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17

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Summary

1. Researches dealing with insect eggs are limited to hatchability, hatching conditions, elemental analysis and egg viability. Little is known about the influence of different elements on the insect eggs.
2. In the present study, the elemental analysis for two honey bee races was studied using energy dispersive X-ray spectroscopy.
3. Carbon, nitrogen, oxygen, magnesium, phosphorus, sulfur, calcium and zinc were found in eggs of the both races. Significant differences between races were however found for some of the elements, such as oxygen, phosphorus, sulfur, calcium and zinc.
4. The pattern of elemental analysis was not stable in the studied races; this open a possible new trend in insect classification and phylogeny based on the egg elemental analysis. Moreover, elemental analysis could help researches on egg cryopreservation and viability.

Key-words: *Apis mellifera*, honey bees, eggs, elemental composition

Introduction

There are various factors affecting successful egg hatching in insects including environmental factors, such as relative humidity and temperature, parasitism conditions, genetic factors and diseases. A little is known about eggs hatchability in most insects. Insect eggs (*e.g.*, honey bees) consist of micropyle, chorion, delicate Vitelline membrane, cortical layer of cytoplasm (Snodgrass and Erickson 1992). The vitellogenin is the major egg yolk protein (Hagedorn and Kunkel 1979). Different resistance to dehydration has been found among different egg types (Wegener et al. 2010).

The common compound of the insect egg chorion is polycrystalline material, which has been found in insects belonging to orders Orthoptera, Odonata, Neuroptera, Hemiptera, Hymenoptera and Coleoptera (Furneaux and Mackay 1972). Although the elemental compositions of some insect eggs have been previously investigated (Nickles et al. 1995; Szewczuk et al. 2010), the available data on eggs elemental analysis of insects are still very poor.

Honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), is a common model for biological and physiological studies of insects. There are about 24 different races of *A. mellifera* around the world (Ruttner 1992). The egg hatch of *A. mellifera* takes approximately three days to happen (Laidlaw 1979). The special conditions are

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needed for *A. mellifera* eggs to hatch; hatching process depends on dissolving the chorion by hatching fluid (Collins 2004). In particular, humidity is an important factor for egg hatchability (Doull 1976; Koniger 1978; Bisht et al. 1979; Kraus et al. 1998). Honey bee queens mark their eggs with a chemical signal, which helps honey bee workers to identify the queen eggs (Oldroyd and Ratnieks 2000); it also helps protect eggs from desiccation (Martin et al. 2004).

The previous studies on honey bee eggs have focused on the morphology (Woyke 1998; Gençer and Woyke 2006) ultrastructural and chemical characterization (Katzav-Gozansky et al. 2003) and surface chemicals (Martin et al. 2004). The present study, however, aimed to provide insights about some of the elemental analysis of the eggs. In addition, the differences between honey bee races in their elemental analysis pattern were investigated.

Materials and Methods

Samples of 1-day-old eggs of two honey bee colonies, Yemeni (*A. mellifera yemenitica*) and Carniolan (*A. mellifera carnica*) races, were collected from the apiary of Bee Research Unit (King Saud University).

The preparation of the eggs for scanning electron microscopy was performed as follows. The eggs were then fixed in buffered aldehyde (2.5% glutaraldhyde in phosphate buffer) for 3 h, drained in glutaraldehyde, rinsed 3 times in sodium cacodylate solution buffer (each time for 5 min), rinsed in distilled water, post-fixed in osmium tetroxide for 1 h, dehydrated using a graded ethanol series (25, 50, 75, 100 and 100%; each for 10 min), rinsed in distilled water, mounted on specimen stubs and coated with gold.

The elemental composition of eggs was identified by energy dispersive X-ray spectroscopy (EDS), which was added to the scanning electron microscope (SEM; Jeol JSM-6380LA). The elemental contents at three different sites of the eggs (Figure 1) were recorded. The data were analyzed using Student's *t*-test.

