

Size change of *Apis mellifera* eggs during the incubation period

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SUMMARY

Nine queen honey bees (*Apis mellifera*) were restricted for 4 h 30 min under a queen-excluder cap on an empty comb in the centre of the broodnest. 25 eggs were removed from each comb and their length and width were measured, as well as the length of the embryo. The egg removal and measurements were repeated every 7 h 30 min. In total, 2475 eggs were measured, on which 7425 measurements were made. In addition, groups of 25 eggs were weighed in four determined periods. Results showed that the length, width and volume of eggs 7 h 30 min–12 h old reduced to 98%, 98% and 94% of their initial dimensions, respectively. Eggs 15–19 h 30 min old increased the three dimensions to 99%, 98% and 96%, respectively. Next the eggs decreased to 91%, 90% and 73% at the age of 52 h 30 min–57 h. Subsequently a peculiar phenomenon occurred, namely the eggs increased to 93%, 92% and 78% at the time of hatching. The embryo changed its length similarly to the egg. The correlation coefficient between the length of embryo and egg was r = 0.92. The weight of eggs decreased continuously to 65% of their initial value at the hatching time. Thus, the eggs change their size and weight during the whole incubation period. It is suggested that the size and weight changes of eggs are due to metabolic processes.

Keywords: honey bees, *Apis mellifera*, egg size, embryo size, incubation period, development

INTRODUCTION

Thousands of honey bee (*Apis mellifera*) eggs have been measured (Reinhardt, 1960; Hejtmanek, 1961; Jordan, 1961; Maul, 1967; Henderson, 1992). Egg size varies for different conditions of egg laying. Sizes of eggs laid by queens or laying workers of the same species of Asian or European honey bees differ significantly (Woyke, 1992, 1994). The weight of eggs laid by different queens differs significantly, as well as weight of eggs laid in various periods (Taber & Roberts, 1963; Roberts & Taber, 1965, Król, 1996). Size changes of embryos were reported by Nelson (1915) Fleig and Sander (1986) and Milne *et al.* (1988). Incubation times of eggs were recorded by Nelson (1915), DuPraw (1961), Harbo and Bolten (1981) and Milne *et al.* (1988). According to Borodacheva (1973), queens reared from heavier eggs were heavier and had more ovarioles than those from lighter eggs. Weight of eggs also affected the external characters of workers to some extent. Bilash *et al.* (1985) showed that colonies headed by queens reared from heavier eggs produced more honey than those headed by queens hatched from lighter eggs.

Beekeepers generally believe that the eggs do not change their size during the incubation period. However, Woyke (1993) found that eggs of three species of Asian honey bees changed their size during incubation period. Therefore, changes of size and weight during the incubation period of *Apis mellifera* eggs were investigated. Because the age of the eggs investigated till now was not determined, or only young ones were measured, means for eggs of different ages whose frequency was equally distributed are presented. The incubation period of eggs was also recorded.

MATERIALS AND METHODS

Nine queens, three each of *A. m. carnica, A. m. caucasica*, and *A. m. ligustica*, were used in this investigation. Empty comb (36 × 26 cm) confined in queen excluder was placed for 1 day in the centre of the broodnest in preparation for egg laying. Next, the queens were restricted by a queen excluder cap (15 × 15 cm) on that comb for 4 h 30 min. Afterwards, they were moved with the cap on to the opposite side of the same comb for the same period. They were then put on another comb in a queen excluder isolator. Thus, several comb areas containing eggs of known age were obtained. Twenty-five eggs were removed with the aid of a needle at the end of a queen-restriction period. The comb was returned to its previous place within the colony. Every 7 h 30 min a new set of 25 eggs was removed and measured. Egg length and width was measured under a stereomicroscope with the aid of an eyepiece micrometer (magnification 62.5 × for length and 100 × for width). Width was measured at the mid-point of the length. To measure the length of embryos, the eggs were covered with a layer of light paraffin oil, which caused the eggs to be transparent. In total, 7425 measurements of 2475 eggs were made. The volume of eggs was calculated as an ellipsoid. In addition, groups of 25 eggs were removed from comb cells and were weighed together on a scale (± 0.1 mg). The eggs were weighed at 4 h 30 min, 57 h, 72 h and 79 h 30 min after the queen was restricted on the comb. A total of 900 eggs were weighed. Analysis of variance was applied, and an LST test was used to determine significant differences between the means.

