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Hatching rates and characteristics of Carniolan Honey Bee eggs

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ABSTRACT

Few studies have been performed on honey bee eggs to date, particularly on egg hatchability and other egg characteristics. In the harsh environmental conditions of Saudi Arabia, it has been found that honey bee eggs from different subspecies are impacted by low relative humidity (RH). Therefore, the hatching rates of eggs of two subspecies, Yemeni (*Apis mellifera jemenitica*) and hybrids of Carniolan honey bees (*Apis mellifera carnica*), were studied under different RH gradients, and various egg characteristics (morphology, egg surface chemicals and egg water content) were described for these two subspecies. The results of these analyses demonstrated that Yemeni honey bee eggs displayed higher hatching rates than Carniolan honey bee eggs across the humidity gradient, although no eggs were able to hatch at a relative humidity of 30%. Differences in egg morphology were detected between the two subspecies. The egg surface chemicals were approximately the same for Carniolan and Yemeni honey bee eggs, while the Yemeni honey bee eggs exhibited a higher water content. The differences between the two subspecies in egg hatching rates could be attributed to differences in egg water content as well as to some internal factors within the eggs.

Keywords: Honey bees, Eggs, Carniolan, Yemeni, hatching

INTRODUCTION

Various factors impact honey bee colony activity and performance, the most important of which are temperature and relative humidity. Within colonies, bees are typically able to maintain a temperature between 33°C and 36°C [1] and a relative humidity above 75%. The regulation of relative humidity is particularly important for egg hatching. If the relative humidity is too high [2] or if it falls below 50%, egg hatching may fail [3]. Although honey bee workers are able to control the relative humidity within the colony [4] through different methods, including the collection of water from the environment [5], the problem of low egg hatching rates has been noted previously in Saudi Arabia. Saudi Arabia endures harsh environmental conditions of elevated temperature and limited rainfall, especially during the summer months. In central Saudi Arabia, the mean relative humidities within colonies of *A.m. jemenitica* and *A. m. carnica* was found to be 34.7 and 37.8%, while egg hatching rates were 69.2 and 67.4%, respectively, when the ambient temperature was 42°C [6]. Due to the drought conditions in Saudi Arabia, it is likely that a low relative humidity within colonies is the main reason for these low egg hatching rates. Therefore, egg hatching rates under different relative humidity gradients were studied.

In addition to low egg hatching rates, Yemeni honey bees, which are native to Saudi Arabia, were found to display relatively higher egg hatching rates than hybrid Carniolan honey bees, which have been imported into Saudi Arabia [6]. Notably, Yemeni honey bees are more tolerant of harsh conditions than Carniolan honey bees [7]. The difference between the egg hatching rates of the two subspecies under the same environmental conditions may be attributed to specific characteristics of the eggs (e.g., their morphology and surface chemicals).

Various egg types exhibit different levels of resistance to dehydration [8]. Typically, honey bee eggs are cylindrical in shape[9] and maintain their white color and shape throughout development [10]. Relatively few investigations have been performed comparing honey bee eggs from different subspecies. Although no difference in egg size was detected between fertilized and unfertilized queen-laid eggs [11], the size of queen-laid eggs differed significantly from that of worker-laid eggs [12]. Showed that the size and the weight of honey bee eggs are changed during the whole incubation period [13, 14]. Differences between eggs in their dimensions (e.g., their length and width) may predict the viability of honey bee eggs and, thus, egg hatching rates.

Moreover, the surface of honey bee eggs contains various chemical compounds. These chemical compounds are likely to be used by honey bee workers to discriminate between queen-laid eggs and worker-laid eggs [15].

Additionally, these chemicals, especially the hydrocarbon components, protect eggs from desiccation [16]. Three primary components (hydrocarbons, eicosanol and esters) have been identified on the egg surface [16]. Hydrocarbons are the most abundant chemicals on the egg surface [17] and may serve as an anti-dehydration barrier. Thus, egg surface chemicals may participate indirectly in egg hatching rates.

In this study, the impact of relative humidity on egg hatching rates for Carniolan and Yemeni honey bees was investigated. Also, any differences in egg characteristics between the two subspecies were detected to identify potential explanations for the variations in their egg hatching rates.

