

## STABILITY OF HONEY BEE MORPHOLOGICAL CHARACTERS WITHIN OPEN POPULATIONS

### Açık Populasyonlarda Balarısı Morfolojik Karakterlerin Değişmezliği

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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**Anahtar Kelimeler:** Bal arısı, *Apis mellifera*, morfoloji, değişmezlik, bölgeler, çevre.

#### ABSTRACT

Honey bee (*Apis mellifera*) is being kept in different parts in the world. There are many practices which are done on honey bee colonies by beekeepers. Such practices (e.g. requeening and migratory beekeeping) lead to differences in the characteristics of honey bee colonies in the course of time. Morphological characters of honey bees can be measured to characterize honey bee populations and to be used as an indicator for productivity of honey bee colonies. To characterize honey bee populations, the known method depends on the collection of random samples of honey bee workers from different hives and locations. However, there are different factors that can affect morphological characters. Thus, studying the stability degree of these characters is required to identify fluctuation levels within open populations of honey bees and to recommend the suitable method for its characterization. Morphological characters of 96 honey bee colonies and 1440 honey bee workers in six districts were studied for two successive years and obtained results were compared. Morphological traits of the second year were lower than the first year in most of studied characters, especially cubital index, in studied districts except for tongue length which increased in all studied districts by 0.19 to 0.69 mm. Obtained results showed that, for a fast screening for alterations happened in bee populations, it is sufficient to measure cubital index and tongue length. Also, taking the mean of morphological measurements for at least two years is considered sufficient to characterize open honey bee populations.

#### INTRODUCTION:

The honey bee, *Apis mellifera* L., is widespread in Africa, Europe, and parts of Asia with a wide diversity of subspecies that can be classified with Morphometric tools (Ruttner, 1975; Ruttner *et al.*, 1978). Honey bees differ in their morphology, behavior and physiology according to the environmental conditions they have adapted to (Ruttner, 1992). Based primarily on morphological

characters, more than two dozen subspecies have been described within the lineages (Ruttner, 1992; Sheppard *et al.*, 1997). Morphological studies have provided a large amount of information on the structure of *A. mellifera* L. species (Garnery *et al.*, 2004). The discrimination between honey bee subspecies is important for beekeeping and the preserving of honey bee biodiversity (Tofilski, 2004). Most efforts to differentiate honey bee groups, based on morphological data, have used

multiple body characteristics, including worker body size, hair length, wing length and width, and proboscis length (Bucó *et al.* 1987; Rinderer *et al.* 1993; Crewe *et al.*, 1994; Ftayeh *et al.* 1994; Diniz-Filho and Malaspina, 1995; Quezada-Euan *et al.*, 2003). Wing measurements are very important for honey bee classification (Nielsen *et al.*, 1999). The most simple method in honey bee classification is measuring the fore wing characters (Kauhausen-Keller and Keller., 1994). Cubital index (a ratio of lengths of two wing veins) has been considered the most important character used for honey bee classification. Many subspecies of *A. mellifera* have been described and discriminated mainly according to their Cubital index values (Tofilski, 2004; Rostecki *et al.*, 2007).

Morphological characters of honey bees were found to be correlated with other colony productive characteristics. Poklukar Kezic, (1994) found that the Cubital index of Carniolan honey bees was related positively to swarming tendency and negatively to aggressiveness. Honey bees with bigger leg and wing have higher flight power and could gather more pollen and nectar (Mostajeran *et al.*, 2006). There was a correlation between honey production and overall size, corbicular area, wing measurements and tongue length (Cobey and Lawrence, 1988; Kolmes and Sam, 1991; Milne and Pries, 1984 and Mostajeran *et al.*, 2002). Wing size influences flight ability (Mattu and Verma, 1989). Honey production can be improved by selection for the fore wing width (Edriss *et al.*, 2002). Beekeeping practices such as honey bee stock importation and migratory beekeeping might induce high levels of introgression within populations (Dražić *et al.*, 2004 and Rortais *et al.*, 2004). The introduction of honey bee subspecies into different geographic areas by beekeepers has produced subspecies admixtures in many parts of the world (Arias *et al.*, 2006). Several works with *A. mellifera* involving morphological characters showed that there is a strong influence of the environment in the morphology of honey bees (Eischen *et al.*, 1982 and Milne *et al.*, 1986).