RESULTS

Incubation period of eggs

In the conditions described, no larvae were found in cells 72 h after the queens were restricted on a brood comb. In six colonies out of nine (67%), some larvae were present among eggs 79 h 30 min after queen restriction. Thus, the larvae hatched between 72 h and 79 h 30 min (average 76 h) after egg laying. In three colonies, no larvae were found 79 h 30 min after queen restriction. Thus, the larvae hatched later than 75 h–79 h 30 min (average later than 77 h) after egg laying. No more eggs were found 7 h 30 min later.

Length of eggs

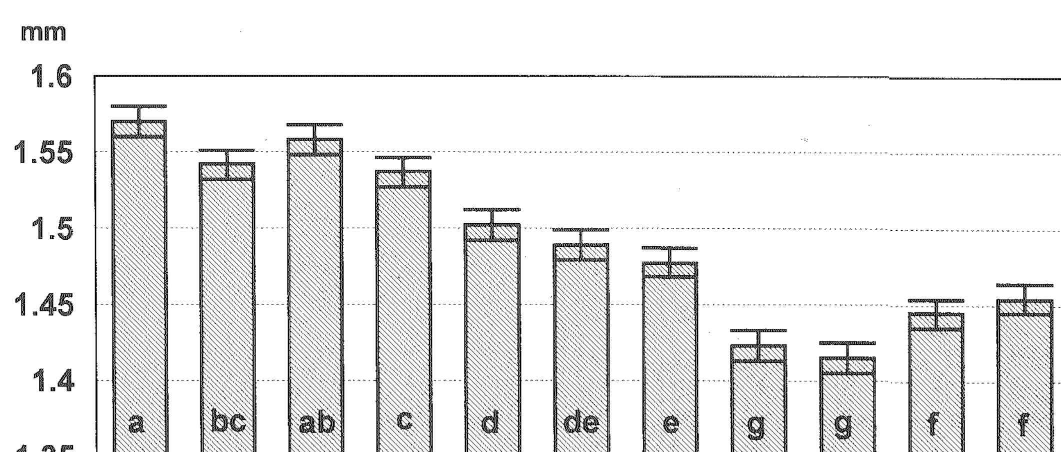
Table I shows that the length of 2475 eggs ranged between 1.196 mm and 1.846 mm. The longest were 54% longer than the shortest. The mean was 1.492 mm (s.d. ± 0.119 mm). The mean length of eggs deposited by different queens of the same subspecies differed significantly. The mean length of all *A. m ligustica* eggs was significantly longer than that of the two other subspecies.

Figure 1 shows that at the end of the 4 h 30 min queen confinement periods, the youngest eggs 0 h–4 h 30 min old were on average 1.570 mm long (n = 225). Seven and a half hours later, eggs 7 h 30 min–12 h old reduced their length significantly to 98%. This means that in the honey bee, biological activity occurs immediately after egg deposition (contrary to many other insects). However, after the next period, eggs 15 h–19 h 30 min old increased their length to 99.2%. No significant difference was found between means for the last two periods. After separate analysis was conducted for every queen, the older eggs were significantly longer than the younger ones in seven colonies out of the nine investigated. This indicates that significant differences may be expected in about 80% of bee colonies. Next the length of eggs shortened continuously up to the age of 60 h–64 h 30 min, reaching 90.1% (1.415 mm) of their original length. Afterwards, a peculiar phenomenon occurred, namely the eggs increased in length reaching, at the time of hatching, 92.6% (1.454 mm) of their initial size. In individual queens, the shortest younger eggs (15 h–19 h 30 min old), were longer than the longest eggs older than 42 h. Thus, the eggs change their length during the whole incubation period.

TABLE 1. Length of eggs (mm) in different bee colonies and bee subspecies.

