

Measuring the Morphological Characters of Honey Bee (*Apis Mellifera* L.) Using A Simple Semi-Automatic Technique

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Abstract: Measuring of morphological characters of honey bees was carried out using a simple technique depends on the combination between Scanner and Photoshop program. This technique was called Scan Photo technique (SPT). The main idea is to dissect the body parts of honey bee worker, and then the separated parts were scanned as images. The images were opened at Photoshop program, and then the ruler of the program was used to measure the characters. Comparison between SPT and using Binocular with unocular micrometer showed no significant difference between the two methods in measuring the chosen morphometric characters. The measurements of 11 morphological characters of honey bee workers from Local colonies (Parents) were compared with those of their F_1 colonies, in which queens have been mated under natural mating conditions. The overall means of Parents (P) and their F_1 were 5.95 mm and 6.05 mm for tongue length; and 8.91 mm and 8.64 mm; 3.05 mm and 3.22 mm; 6.28 mm and 6.05 mm; 1.81 mm and 1.71 mm for fore wing length; fore wing width; hind wing length and hind wing width, respectively. Also, the overall mean values of P and F_1 were 2.89 and 3.25 for cubital index; and 20.8 and 20.6 for number of hooks, in respect. On the other hand, the overall means were 2.25 mm and 2.22 mm; 2.83 mm and 2.80 mm; 2.10 mm and 2.04 mm; 1.08 mm and 1.06 mm for femur length; tibia length; basitarsus length and basitarsus width of P and F_1 , in respect. Significant differences were found only between workers of parent and F_1 mean in tongue length, hind wing length and basitarsus length. No significant differences were found between the other tested characters.

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Key words: Honey bee, *Apis mellifera*, Morphological characters, Scan Photo technique.

1. Introduction:

In the last years, morphometric analyses are being tool for characterization of genetic materials (Andereb et al., 2008).

Measuring of morphological characters honey bee body parts, separated and laid fixed or mounted on glass slides were performed using projectors (Ruttner et al., 1978), a glass magnifying leica, equipped with ocular millimetrical ruler (Oliveira-Jr et al., 2000), TV screen (Kandemir et al. 2000), a camera that projected their images on to a computer monitor with TV program (Schneider et al., 2003), CCD camera combined with an on – screen measuring system (Bee2) (Shaibi et al., 2009), or a digital camera (Leica DC 100) attached to a stereomicroscope (Gencer and Firatli, 2005). All body parts of honey bee workers were investigated under an Olympus optical binocular microscope, magnification scale 25 (Jevtic et al., 2007). Marghitas et al. (2008) used Olympus stereomicroscope with a camera connected to computer with Quick Photo Micro 2.2 program. A binocular microscopy with a micrometer eyepiece and a mill metrical slide and a microscope were used to measure some morphological characters (Al-Buraki and Al-Buraki, 2008).

Morphological characters of body parts of honey bee workers were measured using a micrometer eye-piece fixed within a stereomicroscope. (El-sarrag et al., 1992; Mostajeran et al., 2002; and Adl et al., 2007) or with a computer –aided measuring system based on a video system and measuring program (Arias et al., 2006; Cakmak et al., 2006; and Meixner et al., 2007;). Also, the image analyzer IMAGEPRO plus version 3.0.1 and Media Cybernetics were used (Andereb et al., 2008). Discriminant analysis with Numerical Output (DAWINO) method based on discriminate analysis of 30 wing characters was used to determine to what race the samples were closest with the greatest probability. (Chlebo and Kopernicky 2004). Mazeed (2004) used a Slide-Scan connected to a computer that display the worker honey bee fore wing on the monitor, 17 wing intervention points were chosen to establish a coordinate system representing 17 coordinate points to perform microtaxonomy of honey bees using wing venation pattern .

The preparations of bee body parts were scanned on a glass of flat office scanners (with different resolution 600, 2400 and 4800 dots per inch (dpi)). The cubital index was determined using an

Mst 131 microscope with micrometric eyepiece or by means of a computer consisted in sending the scanned picture into Flugelindex 2 software, in which measurement points were marked (Rostecki et al., 2007).