The common method that was used in honey bee population characterization is based mainly on the collection of random honey bee samples of about 15 honey bee workers from a different random number of colonies and locations. Taking into account the presence of different factors that affect on the morphological characters of honey bees, this research aimed to: study the stability degree of

honey bee morphological characters within open populations; test the traditional methods of the characterization of honey bee populations by doing the characterization of different honey bee populations for two successive years; identify the most stable characters, and the fluctuation degree within characters; recommend a suitable method for the characterization of honey bee populations.

### MATERIALS AND METHODS:

Morphological characters of Carniolan honey bee workers were measured for six districts (1- Damanhour, 2-Etay El-Baroud, 3- El-Mahmoudia, 4-Hosh Esa, 5- El-Dalangat, and 6- Kafer El-Dawar) in Egypt for two successive years (2006 and 2007). These morphological characters were compared and statistically analysed.

For measuring morphological characters, samples of honey bee workers were collected during autumn seasons (September to November) of 2006 and 2007. Eight colonies were chosen randomly per district with a total of (96 colonies/ 2 years). Each colony was represented by 15 honey bee workers according to the methods of Ruttner *et al.* (2000), Sheppard and Meixner (2003) and Meixner *et al.* (2007). Samples were collected directly from brood comb according to Padilla *et al.* (1992) by shaking bees in a jar. A total of 120 honey bee workers were collected from each district per year (1440 honey bee workers/ 2 years).

Collected bees were killed in a deep freezer. The carrying out of measurements such as tongue length was easier when samples were frozen. Honey bee workers were dissected using forceps to separate body parts (tongue, right fore wing, right hind wing, and right hind leg). Studied morphological characters were head characters (tongue length) and thorax characters (fore wing length, fore wing width, number of hooks, Cubital A length, Cubital B length, Cubital index, Distance C and D value, hind wing length, hind wing width, hind leg femur length, hind leg tibia length, hind leg basitarsus length and hind leg basitarsus width). All studied characters were measured by Scan Photo method (Abou-Shaara, *et al.*, 2011) as separated body parts were scanned by using scanner and then were measured by Photoshop Program.

The characterization of honey bee workers of each district was done for the two years and differences were identified. The data were statistically analyzed by analysis of variance (ANOVA) and means were

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compared using the Least Significant Difference test L.S.D.0.05.

### RESULTS:

The mean values of studied morphological characters of honey bee workers from the six studied districts showed that there were differences in the measurements of all the studied morphological characters among all of the studied districts for 2006 and 2007 (Table1 and Table 2).

Statistical analysis for 2006 measurements showed that, except for basetarsus width, there were significant differences among districts ( $P < 0.05$ ) in all studied morphological characters. On the other hand, statistical analysis for 2007 revealed the presence of significant differences among locations ( $P < 0.05$ ) in all studied morphological characters except: fore wing length, hind wing length, distance D, femur length, and basetarsus length.