Colony no.[1]	No. eggs	Range	Mean	s.d.	Coefficient of variance (%)
Apis mellifera carnica					
4	275	1.274–1.560	1.401b*	0.068	4.88
44	275	1.326–1.586	1.447c	0.066	4.59
2	275	1.430–1.638	1.560f	0.042	2.70
Total	**825**	**1.274–1.638**	**1.469α[2]**	**0.090**	**6.13**
Apis mellifera caucasica					
78	275	1.196–1.482	1.316a	0.062	4.73
43	275	1.274–1.612	1.446c	0.069	4.79
37	275	1.534–1.846	1.672h	0.070	4.20
Total	**825**	**1.196–1.846**	**1.478α**	**0.162**	**10.94**
Apis mellifera ligustica					
3	275	1.378–1.612	1.469d	0.047	3.21
66	275	1.430–1.664	1.518e	0.056	3.66
64	275	1.300–1.716	1.599g	0.063	3.91
Total	**825**	**1.300–1.716**	**1.528β**	**0.077**	**5.04**
Overall	**2475**	**1.196– 1.846**	**1.492**	**0.119**	**7.94**

[1]The colonies are arranged according to increasing mean length of eggs
[2]Means for subspecies α are compared with means for other subspecies and not for colonies
*Different letters after the means indicate statistically significant differences between them



H	4.5	12	19.5	27	34.5	42	49.5	57	64.5	72	79.5
High I	1.58	1.551	1.568	1.546	1.512	1.499	1.487	1.433	1.425	1.454	1.464
Low I	1.56	1.532	1.548	1.527	1.492	1.479	1.468	1.413	1.405	1.435	1.445
mm ▨	**1.57**	**1.542**	**1.558**	**1.537**	**1.502**	**1.489**	**1.477**	**1.423**	**1.415**	**1.445**	**1.454**

FIG. 1. Length of *Apis mellifera* eggs during different incubation periods. Abscisa (H) presents time after restriction of queens on empty combs for 4.5 h. Error bars present 95% LSD.

No significant relationship was found between the length of eggs at different ages and the race of the bees ($F = 1.12$, $P = 0.32$). Figure 2 shows that the trends in egg-length changes were similar among all three subspecies.

Width of eggs

The width of eggs of different ages ranged from 0.298 to 0.403 mm. Thus, the widest were 35.2% wider than the narrowest. The overall mean was 0.350 mm

