Article

Morphometric study of newly emerged unmated queens of honey bee *Apis mellifera* L. in Ismailia Governorate, Egypt

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Abstract
Recently, morphometric analysis is being a very good tool for identification of honey bee races and characterization of genetic materials. This fact has motivated the present work to investigate the effects of two grafting methods, three types of artificial queen wax cups and four periods of queen rearing on some morphological characters of newly emerged queens. The developed technique used in the present study depends on the integration between Scanner unit and Photoshop program, called Scan Photo Method (SPM). The measurements of 23 morphological characteristics of reared queens were estimated by using SPM. Results indicated significant differences between periods in the measurements of the studied characteristics on forewing such as cubital index, distance C, distance D, radial field, inner wing length, inner wing width, dumb bell index, distance I, II, III and IV, except cubital A and cubital B. Regarding the effects of cup types and grafting methods, data also revealed significant differences in all measurements of studied characteristics, except tibial length, hind wing length, cubital B, radial field and distance IV. Further works in this area were recommended to find out a relation between some morphometric characters and important of some quantitative characters.

Keywords *Apis mellifera*; morphological characters; queen rearing; morphometric analysis.

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1 Introduction

Morphometric analysis is being a very good tool for identification of honey bee races (Rinderer et al., 1995). With its 42 measured morphological parameters, honeybee has become one of the most studied insects (Mladenovic et al., 2011). Several works with *Apis mellifera* involving morphological characteristics showed that there is a strong influence to the environment in the morphology of the same ones (Eischen et al., 1982; Milne and Friars, 1984; Milne et al., 1986). One of these characteristics is the corbicula, whose area and length meet positively correlated with the productivity (Eischen et al., 1982; Milne and Pries, 1984; Kolmer and Sam, 1991). Morphometric methods are based on multiple measurements of many individuals (Alpatov, 1929). Interpretation of the measurements was difficult without multivariate statistical methods, which were

introduced by DuPraw (1964). Several methods were used in measuring the morphological characteristics of both workers and queens of honey bee. These methods were developed from the use of microscopes, binocular, projectors even computer software. Slide scan connected to a computer which displays the forewing of worker honey bee, 17 wing intervention points were chosen to establish a coordinate system for microtaxonomy of honey bee (Mazeed, 2004).

The queen holds the most important position in the colony of honeybee. To put it in more explicit terms, the yield of performance of a colony is associated with the combined effect of the climate, vegetation and its breeding value to large extent. Many techniques of rearing queen bees have been developed to allow beekeepers to reproduce good stock to replace old or undesirable queens in their colonies, or to start new colonies (Genc, 1992). Successful queen rearing demands suitable conditions. Attempting to rear queens at the wrong time of year will result in poor quality queens. The quality of the queen bee determines the benefits received from honey bee colony as through the queen, via its progeny, the productivity, temperament and behavior of the colony can be manipulated by the beekeeper. Various environmental factors affect the quality of the queen bees, as well as the rearing season and meteorological conditions influenced the rate of queen development (Mahbobi et al., 2012; Fresnaye, 1966).

The objective of this study was to verify the changes on some morphological characters of newly emerged reared queens in order to improve their quality characteristics.

2 Materials and Methods

This research was conducted at Bee Research Unit Apiary, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Carniolan honeybee hybrid *Apis mellifera carnica* was used in the present investigation. Unmated queens were reared based on a standard commercial Doolittle method (Laidlaw and Eckert, 1962). To examine the effect of rearing time on morphometric parameters, queens were reared during four months (March, April, May and July) in 2010.

2.1 Types of queen cups

Three types of artificial queen cups which made from honey bee wax were used in the present work. The first one was 10 mm in depth and had two layers of wax (ordinary cup), the second one was 10 mm in depth and had four layers of wax (thick cup) and the last one was 20 mm in depth with two layers of wax (long cup) (Abrol et al., 2005).

2.2 Types of grafting

Two types of single grafting method were used in this experiment, dry and wet grafting (Laidlaw, 1975). Ninety cups were distributed and fixed on six wooden bars, using melted wax. The bars fitted into two frames, three bars in each frame and five cm between each other. Forty-five larvae less than one-day-old transferred into tested queen cups using the two grafting methods. The grafted frames immediately introduced to queenless colony to initiate queen rearing. On the fourteenth day of grafting, queens emerged from the succeeded cups inside the aluminum wire-mesh cages. These treatments were repeated four times in every month once every week and then data were taken as an average through the time of experiment.