**Table 1. Morphological characters for studied districts of 2006.**

Morphological character	District (Mean $\pm$ S.D.)**					
	1	2	3	4	5	6
Tongue length (Ton L)	5.46 $\pm$ 0.15 c*	5.47 $\pm$ 0.12 c	5.24 $\pm$ 0.06 d	5.60 $\pm$ 0.26 bc	5.68 $\pm$ 0.17 ab	5.79 $\pm$ 0.09 a
Fore wing length (FWL)	8.86 $\pm$ 0.09 a	8.82 $\pm$ 0.09 ab	8.76 $\pm$ 0.10 bc	8.73 $\pm$ 0.06 cd	8.62 $\pm$ 0.04 e	8.65 $\pm$ 0.10 de
Fore wing width (FWW)	3.00 $\pm$ 0.07 ab	2.98 $\pm$ 0.06 abc	3.03 $\pm$ 0.04 a	3.02 $\pm$ 0.03 ab	2.92 $\pm$ 0.05 c	2.96 $\pm$ 0.06 bc
Hind wing length (HWL)	6.18 $\pm$ 0.10 a	6.13 $\pm$ 0.04 ab	6.16 $\pm$ 0.04 a	6.12 $\pm$ 0.06 ab	6.05 $\pm$ 0.06 c	6.09 $\pm$ 0.03 bc
Hind wing width (HWW)	1.82 $\pm$ 0.08 ab	1.72 $\pm$ 0.05 c	1.79 $\pm$ 0.07 b	1.85 $\pm$ 0.04 a	1.71 $\pm$ 0.04 c	1.84 $\pm$ 0.03 ab
Cubital Index (CI)	2.93 $\pm$ 0.74 bc	2.54 $\pm$ 0.14 c	3.09 $\pm$ 0.50 b	3.38 $\pm$ 0.86 ab	3.79 $\pm$ 0.35 a	2.87 $\pm$ 0.37 bc
Distance C (DC)	0.80 $\pm$ 0.02 b	0.81 $\pm$ 0.01ab	0.79 $\pm$ 0.02 b	0.81 $\pm$ 0.01 ab	0.83 $\pm$ 0.03 a	0.83 $\pm$ 0.02 a
Distance D (DD)	1.85 $\pm$ 0.03 c	1.91 $\pm$ 0.03a	1.89 $\pm$ 0.04 ab	1.87 $\pm$ 0.03 bc	1.89 $\pm$ 0.02 ab	1.90 $\pm$ 0.02 ab
Number of hooks (NH)	20.85 $\pm$ 0.48 a	20.36 $\pm$ 0.33 ab	20.24 $\pm$ 0.79 b	20.59 $\pm$ 0.33 ab	19.41 $\pm$ 0.29 c	19.55 $\pm$ 0.26 c
Femur length (FL)	2.28 $\pm$ 0.04 ab	2.24 $\pm$ 0.06 bc	2.29 $\pm$ 0.06 a	2.25 $\pm$ 0.02 abc	2.22 $\pm$ 0.01 c	2.22 $\pm$ 0.05 c
Tibia length (TL)	2.82 $\pm$ 0.06 bc	2.80 $\pm$ 0.04 bc	2.91 $\pm$ 0.04 a	2.83 $\pm$ 0.04 b	2.78 $\pm$ 0.04 c	2.81 $\pm$ 0.04 bc
Basitarsus length (BL)	2.1 $\pm$ 0.03 b	2.08 $\pm$ 0.02 cd	2.18 $\pm$ 0.04 a	2.11 $\pm$ 0.03 bc	2.07 $\pm$ 0.03 d	2.07 $\pm$ 0.04 d
Basitarsus width (BW)	1.09 $\pm$ 0.04 a	1.10 $\pm$ 0.03 a	1.11 $\pm$ 0.01 a	1.11 $\pm$ 0.01 a	1.12 $\pm$ 0.02 a	1.12 $\pm$ 0.02 a

\*Means in the same row followed by the same letter(s) are not significantly different according to L.S.D.0.05.

\*\*All Characters are in length units (mm) except cubital index and number of hooks.

L.S.D. 0.05 values: Ton L= 0.16; FWL= 0.08; FWW=0.06; HWL=0.06; HWW= 0.05; CA=0.02; CB= 0.02; CI=0.56; DC=0.02; DD=0.03; NH=0.49; FL= 0.04; TL= 0.04; BL= 0.03 and BW=0.08.

The overall means of the studied morphological characters of honey bee workers showed variations between the two years of the study, as shown in Table 3. Some characters increased in 2007 while the others decreased. Also, the variations between locations in 2006 were greater than those of 2007. There was one insignificant difference of (basetarsus width) in 2006 versus five insignificant

differences of (fore wing length, hind wing length, distance D, femur length and basetarsus length) in 2007. Statistical analysis for the two years showed that all studied morphological characters were found to show significant differences among districts ( $P < 0.05$ ) except for fore wing width, cubital index and distance C.