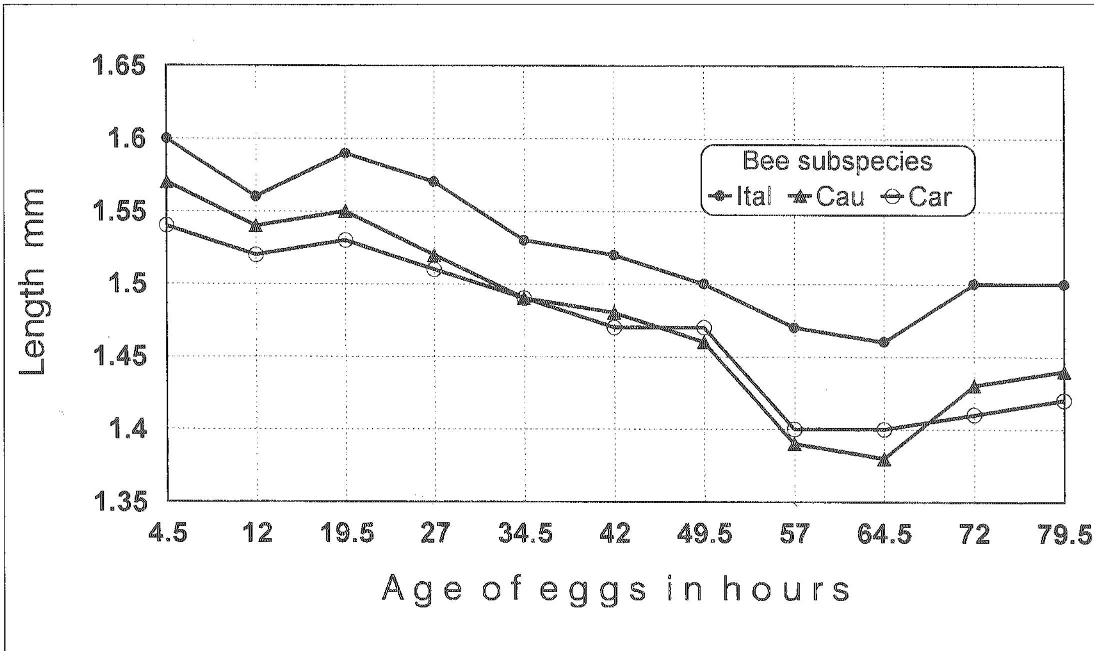
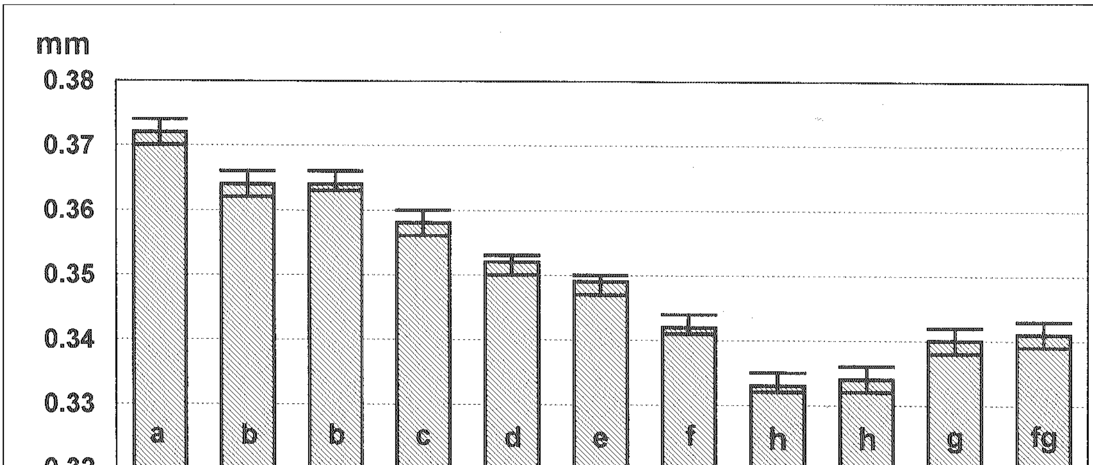


FIG. 2. Changes in length of eggs during incubation period in three bee subspecies: Car = *Apis mellifera carnica*, Cau = *A. m. caucasica*, and Ital = *A. m. ligustica*.