2.3 Morphological studies

The newly emerged queens were collected and kept individually in small glass vials and fixed for three hours in carnoy´s solution, then rinsed by ethanol 70% three times and preserved in it. Ten of queen's samples were dissected using forceps to separate body parts (head capsules, fore-wings, hind-wings and hind-legs), mounted on glass slide covered by another one in order to obtain appropriate samples for morphometric measurements. Integration between Scanner unit and Photoshop program, called Scan Photo Method (SPM). In SPM, scanner connected to personal computer provided with Photoshop software. This technique was used for measuring

morphological characteristics (Fig.1a), (El-Aw et al., 2012). The scanned image saved in personal computer (Fig.1b) and analyzed with measuring tool of Photoshop program that showed the units of measurement in (mm) (Fig. 1c). The measurements of 23 morphological characters of reared unmated queens were estimated using the previously developed technique (SPM).

The selected morphological characteristics were; head characteristics (head capsule length and width), thorax characteristics included forewing length and width, hind wing length and width, and hind leg characteristics (femur length, tibial length and basitarsus length and width). The abovementioned characteristics were selected to study according to Ruttner et al. (1978).

2.4 Statistical analysis

Data obtained from morphological characteristics were statistically analyzed through ANOVA (SAS Institute 2002). When F-test was significant, means were separated using Tukey's Honestly Significant Difference (HSD) Test at the 0.05 level of significance.

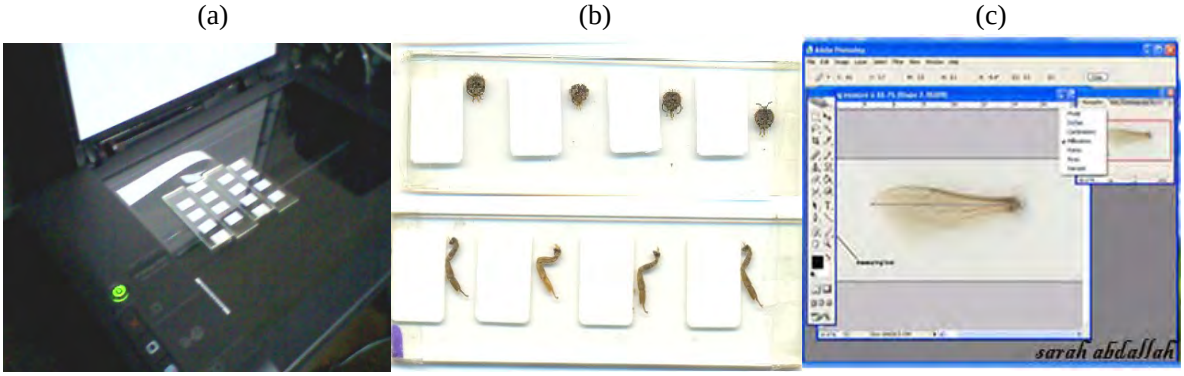

Fig. 1 Steps of measuring morphological characteristics using SPM. The following 23 morphological characteristics had been measured. head capsule length and width, forewing length and width, cubital A length, cubital B length, cubital index, distance D, distance C, radial field, inner wing length, inner wing width, dumb bell index, distance I, distance II, distance III and Distance IV, the hind-wing length and width, the femur length of hind leg, tibial length and the basitarsus length and width.

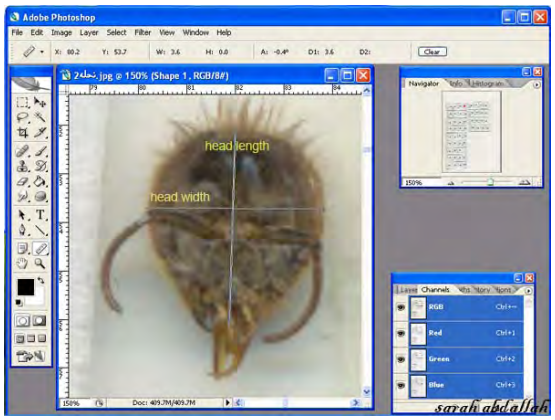

Fig. 2 Measuring of head capsule length and width in mm.