**Table 2. Morphological characters for studied districts of 2007.**

Morphological character	District (Mean±S.D.)**					
	1	2	3	4	5	6
Tongue length (Ton L)	6.05±0.07A a*	5.97±0.08 bc	5.94±0.09 bc	5.92±0.08 c	5.94±0.05b c	5.98±0.07 b
Fore wing length (FWL)	8.71±0.09 a	8.82±0.02 a	8.72±0.06 a	8.73±0.07 a	8.72±0.03 a	8.74±0.01 a
Fore wing width (FWW)	2.98±0.03 ab	2.92±0.03 b	2.88±0.04 b	2.90±0.02 ab	2.96±0.02 ab	3.03±0.09 a
Hind wing length (HWL)	6.05±0.05 a	6.16±0.04 a	6.10±0.04 a	6.11±0.03 a	6.15±0.05 a	6.04±0.06 a
Hind wing width (HWW)	1.71±0.05 bc	1.67±0.01 c	1.76±0.06 ab	1.78±0.04 a	1.77±0.03 a	1.80±0.01 a
Cubital Index (CI)	3.19±0.44 a	3.02±1.15 ab	2.81±1.14 abc	2.58±0.28 bc	2.45±0.20 c	2.64±0.28 bc
Distance C (DC)	0.81±0.02 ab	0.80±0.01 b	0.83±0.002 a	0.81 ± 0.01 ab	0.82±0.01 ab	0.82±0.02 ab
Distance D (DD)	1.86±0.02 a	1.87±0.04 a	1.91±0.01 a	1.82±0.04 a	1.86±0.04 a	1.85±0.03 a
Number of hooks (NH)	21.15±1.05 a	20.12±1.15 b	20.51±1.28 ab	20.47±1.55 ab	20.69±1.36 ab	20.20 ±1.12 b
Femur length (FL)	2.24±0.04 a	2.24 ± 0.03 a	2.22 ± 0.02 a	2.22 ± 0.02 a	2.21±0.02 a	2.28±0.01 a
Tibia length (TL)	2.79±0.03 c	2.79±0.02 c	2.84 ± 0.02 ab	2.85 ± 0.01 a	2.80±0.01 bc	2.82±0.01 abc
Basitarsus length (BL)	2.07±0.04 a	2.14±0.03 a	2.14 ± 0.03 a	2.09 ± 0.03 a	2.12±0.02 a	2.11±0.05 a
Basitarsus width (BW)	1.07±0.03 b	1.11 ± 0.01 ab	1.12 ± 0.03 a	1.11 ± 0.01 ab	1.11±0.01 ab	1.10±0.005 ab

\*Means in the same row followed by the same letter(s) are not significantly different according to L.S.D.0.05.

\*\*All Characters are in length units (mm) except cubital index and number of hooks.

L.S.D. 0.05 values: Ton L= 0.05; FWL= 0.15; FWW=0.10; HWL=0.27; HWW= 0.05; CI=0.52; DC=0.02; DD=0.09; NH=0.82.; FL= 0.07; TL= 0.04; BL= 0.07 and BW=0.04.

**Table 3. Morphological characters for 2006 and 2007 years, and the overall mean of the two studied years.**

Morphological characters	2006 (Mean±S.D.)	2007 (Mean±S.D.)	Overall (Mean±S.D.)
Tongue length (mm)	5.54 ±0.19	5.97 ±0.05	5.76±0.30
Fore wing Length (mm)	8.75± 0.09	8.74± 0.04	8.75±0.01
Fore wing width (mm)	2.99± 0.04	2.93± 0.06	2.96±0.04
Hind wing Length (mm)	6.13±0.05	6.10±0.05	6.12±0.02
Hind wing width (mm)	1.79±0.06	1.74±0.05	1.77±0.03
Cubital index	3.10 ±0.43	2.78 ±0.28	2.94±0.23
Distance C (mm)	0.81±0.03	0.81±0.01	0.81±0.01
Distance D (mm)	1.89±0.01	1.86±0.03	1.88±0.02
Number of Hooks	20.17±0.57	20.52±0.37	20.34±0.25
Femur length (mm)	2.25±0.03	2.23±0.02	2.24±0.01
Tibia length (mm)	2.83±0.04	2.81±0.02	2.82±0.01
Basetarsus length (mm)	2.11± 0.05	2.15± 0.08	2.13±0.03
Basetarsus width (mm)	1.11± 0.01	1.10± 0.02	1.11±0.01

### DISCUSSION:

Characters of studied honey bee workers for 2007 were lower than 2006 in most of the studied characters except tongue length, basitarsus length and number of hooks. These results may be attributed to the beekeeping activities like requeening. Moreover, such differences could be due to the introduction of some honey bee queens belonging to different races. The importation of honey bee subspecies by beekeepers might induce high levels of differences within populations (Garnery *et al.*, 1998 and Rortais *et al.*, 2004) and produced subspecies admixtures in many parts of the world (Arias *et al.*, 2006). Also, the migratory beekeeping may play a key role in forming differences in accordance with Marghitas *et al.* (2008), who showed that the honey bee ecotype genes are mixed due to the migratory beekeeping. In addition, the honey bees differ in their morphology according to the environmental conditions they have adapted to (Ruttner, 1992) and there is strong influence of the environment on honey bee morphology (Eischen *et al.*, 1982; Milne and Pries, 1984; and Milne *et al.*, 1986).