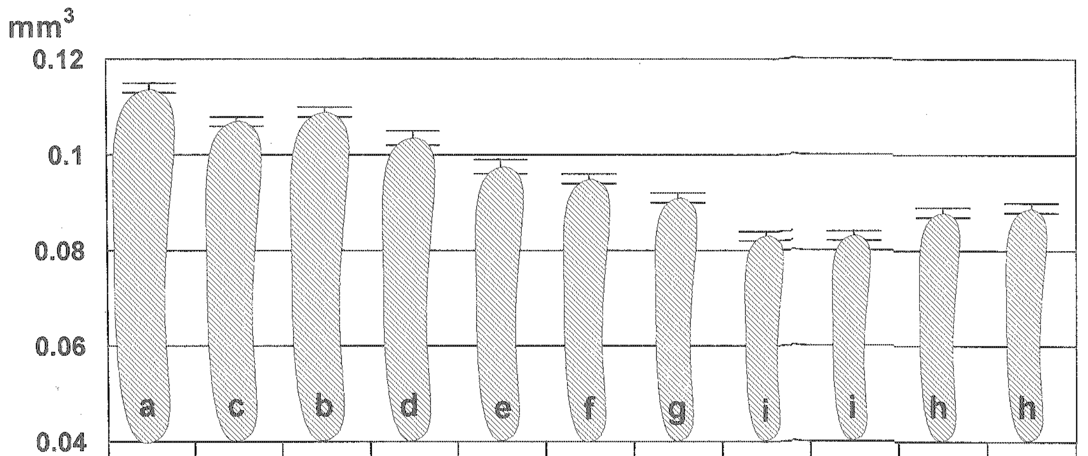


H	4.5	12	19.5	27	34.5	42	49.5	57	64.5	72	79.5
High I	0.374	0.366	0.366	0.36	0.353	0.35	0.344	0.335	0.336	0.342	0.343
Low I	0.37	0.362	0.363	0.356	0.35	0.347	0.341	0.332	0.332	0.338	0.339
mm	**0.372**	**0.364**	**0.364**	**0.358**	**0.352**	**0.349**	**0.342**	**0.333**	**0.334**	**0.34**	**0.341**

FIG. 3. Width of *Apis mellifera* eggs during different incubation periods (see fig. 1 for details).

(s.d. ± 0.019, n = 2475). Figure 3 shows that eggs 0 h–4 h 30 min old were 0.372 mm wide. Eggs 7 h 30 min–12 h old decreased their width to 97.8% of their initial size. Next, the eggs decreased their width to 89.5% (0.333 mm) at the age of 52 h 30 min–57 h. At the time of hatching, the width increased to 91.70% (0.341 mm) of its initial value.

The correlation coefficient between the length and width of all eggs of different ages was r = 0.59



H	4.5	12	19.5	27	34.5	42	49.5	57	64.5	72	79.5
High I	0.115	0.108	0.11	0.105	0.099	0.096	0.092	0.084	0.084	0.089	0.09
Low I	0.113	0.106	0.108	0.102	0.096	0.094	0.09	0.082	0.082	0.087	0.088
mm³	**0.114**	**0.107**	**0.109**	**0.104**	**0.098**	**0.095**	**0.091**	**0.083**	**0.083**	**0.088**	**0.089**

FIG. 4. Volume of *Apis mellifera* eggs during different incubation periods (see fig. 1 for details).

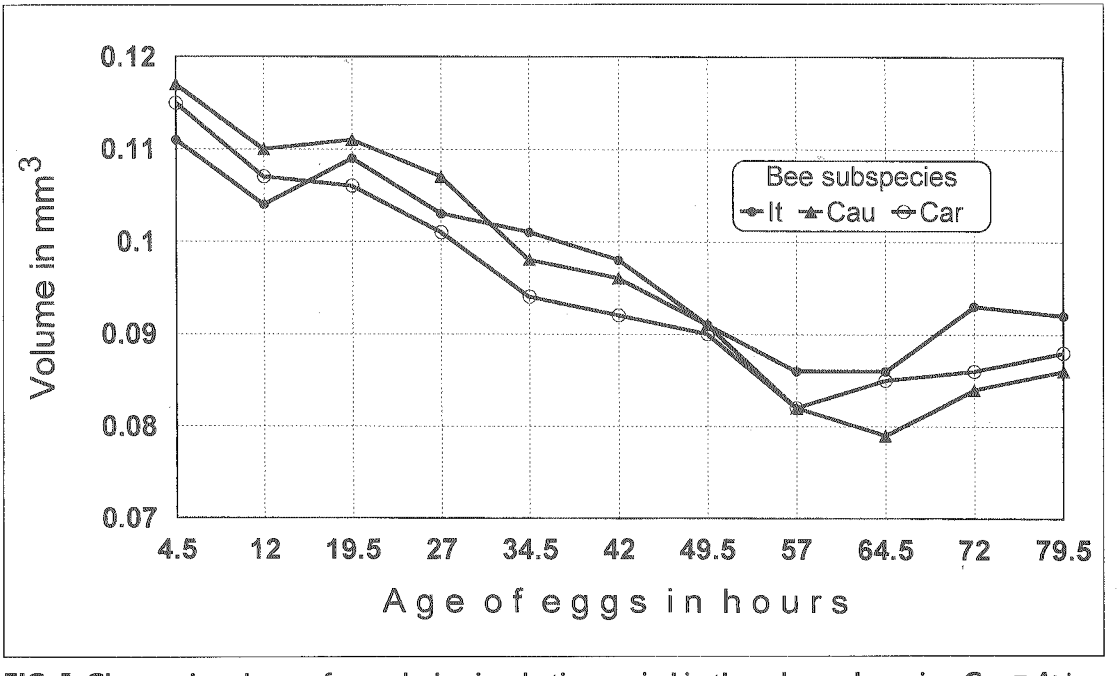
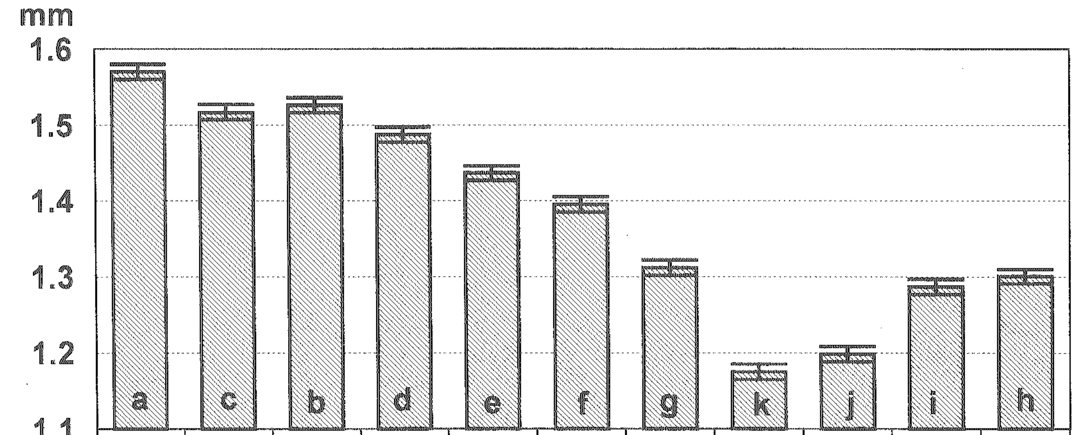