Unfortunately, the most of these methods depend only on measuring fore wing characters and the commonly applied method for measuring morphological characters of honey bees is to use the binocular with an ocular micrometer which is time consuming and less accurate. So, in this work, we aimed to develop a simple semi automatic technique to measure all morphological characters of honey bee, and to study the effects of requeening under natural mating conditions on morphological characters of the first generation using the developed technique. This method depends on the combination between Scanner and Photoshop program and called Scan Photo method (SPT).

2. Material And Methods:

A. Development of a simple semi-automatic technique:

1.1. Preparation of samples:

Sample was taken from a colony in an apiary at Damanhour district where 15 local honey bee workers were collected from combs in a glass jar and then killed under cooling in a deep freezer. Workers were dissected to separate (tongue, right fore wing, right hind wing and right hind leg).

1.2. Morphological characters:

The tested morphological characters were fore wing length and width, hind wing length and width, tongue length, femur length, tibia length, basitarsus length and width. Measurements of the above mentioned characters were taken according to Ruttner (1988).

1.3. Evaluation of the developed technique:

The experiment was conducted to evaluate the developed technique, where morphological characters were measured by Scan Photo method and compared with those obtained by the binocular with unocular micrometer. In Scan Photo method all of the studied morphological characters were measured using scanner (Zoom 100%, color is red green blue (RGB), resolution 2400 points per inch (ppi) and units of mm) connected to laptop (Fujitsu) with screen resolution (1024 by 768 pixels) and provided with measuring program (PhotoShop). The separated body parts of worker bees were put on glass slides and covered with another glass slides. The slides were scanned and moved into the computer as images. The images were opened on the PhotoShop program and characters were measured with measuring tools.

The steps of using the Scan Photo method showed in Fig. (1) as follow:

- 1- Collecting of honey bee worker (Fig. 1a).
- 2- Separation of bee body parts (Fig. 1b).
- 3- Double glass slides (under light lid) contain the separated body parts and scanned with the scanner (Fig. 1c).
- 4- Laptop screen show the double glass slides inserted inside a frame and scanned with the scanner (Fig. 1d).
- 5- Many right fore wings transformed as image after scanning (Fig. 1e).
- 6- Measuring of morphological characters for only one chosen fore wing by using the Photoshop program (Fig. 1f).

To adjust this method, a drone fore wing was scanned beside a ruler, fore wing length was 12 mm by the ruler and the same length was obtained by the Photoshop.

1.4. Measuring of morphological characters:

Measurements of the above-mentioned characters were carried out according to Ruttner (1988). Fore wing length of honey bee workers was measured as a maximum length distance in units of (mm) and forewing width was measured as a maximum width distance in the fore wing in the units of (mm). Hind wing length and width were measured as a maximum hind wing length and width in the units of (mm), respectively. Tongue length was measured as a maximum distance from cardo to the flabellum. Femur length was measured as a maximum distance from the end of the trochanter to the beginning of the tibia. Tibia length was measured as a maximum distance from the end of the femur to the beginning of basitarsus. Basitarsus length was measured as a maximum distance and Basitarsus width was measured as a maximum width distance of the basitarsus. All measurements were in the units of (mm). All measurements were in units of (mm) except cubital index and number of hooks (as a number).

Statistical analysis was carried out and means were compared by using T-test (5%).

B. Worker morphological characters of Parents and F₁ under natural mating conditions:

The beekeepers at El-Beheira Governorate used to requeening by two methods. The first method by rearing virgins related to mother queens under emergency conditions. The second method by purchasing virgin queens from a queen producer and let them to natural mating. Morphological characters of workers related to Parents and F₁ under emergency queen rearing conditions were investigated.

3.1. Experimental colonies

Four Local hybrid colonies were chosen randomly in an apiary at Damanhour city, and considered as Parents. Four virgin queens were

reared (one virgin/parent queen under emergency queen rearing) at mid of June by artificial swarming, and subjected to an open mating system.