MATERIALS AND METHODS

The investigations were performed at the Bee Research Unit laboratory, King Saud University (KSU).

Egg hatching rates

This experiment was conducted under controlled conditions of temperature and relative humidity (RH) in Memeret incubators (Germany). A total of 150 eggs (one-day old) from each race, Yemeni and Carniolan honey bees, were used per treatment. Each race was represented by five colonies, and one or two wax pieces (approximately 3 x 3.5 cm) from each colony containing 30 newly laid eggs were cut out of the wax comb. If more than 30 eggs were present in the extracted wax comb, the extra eggs were removed. Wax pieces containing eggs were used in this experiment to avoid any damage to the eggs. The eggs were maintained in incubators at 30, 50 or 75% RH at a fixed temperature of 35°C for three days (these values of relative humidity were selected to mimic arid conditions). Egg hatching rates were calculated by dividing the number of hatched eggs by the total number of eggs and multiplying this value by 100. The egg hatching rates were then compared for the two subspecies.

Egg size and weight

The morphological characteristics of 150 eggs (one day old) were measured per race (30 eggs per colony and five colonies per race). The eggs were carefully mounted on glass slides, and measurements were obtained with a Hewlett-Packard Deskjet scanner at a resolution of 1200 dpi. During the scanning process the glass slides were covered with petri dishes to avoid eggsdestruction by the scanner cover. The obtained images were then opened in the computer program Photoshop to capture measurements according to the Scan Photo method [18, 19]. Egg length, width and the slenderness index were determined for all eggs from both subspecies. The measurements were performed as indicated in Figure 1 according to [20]. Egg length was measured as the maximum distance from the anterior pole to the posterior poleof the egg, and egg width was measured as the widest distance in the middle of the egg. The slenderness index was calculated as the egg length divided by the egg width, according to [21]. The egg weight was calculated by combining 100 eggs from five colonies per race (20 eggs per colony), which were placed on a glass slide with a known weight (W0) and weighed (W1) using a GR 200 balance (A & D Company Limited, Japan). Then, the egg weight was calculated as W1 – W0. The resulting weights weretransformed from mg to µg units.

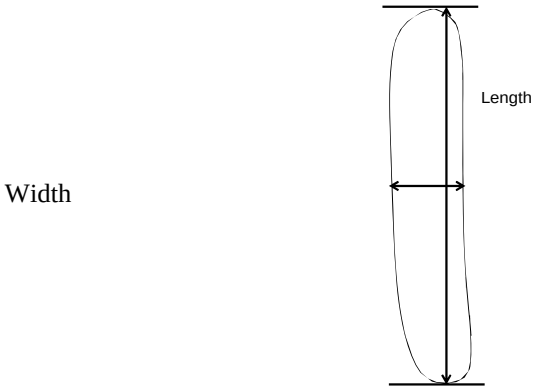

Fig 1: Egg length and width

Egg surface compounds

The qualitative chemical analysis of egg surface compounds was performed as described by [22]. A total of 50 eggs (one-day old) were analyzed per race. Eggs were pooled and washed for 1 min in 5 ml dichloromethane. Then, egg particles were removed using glass wool, and the extract was concentrated to 70 µl. The samples were analyzed with an Agilent 7890A GC system coupled to a 5975C MS (Triple-Axis detector) using an Agilent 19091S column (250 µl ID x 30 m, film thickness: 0.25 µl; Agilent Technologies Inc., USA). The injection port temperature was 300 °C. The samples were injected in splitless mode, and helium was used asthe carrier gas (flow rate of 1 ml/min). The GC oven was programmed with an initial temperature of 260 °C. This temperature was increased by 10°C/min to a final temperature of 325°C, which was then held for 2 min. The mass spectra were recorded, and an MS database (NIST 08. L) was used for compound identification (only compounds with a probability above 80%

were considered). Also, only the compounds which have been previously identified on honey bee egg surface were considered.

Egg water content

The egg water content was determined in 100 eggs from five colonies per race (20 eggs per colony). The eggs were first weighed (W0) using a GR 200 balance (A & D Company Limited, Japan) and then placed in an incubator at 70°C for 24 hours and dried completely. The dry weight of the eggs was subsequently measured (W1), and the egg water content was calculated as a percentage using the equation W0-W1/W0 x 100. Finally, the egg water content determined for the two subspecies was compared.