FIG. 5. Changes in volume of eggs during incubation period in three bee subspecies: Car = *Apis mellifera carnica*, Cau = *A. m. caucasica*, and It = *A. m. ligustica*.



H	4.5	12	19.5	27	34.5	42	49.5	57	64.5	72	79.5
High I	1.58	1.527	1.536	1.497	1.446	1.405	1.322	1.185	1.208	1.297	1.31
Low I	1.56	1.507	1.516	1.477	1.427	1.385	1.302	1.165	1.188	1.277	1.291
mm	**1.57**	**1.516**	**1.526**	**1.487**	**1.437**	**1.395**	**1.312**	**1.175**	**1.198**	**1.287**	**1.301**

FIG. 6. Length of *Apis mellifera* embryo during different incubation periods (see fig. 1 for details).

($P < 0.001$). This indicates a moderately strong relationship between the two variables. The r^2 statistic indicated that the model explained only 34.92% of the variability in the width of eggs. Thus, the longer eggs were also wider; however, the variability in egg width was not explained only by the length variability. Therefore, a correlation was also examined between the variability of length and width influenced by the age of the eggs. For that purpose, the correlation was calculated between the mean lengths and mean widths of eggs during different incubation periods (presented in figs 1 and 3). The correlation coefficient was $r = 0.98$ ($P < 0.001$). This indicated a strong relationship between the two variables. The r^2 statistic indicated that this model explained as much as 97.0% of the variability in egg width. This shows that eggs were wider at the ages when they were longer, and were narrower at ages when they were shorter. Thus, the eggs did not shorten at the same ages because they became wider.

Volume

The volumes of eggs calculated as ellipsoids may differ from exact egg volumes, however, they are useful for comparison purposes. Such volumes of different aged eggs ranged from 0.059 mm^3 to 0.150 mm^3. Thus, the largest eggs were 2.5 times larger than the smallest. The overall mean was 0.096 mm^3 (s.d. ± 0.016, n = 2475). Figure 4 shows that the mean volume of eggs 0 h–4 h 30 min old was 0.114 mm^3. Seven and a half hours later (age 7 h 30 min–12 h), egg volume decreased significantly to 93.9% of the initial size. After the next period (15 h–19 h 30 min), they increased significantly to 95.6%. Next at the age of 52 h 30 min–64 h 30 min, the eggs decreased significantly to 72.8% (0.083 mm^3). Afterwards, the volume of eggs increased significantly to 78.1% (0.89 mm^3) at the hatching time.