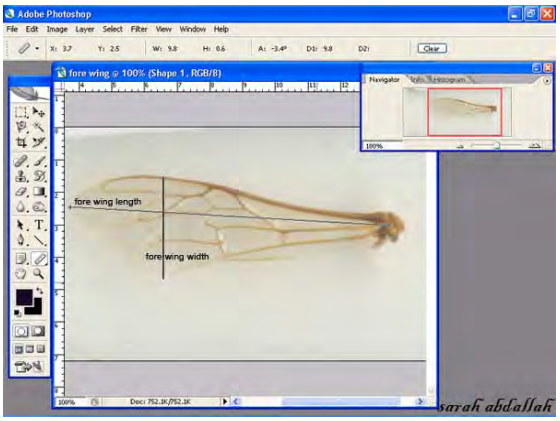

Fig. 3 Measuring of forewing length and width in mm.

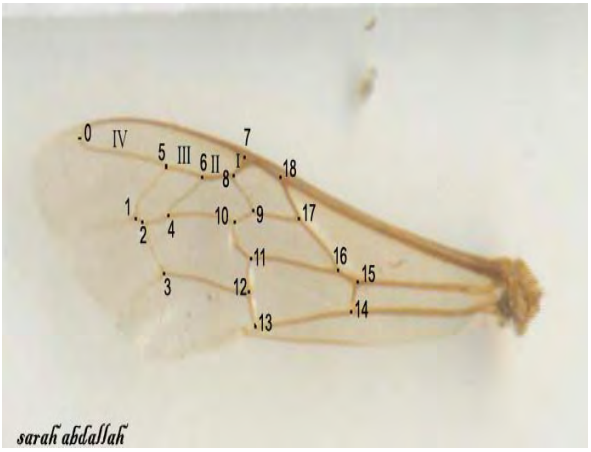

Fig. 4 Forewing venation pattern showing wing points (0-18). (Figure described by Bee Research Institute, Dol, Czech Republic)

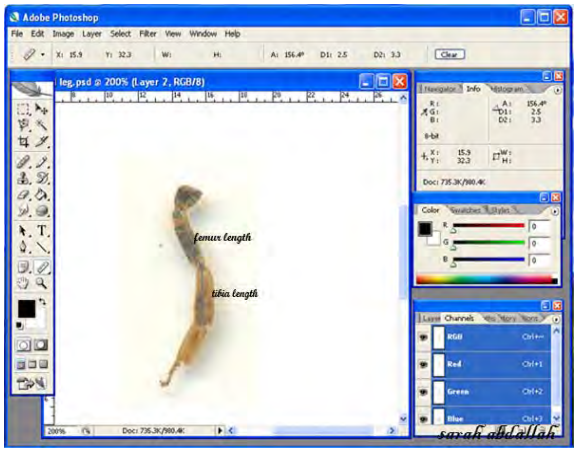

Fig. 5 Measuring of hind leg characteristics in mm.