Statistical analysis

For the above-mentioned experiments, the mean ± standard error was calculated. For the egg hatching rates, a factorial experiment, the data were statistically analyzed via analysis of variance (ANOVA), and the mean values were compared with the Tukey test at a probability of 5%. For the other experiments, except egg surface compounds, the mean values were compared using a *t*-testat a probability of 5%. The statistical analysis was done using the SAS 9.1.3 program [23].

RESULTS

Egg hatching rates

As shown in Table 1, it was clear that at 30% RH, the eggs of the two subspecies were not able to hatch successfully (Figure 2). At 50% RH, more eggs of Yemeni honey bees hatched than did Carniolan honey bee eggs. The hatching rates for both subspecies at an RH of 50% were approximately 50%. At an RH of 75%, most of the Yemeni honey bee eggs were able to hatch, while only approximately half of the Carniolan honey bee eggs hatched. In general, the Yemeni honey bee eggs showed a greater ability to hatch under moderate relative humidity conditions than did the Carniolan honey bees.

The Two-factor ANOVA showed significant effect of the RH factor on the egg hatching rates (F=362.31 and *P*= 0.0001<0.05). Also the race factor affected the egg hatchability significantly (F=13.04 and *P*= 0.0014<0.05) as well as the interaction between both factors was significant (F= 3.87 and *P*= 0.0349<0.05). No significant differences were detected between the hatching rates of Carniolan and Yemeni honey bee eggs at RHs of 75% and 30%, whereas a significant difference was found between the two subspecies at a relative humidity of 50% according to the Tukeytest (*P* = 0.05 and DF = 24). A strongly positive and significant correlation (r = 0.92, *P* = < 0.0001) was found between the hatching rates and relative humidity.

Table 1: Egg hatching rates for Yemeni and Carniolan honey bees under different humidity levels.

RH (%)	Mean of egg hatching (%) ± S.E.	
	Carniolan honey bees	Yemeni honey bees
30	0.00 ± 0.00 d	0.00 ± 0.00 d
50	39.33 ± 3.39 c	52.66 ± 2.87 b
75	58.67 ± 3.09 ab	66.67 ± 2.35 a

*: Means followed by the same letter are not significantly different according to Tukey test $_{0.05}$.

Table 2: Egg size and weight for Yemeni and Carniolan honey bees.

Egg character*	Mean ± S.E.	
	Carniolan honey bees	Yemeni honey bees
Length (mm)	1.54 ± 0.004b	1.58 ± 0.005a
Width (mm)	0.36 ± 0.003a	0.35 ± 0.004a
Slenderness index	4.31 ± 0.050b	4.55 ± 0.06 a
Egg weight (µm)	124 ± 4.00a	121 ± 6.40a

*: Means in the same row followed by the same letter are not significantly different according to *t*-test.

Egg surface compounds

Table (3) shows 13 alkanes detected of the eggs. Hentriacontaneand Tridecane were only detected in Yemeni honey bee eggs, whilepentetriacontene was detected only in Carniolan honey bee eggs.

Approximately the same compounds were detected for the tworaces.

Table 3: Egg surface chemicals for Yemeni and Carniolan honey bees (+: present and --: absent).

Compounds	Race	
	Carniolan honey bee eggs	Yemeni honey bee eggs
Alkanes	--	+
Hentriacontane		
Eicosane	+	+
Pentacosane	+	+
Heptacosane	+	+
Dodecane	+	+
Tetracosane	+	+
Pentadecane	+	+
Heptadecane	+	+
Octacosane	+	+
Octadecane	+	+
Tridecane	--	+
Heneicosane	+	+
Nonadecane	+	+
Alkenes		
Octadecene	+	+
Tetradecene	+	+
pentetriacontene	+	--

Egg water content

The mean water content of the Carniolan honey bee eggs was 88.70 ± 1.94 (mean ± SE), while that of the Yemeni honey bee eggs was 90.11± 1.43 (mean± SE). Thus, Yemeni honey bees displayed a relatively higher water content than did Carniolan honey bee eggs. However, no significant differences were found between the two subspecies according to the *t*-test ($P = 0.57 > 0.05$).