The mean volumes of different aged eggs of the three subspecies were as follows: *A. m. carnica*, 0.095 mm^3; *A. m. caucasica*, 0.096 mm^3; and *A. m. ligustica*, 0.098 mm^3 (n = 825 eggs for each subspecies). *A. m. carnica* eggs were statistically smaller than *A. m. ligustica*. The *A. m. caucasica* eggs were medial and no significant difference was found between them and the two others. A relationship was found between egg volume and bee race during the incubation period. Figure 5 shows that eggs 0 h–4 h 30 min old were the largest in *A. m. caucasica* and the smallest in *A. m. ligustica*. However, the opposite occurred with eggs older than 60 h till the time of hatching, when *A. m. ligustica* eggs were the largest and *A. m. caucasica* the smallest. The largest volume changes occurred in *A. m. caucasica* eggs and the smallest in *A. m. ligustica* ones.

Embryo length

Figure 6 shows that the mean length of embryos 0 h–4 h 30 min old was 1.570 mm (n = 225). Seven and a half hours later, at the age of 7 h 30 min–12 h, the embryos shortened significantly to 96.6%. After the next period, embryos 15 h–19 h 30 min old increased their length significantly to 97.2%. Next the embryos shortened significantly up to 74.8% at the age of 52 h 30

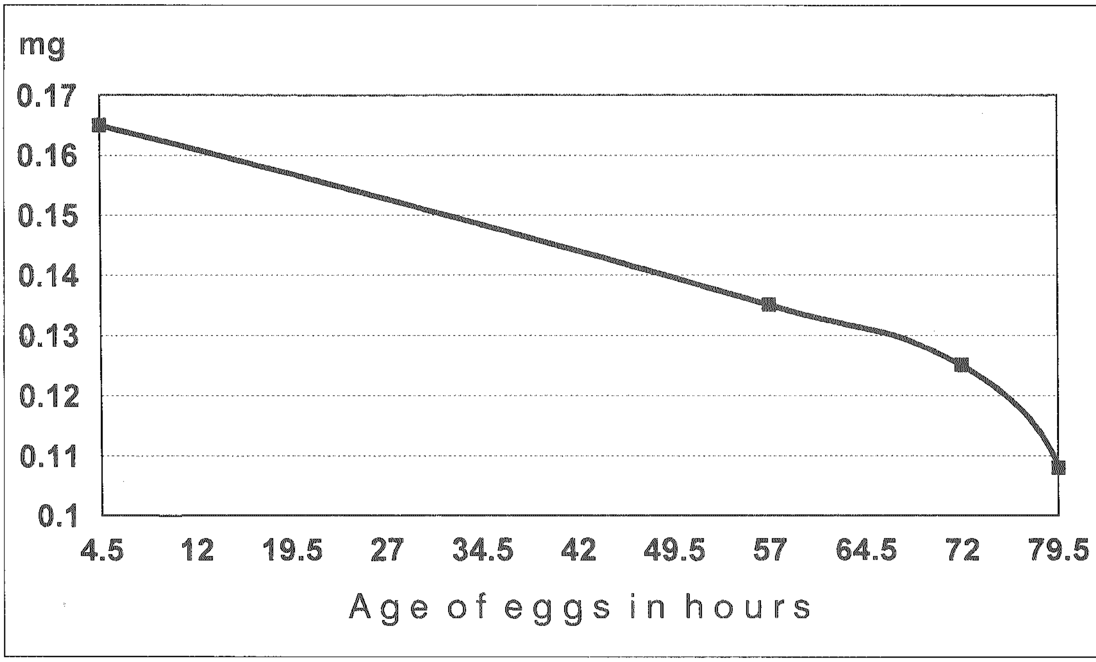


FIG. 7. Weight of eggs during different incubation periods. The marks show means for 225 (25 × 9) eggs.

min–57 h. Afterwards, the embryos increased their length significantly up to 82.9% at the time of hatching.