3 Results and Discussion

3.1 Effects of rearing time on morphological characteristics of reared unmated queens

The measurements of 23 morphological characteristics of reared queens under the environmental conditions of Ismailia during four months of 2010 showed in Table 1. The maximum length of femur, tibia and basitarsus in hind leg were recorded during April as 2.72, 3.33 and 2.45 mm, respectively. While the shortest length was 2.65 and 2.33 mm during May for femur and basitarsus, and 3.21 mm during March for tibia. Also, the maximum width of basitarsus was recorded during April and May as 1.19 and 1.188 mm, while the shortest width was 1.12 mm during July. Concerning hind wing, the maximum length was recorded as 6.97 and 6.95 mm during July and April, respectively, while the shortest length was 6.78 mm during March. The maximum width of hind wing was 2.03 mm in March, while the lowest width was 1.97 mm in July. Regarding the measurements of forewing, the maximum length was 9.7 mm during July, while the shortest length was 9.5 mm during April. The width of forewing was recorded in maximum average of 3.05 mm during March and July, while the minimum width was 2.90 mm during May. Results in Table 1 showed significant differences ($P<0.05$) between periods in the measurements of the studied characteristics on forewing such cubital index, distance C, distance D, cupital index, radial field, Inner wing length, inner wing width, dumb bell index and distances I, II, III and IV, except cubital A and cubital B. The maximum distance of cubital A was recorded during March and April as 0.54 mm, while the minimum distance was 0.53 during May and July. Also, the maximum distance of cubital B, distance C, distance D and cubital index were 0.255, 0.81, 2 and 2.5 mm during July, March, July and April, respectively. The minimum length distance of these parameters were 0.23, 0.77, 1.91 and 2.1 mm during March, July, May and July, respectively. Also, the highest value for radial field was 3.31 mm in July, but the lowest value was 3.15 mm in May. The maximum length and width of Inner forewing were 4.32 and 2.20 mm in July, while the shortest length and width were 4.16 and 2.09 mm in May and April, respectively. The longest distance of dumb bell index was 1.02 mm in April, while the shorter length was 0.97 mm in March. The longest distances of I, II, III and IV were 0.39, 0.51, 0.77 and 1.82 mm during March, July, (March, May and July) and April, respectively. While the shortest lengths of these characteristics were 0.35, 0.45, 0.37 and 1.73 mm during April, May, April and May, respectively. The maximum length of head capsule was 4.01 mm in April, while the minimum was 3.86 mm in July. The maximum width of head capsule was 3.81 mm in July, while the shortest width was 3.67 mm in May.

These results revealed highly significant differences ($P<0.05$) between periods in the measurements of

studied characters of reared queens. Highly significant difference (P<0.05) was observed in the measurements of hind leg characters, cubital index and head capsule length during April compared to the rest periods. Also, highly significant differences (P<0.05) were recorded especially, for the length of fore and hind wing during July.

Morphometrics were used previously as a tool for the study of genetic variability of honeybees. Previous studies focused on studying the morphological characteristics of workers for investigating biodiversity of honey bee (Kekeçoğlu, 2007). However, for the first time in the current study, newly emerged honeybees queens were studied using 23 morphometric characters in an attempt to determine the quality of reared queens in different periods in such area of experiment.

The effect of different periods on the quality of reared queens has been previously studied by measuring traits other than morphological characters such as weight of queens, preoviposition period and numbers of sperms in the spermathecae (Koç and Karacaoğlu, 2011). The present results are in agreement with those reported by Shawer et al. (1980) who found that the body weight of virgin queens differed significantly depending on the rearing season. They also reported that the queens produced in Egypt during May and August were more body weight than those reared in other months. Moreover, Koç and Karacaoğlu (2004) mentioned that the queens of honey bee can be reared in the Aegean Region in Turkey from the end of March to September, but better quality of produced queens was obtained from the end of March until the end of April. Also, the same way by El-Sarrag (1993) who observed in Saudi Arabia that, honeybee queens were successfully reared in February (92%) and during March – June (82%), and decreased sharply during August-September (48%) to reach the minimum (28%) during December – January.

Table 1 Effects of rearing time on morphological characteristics of reared unmated queens.

Months / Morphological characteristics (mm)	March	April	May	July
Femur Length	2.69 a	2.72 a	2.65 a	2.67 a
Tibia Length	3.21 b	3.33 a	3.23 b	3.27 b
Basitarsus Length	2.37 b	2.45 a	2.33 b	2.39 b
Basitarsus Width	1.17 a	1.19 a	1.188 a	1.12 b
Hind Wing Length	6.78 b	6.95 a	6.79 b	6.97 a
Hind Wing Width	2.03 a	2.01 ab	2.01 ab	1.97 b
Fore Wing Length	9.4 b	9.5 b	9.2 c	9.7 a
Fore Wing Width	3.05 a	3.01 ab	2.9 b	3.05 a
Cubital A	0.54 a	0.54 a	0.53 a	0.53 a
Cubital B	0.23 a	0.225 a	0.226 a	0.255 a
Distance C	0.81 a	0.78 ab	0.78 ab	0.77 b
Distance D	1.93 ab	1.94 a	1.91 b	2 a
Cubital index	2.46 a	2.5 a	2.44 a	2.1 b
Radial field	3.21 b	3.23 b	3.15 c	3.31 a
Inner Wing Length	4.22 b	4.25 ab	4.16 b	4.32 a
Inner wing width	2.13 b	2.09 b	2.12 b	2.20 a
Dumb bell index	0.97 a	1.02 a	0.98 a	1.00 a
I	0.39 a	0.35 b	0.36 b	0.36 b
II	0.46 bc	0.47 b	0.45 c	0.51 a
III	0.77 a	0.37 b	0.77 a	0.77 a
IV	1.81 a	1.82 a	1.73 b	1.75 a
Head Capsule Length	Ms**	4.01 a	3.93 a	3.86 b
Head Capsule Width	Ms**	3.72 b	3.67 b	3.81 a