DISCUSSION

Egg hatching rates

Relatively few studies have been performed on the relationship between the hatching rate of honey bee eggs and relative humidity. As observed in the present study, [3] found that no eggs hatched when the RH was less than 50%, while 54% of eggs were able to hatch at 50% RH. The low hatching rates recorded under conditionsof low RH may be caused by the rapid drying of the hatching fluid [3]. Increasing RH promoted high egg hatching rates. Therefore, humidity is a key factor impacting egg hatching. It is known that Yemeni honey bees are more tolerant of elevated temperatures and low humidities than Carniolan honey bees [7]. In the present study, also, Yemeni honey bee eggs showed higher egg hatching rates than did Carniolan honey bees. These results are in accordance with the results published by [6], who found that Yemeni honey bee colonies displayed a 1.8% higher egg hatching rate than Carniolan honey bee colonies under a relative humidity of approximately35%.

Egg size and weight

Honey bee eggs typically exhibit a cylindrical shape [9] and white color. Almost all of the eggs from the various honey bee subspeciesshare the same structure. However, some differences have been detected between the eggs of some honey bee subspecies and species. In the present study, Yemeni honey bee eggs were found tobe longer than Carniolan honey bee eggs and exhibited a higher slenderness index, although no clear difference was detected in egg width between the studied subspecies. These results are similar to results from other studies [11] observed no difference in egg size between fertilized and unfertilized eggs laid by bee queens. The width of honey bee eggs has been reported to be approximately 0.35 mm [24], which is in accordance with the results of the present study. However, the egg length measured in the present study was shorter than that recorded in other studies for different honey bee species, including *A. cerana* queens, *A. dorsata* queens, *A. cerana* egg-laying workers, *A. andreniformis* [20] and *A. m. mellifera* [12]. Additionally, the egg length, width and slenderness index obtained for the studied subspecies were higher than those reported for *A. m.caucasica* [21]. No clear difference in egg weight was detected between the two subspecies. The obtained egg weights were closeto those found by [21] for queen-laid eggs of *A. m. caucasica*. The differences between the egg measurements recorded for the two subspecies examined in this study cannot sufficiently explain any differences between the two subspecies with regard to egg hatchingrates.

Egg surface compounds

Several different compounds cover the surface of honey bee eggs, including alkenes, alkanes, methylalkanes, alcohols, aldehydes, esters and terpenes [9, 16] identified three main components of egg surface chemicals (hydrocarbons, eicosanol and esters), and the proportions of these components for queen-laid eggs were found to be 98.2% for hydrocarbons, 0.1% for eicosanol and 1.7% for esters. Thus, hydrocarbons are the major egg surface compounds. The presence of significant amounts of hydrocarbons on egg surfaces has been observed by other researchers as well [22, 25]. The compounds detected in the present study are very important for eggs. For example, alkenes and alkanes protect eggs fromdesiccation [16] and thus ensure normal egg hatching. The few differences in egg surface compounds detected between the two subspecies may be due to differences in egg marking signals. As honey bee queens mark their eggs with a specific signal to discriminate between queen-laid eggs and those laid by nurse honey bee workers [15]. In general, the detected differences betweenthese compounds can not explain any differences between egg hatching rates. In a previous study by [26] the elemental analysis of Yemeni and Carniolan honey bee eggs was studied. They detected carbon, nitrogen, oxygen, magnesium, phosphorus, sulfur, calcium and zinc in the eggs. The elemental composition patterns were not fixed for the two subspecies which suggest the existence of internaldifferences between them (e.g., in terms of genetic characteristics and embryo viability).

Egg water content

Yemeni honey bee eggs contained approximately 1.41% greater amount of water than Carniolan honey bee eggs. This difference in egg water content may suggests higher hatchability of Yemeni than Carniolan honey bee eggs under conditions of low relative humidity. Due to the very small size of honey bee eggs, any difference between eggs may contribute to egg hatching. It is known that bee eggs can tolerate dehydration [8], and according to the present study, this ability could be attributed to the egg water content.

CONCLUSION

The size and weight of eggs do not explain the higher egg hatching rates of Yemeni honey bees than the Carniolan honey bees. The water content, 1.4% higher in eggs of Yemeni honey bees than of Carniolan honey bees may suggests the higher hatchability in Yemeni honey bees.

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