3.2. Honey bee worker samples collection:

Fifteen honey bee workers were collected from each colony. Total of 60 workers for Parents and for F1. Collected samples were killed in deep freezer then dissected to separate fore wing, hind wing, hind legs and tongue. Morphological characters were measured as previously mentioned.

3.3. Morphological characters of workers:

The studied morphological characters were head characters [tongue length], thorax characters [fore wing length, fore wing width, Cubital vein A, Cubital vein B, Cubital index, hind wing length, hind wing width, number of hooks, hind leg (femur, tibia, basitarsus length and basitarsus width)]. All measurements were in units of mm except hooks number and cubital index.

The data of this trail were statistically analyzed by analysis of variance and means were compared using L.S.D.0.05 according to Steel and Torrise (1984).



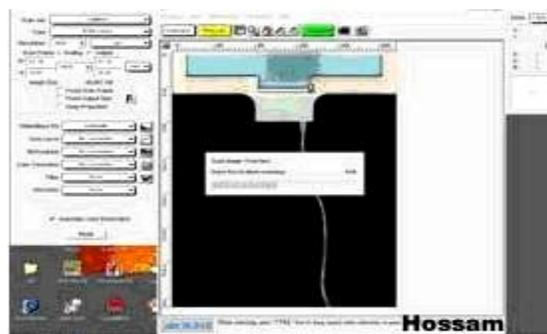
(a)



(b)



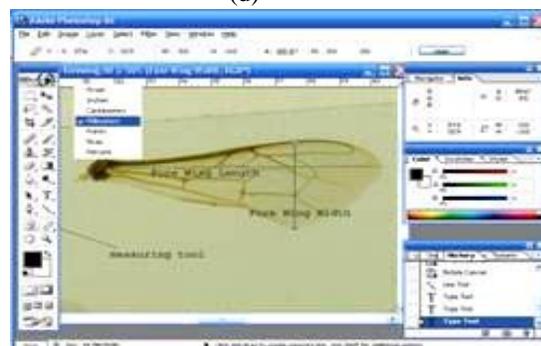
(c)



(d)



(e)



(f)

Fig. (1): Steps of measuring morphological characters using the Scan Photo technique: a) Collecting of bee sample, b) Separation of body parts, c) Using the scanner to scan the separated parts, d) Laptop screen, e) Right fore wings after scanning, f) Measuring of morphological characters by Photoshop program.

3. Results And Discussion:

Samples of 15 honey bee workers were collected from a colony in an apiary at Damanhour district (El-Beheira Governorate).

1. Comparison between Scan Photo method and the Binocular method.

Data in Table (1) show that all morphometric characters measured with Binocular method were higher than the same characters measured with Scan Photo method. The differences ranged from 0.05 to 0.15 mm.

Statistical analysis showed that there was no significant difference between the two methods in measuring the chosen morphometric characters. This result indicated that the simple developed method (Scan Photo method) could be applied to measure most of morphometric characters of honey bees.

Data showed that the measurement values using Scan Photo method were relatively less than those obtained by the Binocular method. These results agree with Samborski et al. (2002), who obtained variations in Cubital index values (from 0.05 to 0.06) when three methods were used in measuring Cubital A and B; the highest values were obtained from microscope then microfilm viewer then the computer.

On the contrary with Rostecki et al. (2007), who found that Cubital index value obtained from a computer measurement at the resolution of 4800 dpi and LCD type monitor was higher than that obtained in the microscopic measurements with a variation of 0.07.

When Scan Photo method was compared with the Binocular method, it was clear that the length of a character can be measured in different units (mm, cm, inch and pixel) by Scan Photo, while it usually in mm by binocular method. Also, Scan Photo method requires only a scanner with high resolution and a computer with Photoshop program but the other method requires a binocular and unocular micrometer. In Binocular method, counting the numbers of units and calculating the lengths may result in mistakes and requires long time depending on the person who measures the characters. On the contrary, the ruler of Photoshop program measures the lengths automatically, so the Scan Photo method is more accurate and less time consuming.