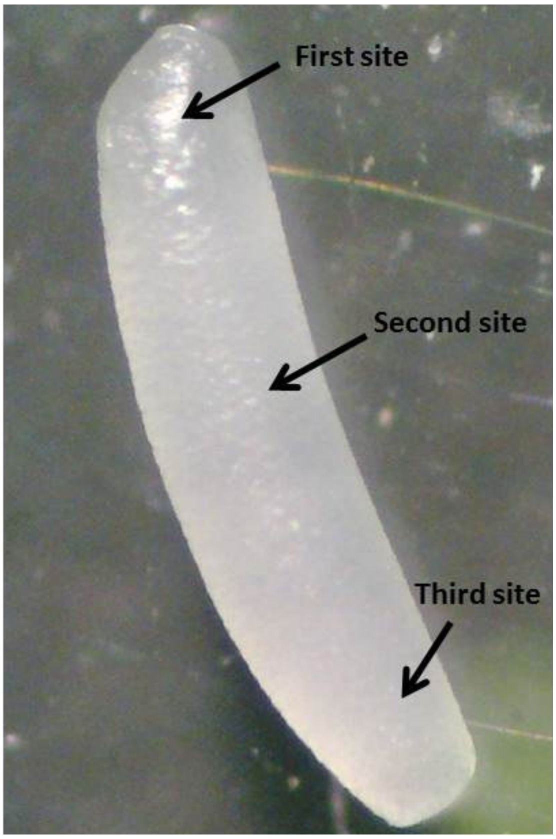

Figure 1. The sites on *A. mellifera* eggs studied for elemental composition

Results and Discussion

In total eight elements, including carbon, nitrogen, oxygen, magnesium, phosphorus, sulfur, calcium and zinc were found (Table 1). However, there were similarities and differences in elemental composition of different egg sites within and between races; carbon, oxygen and sulfur were found in all sites of both the races. In both the races, carbon presented the highest amount of the egg elements, followed by nitrogen.

Table 1. Eggs elemental analysis of the two races of *A. mellifera*

	Mass (%)							
	Site 1[1]		Site 2		Site 3		Mean ± SE	
Elements	Y[2]	C	Y	C	Y	C	Y	C
Carbon	74.91	83.99	63.82	95.96	92.73	60.63	77.15±8.42 a[3]	80.19±10.37 a
Nitrogen	23.42	10.08	34.16	-	3.07	36.76	20.22±9.11 a	15.61±10.96 a
Oxygen	0.98	2.58	1.16	2.06	2.19	1.54	1.45±0.37 a	2.06±0.30 b
Magnesium	-	-	-	0.84	0.79	0.08	0.26±0.26 a	0.31±0.26 a
Phosphorus	0.21	-	0.63	-	-	-	0.28±0.18	-
Sulfur	0.47	1.32	0.23	0.96	1.10	0.99	0.6±0.25 a	1.09±0.11 b
Calcium	-	0.73	-	0.19	-	-	-	0.31±0.21
Zinc	-	1.30	-	-	0.12	-	0.04±0.040 a	0.43±0.43 b

[1] The sites on *A. mellifera* eggs (as shown in Figure 1).
[2] Y and C denote Yemeni (*A. mellifera yemenitica*) and Carniolan (*A. mellifera carnica*) races, respectively.
[3] Different letters within rows show a significant difference ($P < 0.05$, Student's *t*-test).

The pattern of the elemental composition was not fixed for races and within examined sites. In Yemeni race, nitrogen were found in all three sites, phosphorous in only two sites, and magnesium and zinc in only one site; but no calcium were found. In Carniolan race, nitrogen, magnesium and calcium were found in only two sites, and zinc only in one site; but no phosphorous were found.

There was no significance difference between races for the mean percentage of carbon, nitrogen and magnesium contents. However, the mean percentage of oxygen, sulfur and zinc in honey bee eggs were significantly different between the races.

The previous studies on the eggs of *Lycaeides melissa samuelis* (Lepidoptera, Lycaenidae) have shown the presence of oxygen, carbon, nitrogen, phosphorus, sodium, sulfur, magnesium, calcium, silicon, chlorine and potassium in the eggshell, with some differences according to the examined locations (Nickles et al. 1995; Nickles et al. 2002).

There are similarities and differences between the elements found in the present and above-mentioned studies; such differences may be attributed to the differences in taxonomical characteristics.

The elements such as phosphorus, sulfur, potassium and calcium have been also found in the eggs of another arthropod, a crustacean, *Daphnia magna* Straus (Diplostraca, Daphniidae) (Kawasaki et al. 2004). This might be indicative of the presence of common elements in closely-related taxa; this is important because elemental analysis could help in understanding the evolutionary relatedness between insect orders.

In the present study, carbon presented the highest percentage (about 78%) for both the races, followed by nitrogen (about 17%). In a study on the eggs of *Sitophilus granarius* (L.) (Coleoptera, Dryophthoridae), Szewczuk et al. (2010) found high content of nitrogen (about 9%) during elemental analysis. While Nickles et al. (2002) found plenty of magnesium and phosphorus on egg tops, silicon on the sides, sulfur in the vitelline membrane, and calcium and phosphorus in the micropyle of *L. melissa samuelis* eggs. It seems that the highest amount of carbon and nitrogen may be attributed to the organic compounds and egg proteins (*e.g.*, vitellogenin).

The present study indicated that the elemental analysis pattern was not stable in the studied races of honey bee; this open a possible new trend in insect classification and phylogeny based on the egg elemental analysis. In addition, the correlation between egg elemental analysis and egg viability may help egg cryopreservation studies; further studies, however, are required to cover this point.

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