The correlation coefficient between the length of embryos and the length of eggs was r = 0.87. (P < 0.001). Thus, a moderately strong relationship between the two variables was found. The length of eggs was equal to 0.6466 ± 0.6116 times the embryo length. The r^2 statistic indicated that the model explained 75.2% of the variability in egg length. Correlation was examined between length of egg and embryo at determined incubation periods. For this purpose, correlation was calculated between the means of egg and embryo lengths at known ages presented in figures 1 and 6. The correlation coefficient between the means of embryo and egg lengths was r = 0.92 (P < 0.001). Thus, a statistically significant relationship was found between the length of embryos and eggs at the 99% confidence level. The mean length of eggs at known age was 1.0710 ± 0.3007 × mean embryo length at this age. The mean length of embryo at known age was –2.7837 ± 2.8043 × mean egg length. The r^2 statistic indicated, that the model explained 84.3% of the variability in egg length. Thus, it is clear that the length of eggs is strongly related to the length of embryos.

Weight

Eggs were weighed in batches of 25, thus, the weight of individual eggs is presented as a mean of 25. The weight of eggs ranged from 92 mg to 175 mg. The heaviest were 1.9 times heavier than the lightest. The mean weight of different aged eggs was 0.133 mg (n = 900). However, the eggs were collected during four periods not equally distributed over the incubation period. Thus, the 0.135 mg (n = 225) weight of eggs collected closer to the middle of the incubation period, at the age of 52 h 30 min–57 h, is probably closer to the overall mean of different aged eggs. Figure 7 shows that the mean weight of 225 eggs, 0 h–4 h 30 min old originating from nine queens, was 0.167 mg. Eggs 52 h 30 min–57 h, and 67 h 30 min–72 h old decreased their weight to 82% and 76% (0.135 and 0.125 mg) of their initial value, respectively. Figure 7 shows that the weight decreased proportionally up to the age of 72 h. No depression characteristic for the size of eggs at the age of 57 h was observed for egg weight. During the last hours before hatching, a sharp weight decrease down to 65% (0.108 mg) was observed. Thus, contrary to the size increase observed during the terminal incubation period, weight decrease occurred during the same final development stage

The mean weights of eggs at the four different ages all differed significantly. Examination of the variation revealed that the lightest eggs shortly after oviposition were heavier than the heaviest at the time of hatching.

DISCUSSION

The sizes presented in table 1 are the first given in the literature for means of eggs of different known ages (11) whose frequencies are equally distributed. Comparison of two or more sets of eggs of different ages may be misleading.

Since significant differences in egg size were found between *A. mellifera* subspecies, egg size may be considered as a specific character of some subspecies.

The results show that not only the overall means of different subspecies should be considered but also results of individual queens. Many times statistically significant differences were found between means concerning individual queens, while, due to high variance, they could not be detected between overall means.

The present investigation demonstrated that the eggs, including the chorion, change their size and weight over the whole incubation period. The weight decreases all the time, while egg size increases during the final incubation stage. The decrease in size and weight could be explained by energy consumption necessary for embryo development. Concerning the final size increase in eggs, special observations revealed that air enters the embryo tracheoles during the final development period, before the embryo hatches from the egg. Probably some air also penetrates into the body fluids. The increase in the amount of air in an embryo results in an undetectable weight increase and a significant size increase. Apart from air penetration some other phenomena must also result in size increase. Tracheoles are not present in eggs 15–20 h old, when a size increase of the yolk and eggs including chorion was also detected. It is known that not only the embryo, but also the egg yolk respires (Melvin, 1928). The synthesis of structural proteins, as well as of lipids from carbohydrates results in a respiratory quotient (RQ) of over 1 (Melampy & Willis, 1939). This means that more CO_2 is produced than O_2 is consumed. If the CO_2 remains inside the egg, its size increases.

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