However, many researchers used 17–19 landmarks on wing to distinguish the different honey bee races. But, pointing the landmarks is time-consuming and often associated with errors because the exact position of a landmark is ambiguous, particularly when veins are wide (Tofilski, 2004).

Table (1): Averages of studied morphological characters of honey bee workers using Scan Photo method and Binocular method.

Characters (mm)	Means \pm		Differences (mm)	T – test Signification
	Binocular	Scan Photo		
Tongue length (Ton L)	6.07 \pm 0.15	6.00 \pm 0.24	0.07	0.48 ⁻
Fore wing length (FWL)	8.85 \pm 0.06	8.80 \pm 0.08	0.05	1.00 ⁻
Fore wing width (FWW)	3.15 \pm 0.05	3.05 \pm 0.06	0.10	1.80 ⁻
Hind wing length (HWL)	6.32 \pm 0.45	6.22 \pm 0.31	0.10	0.36 ⁻
Hind wing width (HWW)	1.9 \pm 0.08	1.85 \pm 0.05	0.05	1.58 ⁻
Tibia length (TL)	2.90 \pm 0.08	2.85 \pm 0.05	0.05	1.00 ⁻
Femur length (FL)	2.35 \pm 0.05	2.20 \pm 0.08	0.15	1.90 ⁻
Basitarsus length (BL)	1.12 \pm 0.05	1.05 \pm 0.06	0.07	1.86 ⁻
Basitarsus width (BW)	2.12 \pm 0.09	2.00 \pm 0.08	0.12	1.88 ⁻

(-): No significant differences were detected between the two methods (T- test 5%). Each mean was obtained from 15 replicates.

It could be concluded that the application of appropriate software such as Photoshop and commonly available office scanners enables considerable simplification of morphological measurements of honey bees. Unfortunately, no data on the use of Photoshop program and scanner, so far, are available in the literatures.

2. Morphological characters of Local honey bee workers from Parent colonies (P) and their First generation (F₁).

Table (2) and Fig. (2) illustrate the measurements of 11 morphological characters of honey bee workers from Local hybrid colonies (Parents) and their F₁ colonies, in which queens have been mated under

natural mating conditions of Damanhour city. The overall means of Parents (P) and their F₁ were 5.95 mm and 6.05 mm for tongue length; and 8.91 mm and 8.64 mm; 3.05 mm and 3.22 mm; 6.28 mm and 6.05 mm; 1.81 mm and 1.71 mm for fore wing length; fore wing width; hind wing length and hind wing width, respectively. Also, the overall mean values of P and F₁ were 2.89 and 3.25 for cubital index; and 20.8 and 20.6 for number of hooks, in respect. On the other hand, the overall means were 2.25 mm and 2.22 mm; 2.83 mm and 2.80 mm; 2.10 mm and 2.04 mm; 1.08 mm and 1.06 mm for femur length; tibia length; basitarsus length and basitarsus width of P and F₁, in respect.

Table (2): Morphological characters of Local honey bee workers from Parent colonies and their First generation.