*Means in the same row followed by the same letter are not significantly different at the P=0.05 level according to Tukey's Honestly Significant Difference ** Ms: Missing data.

3.2 Effect of grafting methods and wax cup types on morphological characteristics of reared unmated queens

Data in Table 2 revealed significant differences in all measurements of studied characteristics, except tibia length, hind wing length, cubital B, radial field and IV. The maximum length of femur and tibia of hind leg were obtained when used thick cups and wet graft with an average of 2.8 and 3.37 mm, respectively. While the shortest length was 2.66 and 3.29 mm for femur by using long cups with wet graft, and tibia using long cups with dry graft, respectively. The maximum length of femur, tibia, basitarsus length and width of hind leg were obtained when used thick cups with wet graft, thick cups with wet graft, ordinary cups with wet graft and ordinary cups with wet graft with an average of 2.8, 3.37, 2.51 and 1.22 mm, respectively. While the shortest length was 2.66, 3.29, 2.41 and 1.17 mm for femur, tibia, basitarsus length and width using long cups with wet graft, long cups with dry graft, long cups with wet graft and thick cups with wet graft, respectively. The longest and widest hind wing was 6.99 and 2.05 mm using long cup with wet graft and thick cups with dry graft, while the shortest one was 6.90 and 1.99 mm using long cups with dry graft and ordinary cups with dry graft. The longest and widest forewing was 9.64 and 3.10 mm using long cups with wet graft and thick cups with wet graft, while the shortest and narrowest was 9.40 and 2.97 mm using thick cups with dry graft and long cups with wet graft. The longest distance of cubital A, cubital B, distance C and distance D was 0.56, 0.23, 0.81 and 1.96 mm using ordinary cups with dry graft and thick cups with wet graft, all types of cups except thick cups with dry graft, thick cups with dry graft, and ordinary cups with wet graft and long cups with wet graft, respectively. While the shortest one was 0.51, 0.21, 0.75 and 1.91 mm for ordinary cups with wet graft, all types of cups except thick cups with dry graft, ordinary cups with wet graft, and long cups with dry graft. The longest distances of cubital index, radial field, inner wing length and width were 2.79, 3.26, 4.31 and 2.17 mm using thick cups with dry and wet graft, while the shorter one was 2.33, 3.20, 4.12 and 2.09 mm using ordinary cups with wet graft. The longest distance of dumb bell index, I, II, III and IV was 1.07, 0.36, 0.50, 0.76 and 1.84 mm using thick cups with wet graft, thick cups with dry graft, ordinary cups with dry graft, long cups with dry and wet graft, and ordinary cups with wet graft. While the shortest distances were 0.99, 0.33, 0.46, 0.70 and 1.79 mm using long cups with wet graft, long cups with wet graft, thick cups with wet graft and long cups with dry and wet graft, ordinary and thick cups with dry graft, and long cups with dry graft, respectively. The longest and widest head capsule was 4.10 and 3.76 mm using ordinary cups with dry graft and thick cups with dry graft, while the shortest one was 3.95 and 3.66 mm using long cups with dry graft and long cups with wet graft.

The obtained results showed significant differences ($P<0.05$) between grafting methods and wax cup types in the measurements of most studied characters of newly emerged queens. The mean values of the 9 morphological characters (femur length, hind wing width, fore wing width, cubital A, cubital B, radial field, inner wing length, inner wing width and dumb bell index) of queens reared from thick cups and wet grafting were higher significantly differences ($P<0.05$) than those of queens reared from other two types of cups and dry grafting. Moreover, the mean value of 6 morphological characters (basitarsus length, basitarsus width, forewing length, cubital B, distance D and distance IV) of queens reared from the ordinary cups and wet grafting were significantly higher ($P < 0.05$) than those of queens reared from the other cup types and dry grafting.