Results revealed that tongue length was the only character that increased in all districts in 2007 by 0.19 to 0.69 mm. This increase in tongue length may be due to the changes in environmental conditions as well as in the studied queens. Marghitas *et al.* (2008) stated that the length of the tongue was considered as a very important character because it shows the geographical variability more accurately than all the other characters. Morimoto (1968) mentioned that tongue length is an important character, showing higher geographic variability and upon which the quantity of nectar gathered depends. Also, Souza *et al.* (2002) stated that the variation between tongue lengths may be important in the exploitation of the environmental resources.

The study points out that distance D, femur length, and basitarsus width can be considered as more stable characters within open populations. These characters were insignificant differences within 2006 or 2007 and differences between districts for these characters were not more than 0.07 mm.

In general, it could be concluded that morphology of open honey bee populations is not stable and under the influence of many factors and that two years of study could be considered sufficient to characterize such populations. Moreover, it could

be sufficient to measure cubital index and tongue length for a fast screening for alterations happened in a bee population. In accordance with previous studies, tongue length reflects environmental factors and cubital index genetic variability. Results of such study could be helpful in the conservation of honey bees as some characters can be measured for honey bee populations periodically to monitor what happens to honey bee characters and to promote the appropriate steps for saving the honey bees.

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### GENİŞLETİLMİŞ ÖZET:

**GİRİŞ:** Bal arısı (*Apis mellifera*) dünyanın farklı bölgelerinde kullanılmaktadır. Arıcılar tarafından farklı uygulamalar balarısı kolonileri üzerinde ya-

pılmaktadır. Bu uygulamalar (örneğin ana değiştirme, ve gezginci arıcılık) zaman içerisinde balarısı karakterlerinin değişmesine neden olmaktadır. Morfolojik karakterler balarısı popülasyonlarını karakterize etmek için ölçülebilirler ve balarısı kolonilerinin üretkenliklerinin bir ölçüsü olarak kullanılabilirler. Balarısı popülasyonlarını karakterize etmek için, bilinen metotlar farklı koloni ve bölgelerden rastgele balarısı örneklerinin toplamasına dayalıdır. Bununla beraber, morfolojik karakterleri etkileyebilecek farklı faktörler de vardır. Dolayısıyla, açık balarısı popülasyonlarındaki bu karakterlerin durağanlığını çalışmak için dalgalanma düzeyinin belirlenmesi ve karakterizasyonu için uygun metotları önermek gereklidir

**MATERYAL VE METOT:** Altı bölgeden 96 balarısı kolonisinden toplanan 1140 balarısı işçi arı örneğinin morfolojik karakterleri birbirini takip eden 2 yıl boyunca çalışılmış ve elde edilen sonuçlar karşılaştırılmıştır. Her koloniden 15 örnek Ruttner ve ark. 2000'e göre ölçülmüştür. Ondört karakter; dil uzunluğu, ön kanat uzunluğu ve genişliği, hamuli sayısı, Kübital A ve B uzunlukları, Kübital indeks değeri, C ve D uzunlukları, arka kanat uzunluğu ve genişliği, arka bacak femur ve tibia uzunlukları, arka bacak basitarsus uzunluğu ve genişliği ölçülmüştür. Elde edilen veriler ANOVA ile test edilmiş ve farklılıklar en az önemlilik farkı ile karşılaştırılmıştır.

**SONUÇLAR:** Her iki yıla ait veriler tablolar halinde Tablo 1 ve Tablo 2'de gösterilmiştir. 12 karakter ve Kübital indeks değeri tablolarda gösterilmiştir. Tablo 3'te ise iki yılın (2006 ve 2007) karşılaştırılması verilmiştir. İkinci yılda elde edilen morfolojik karakterler ilk yıldan tüm ölçülen karakterler açısından özellikle de kübital indeks daha düşüktür, çalışılan bölgelerde dil uzunluğu değeri 0.19 ile 0.69 mm arasında yükselmiştir.

**TARTIŞMA:** Elde edilen sonuçlar balarısı popülasyonlarında meydana gelen değişikliklerin çok çabuk taranması için kübital index ve dil uzunluğunun ölçülmesinin yeterli olacağını göstermiştir. Aynı zamanda en azından 2 yıl morfolojik karakterlerin ortalamalarının balarısı popülasyonlarının karakterize edilmesi için yeterli olabilecektir.