Colonies Character	1		2		3		4		Mean ± S.D. *		L.S.D. 0.05
	P	F ₁	P	F ₁							
Tongue length	5.97*	6.03	5.98	5.99	5.89	6.07	5.96	6.10	5.95±0.04 ^b	6.05±0.05 ^a	0.05
Fore wing length	9.01	8.66	8.98	8.54	8.86	8.75	8.78	8.62	8.91±0.10 ^a	8.64±0.08 ^b	0.12
Fore wing width	3.04	3.01	3.03	2.95	3.07	2.98	3.06	3.95	3.05±0.02 ^a	3.22±0.48 ^a	1.02
Hind wing length	6.40	6.00	6.32	6.06	6.20	6.06	6.19	6.09	6.28±0.10 ^a	6.05±0.03 ^a	0.23
Hind wing width	1.79	1.73	1.81	1.77	1.82	1.68	1.81	1.66	1.81±0.01 ^a	1.71±0.05 ^a	0.23
Cubital index	2.23	2.94	2.66	4.19	3.30	3.00	3.40	2.87	2.89±0.55 ^a	3.25±0.63 ^a	1.78
Number of hooks	22.80	20.00	20.90	21.10	20.90	21.10	18.70	20.50	20.80±1.67 ^a	20.60±0.53 ^a	0.17
Femur length	2.28	2.18	2.18	2.28	2.29	2.21	2.24	2.23	2.25±0.05 ^a	2.22±0.04 ^a	0.37
Tibia length	2.92	2.80	2.73	2.74	2.90	2.83	2.86	2.83	2.83±0.08 ^a	2.80±0.04 ^a	0.84
Basitarsus length	2.14	2.07	2.04	2.06	2.12	2.02	2.12	2.01	2.10±0.04 ^a	2.04±0.03 ^b	0.05
Basitarsus width	1.06	1.04	1.09	1.05	1.10	1.11	1.08	1.05	1.08±0.02 ^a	1.06±0.03 ^a	0.03

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

*: All characters in units of mm except Cubital index and number of hooks (as a number). P: Parents, F₁: First generation.

Statistical analysis illustrated that significant differences were found only between workers of parent and F₁ mean in tongue length, hind wing length and basitarsus length, while insignificant differences were found between them in the other characters.

The obtained results revealed that parent worker means were higher than those of F₁ workers in some characters such as fore wing length, fore wing width, hind wing length, hind wing width, femur length, tibia length, basitarsus length and basitarsus width.

On the other hand, F₁ worker means were higher than those of the parent workers in cubital index and tongue length which show significant differences. In a similar study about the effect of open mating system of different sites in Egypt on the morphological characters of the first single cousins of *A. m. carnica*, Eshbah et al. (2003) found significant increases in the number of hooks, forewing width, proboscis length, and mandible width. Also, Quezada-Euán and Paxton (1999) showed that individual colonies dramatically changed their

worker morphometrics and defensive behavior from one generation to the next. Differences between F_1 and parent workers may be due to the inbreeding, in accordance with Bienefeld (1991) who mentioned that the inbreeding in many cases is the correct reason for the fall off in quality. On other words, the natural mating of virgin queens reared from Local queens and mated from local drones reduced most of the morphological characters of the offspring. On the contrary, characters which affected with environmental condition more than genetic conditions showed a different value like tongue length.

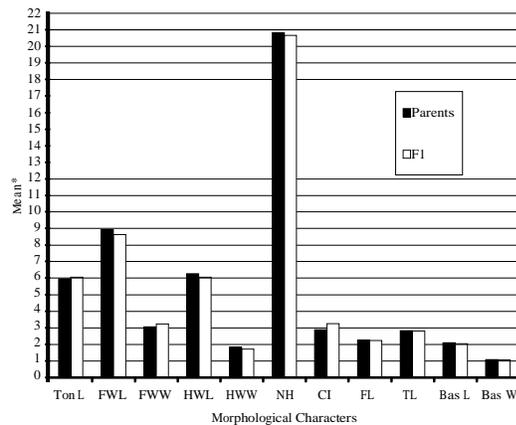


Fig. (2): Morphological characters of honey bee workers from Parent and their F_1 colonies.

*: All characters in units of mm except Cubital index and number of hooks (as a number).

Ton L: Tongue length ; FWL: Fore wing length ; FWW : Fore wing width ; HWL: Hind wing length ; HWW : Hind wing width; NH: Number of hooks; CI: Cubital Index ; FL: Femur length ; TL: Tibia length ; BL: Basitarsus length ; BW: Basitarsus width.

It could be concluded that rearing virgin queens from local queens under natural mating conditions resulted in reducing of morphological characters of first generation offspring. The obtained results may explain the previous results of El-Beheira study during the two years (2006 and 2007) where characters were generally decreased from the first year to the second year (except Ton L , NH and BL) with respect to environmental conditions.

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