Table 2 Effect of grafting methods and wax cup types on morphological characteristics of reared unmated queens.

Cups and grafting types / Morphological characteristics (mm)	Ordinary cup		Thick cup		Long cup	
	Dry	Wet	Dry	Wet	Dry	Wet
Femur Length	2.7 ab	2.7 ab	2.7 ab	2.8 a	2.7 ab	2.66 b
Tibia Length	3.36 a	3.32 a	3.30 a	3.38 a	3.29 a	3.33 a
Basitarsus Length	2.46 ab	2.51 a	2.46 ab	2.45 ab	2.42 b	2.41 b
Basitarsus Width	1.18 b	1.22 a	1.17 b	1.19 ab	1.18 ab	1.2 ab
Hind Wing Length	6.92 a	6.96 a	6.93 a	6.95 a	6.9 a	6.99 a
Hind Wing Width	1.99 b	2.03 ab	2.02 ab	2.05 a	2 ab	1.99 b
Fore Wing Length	9.52 ab	9.57 a	9.4 b	9.51 ab	9.53 ab	9.64 a
Fore Wing Width	3.03 ab	2.99 ab	3.01 ab	3.1 a	3.01 ab	2.97 b
Cubital A	0.56 a	0.51 b	0.54 ab	0.56 a	0.54 ab	0.53 ab
Cubital B	0.23 a	0.23 a	0.21 a	0.23 a	0.23 a	0.23 a
Distance C	0.79 ab	0.75 c	0.81 a	0.79 ab	0.79 ab	0.77 bc
Distance D	1.92 b	1.96 a	1.93 ab	1.94 ab	1.91 b	1.96 a
Cubital index	2.53 ab	2.33 b	2.79 a	2.54 ab	2.64 ab	2.39 ab
Radial field	3.22 a	3.20 a	3.23 a	3.26 a	3.23 a	3.26 a
Inner Wing Length	4.18 bc	4.12 c	4.22 b	4.31 a	4.24 b	4.25 b
Inner wing width	2.14 ab	2.09 c	2.14 ab	2.17 a	2.15 ab	2.11 bc
Dumb bell index	1.06 a	0.96 b	1.02 ab	1.07 a	1.01 ab	0.99 ab
I	0.36 ab	0.35 ab	0.36 a	0.34 ab	0.36 ab	0.33 b
II	0.5 a	0.49 ab	0.49 ab	0.46 b	0.46 b	0.46 b
III	0.70 b	0.74 ab	0.70 b	0.72 ab	0.76 a	0.76 a
IV	1.83 a	1.84 a	1.83 a	1.83 a	1.79 a	1.81 a
Head Capsule Length	4.1 a	4.04 ab	4.04 ab	3.98 b	3.95 b	3.96 b
Head Capsule Width	3.66 b	3.72 ab	3.76 a	3.73 ab	3.66 b	3.76 a

*Means in the same row followed by the same letter are not significantly different at the P=0.05 level according to Tukey's Honestly Significant Difference.

This study was determined the effect of grafting methods and wax cup types on the morphological characters as a qualified evidence of honeybee queens. While the previous studies focused on the impact of these rearing methods on other characters such as larvae acceptance rate, percentage of sealed queen cell, queen emergence, developing time and weight of newly emerged queens (Genc et al., 2005; Nageh et al., 2010; Abd Al-Fattah et al., 2011). In the future more research should be carried out for morphometric analysis and different methods of honey bee queen rearing in order to find a real relationship between some morphometric characteristics and the most important quantitative characters of reared queens.

4 Conclusion

Our results showed that, with the exception of some morphological characters, rearing periods had significantly influenced on the most important morphological parameters of newly emerged honey bee queens under the conditions of Ismailia region. Likewise, the grafting methods and wax cup types had notably differences in most of these characters.

Finally, we concluded that in order to improve the characteristics quality of the produced queens, we should advised beekeepers to select the ideal periods for queen rearing using the most suitable grafting method with wax cup type. According to the conditions of the studied area, the most appropriate time was during April and July using wet graft and thick wax cups, due to the improvements that have been observed on the morphological characteristics of reared queens.

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