0.010 Aa	1.78 ± 0.043 Aa
54–60 h	1.72 ± 0.041 Aa	1.69 ± 0.016 Aa	1.70 ± 0.021 Aa	1.75 ± 0.113 Aa	1.79 ± 0.024 Aa	1.77 ± 0.053 Aa
Mean	1.83 ± 0.045 A	1.83 ± 0.050 A		1.80 ± 0.059 A	1.85± 0.029 A	
Overall Qs – LWs	1.83 ± 0.033 A CV % = 7.55			1.83 ± 0.032 A CV % = 7.47		
% 60h/6h^ Qs – LWs	88.7 -11.3	84.9 -15.1	86.3 -13.7	90.7 - 9.3	93.2 - 6.8	91.7 - 8.3

*$n1$ The number of eggs measured (volume) at each measurement period.

$n2$ The number of egg batch weighed (30 eggs in each batch, total 90 eggs) at each measurement period.

**Coefficient of variation %.

^ percent density at 54–60 h in ratio at 0–6 h, second data – % lost.

Acknowledgments

We wish to thank Yasin Kahya for his assistance during field and laboratory work, Muhip Ozkan and Ozgur Koskan for their guidance in statistical analysis, two anonymous referees and the editor of this paper for their constructive and critical suggestions.

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