Cubital Index Determination of some Honey Bee Races *Apis mellifera subspecies* at El-Manzla Region in El-Dakahlia Governorate, Egypt

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ABSTRACT

Egypt had different honey bee races as a result of import many races in previous years. Here seven locations were choice from isolated place as El-Manzla region to examine the races and their hybrids by using cubital index as one of the important morphometric characters to determine bee races and their hybrids. The results indicated that the general means of cubital indexes were 2.12 with *F value* 12.094 and L.S.D 0.199 in El-Bosrat, 2.44 with *F value* 9.517 and L.S.D 0.217 in El-Kafer El-Geded, 2.66 in Azbat El-Ltaifa, 2.43 with *F value* 12.006 and L.S.D 0.158 in Bazlla, 2.23 with *F value* 21.954 and 0.157 in El-Manzla research station, 2.36 with *F value* 5.607 and L.S.D 0.190 in El-Satayta and 2.40 with *F value* 13.904 and L.S.D 0.189 in Met-Silsil. The seven previous locations illustrated that 49.33% of collecting samples were *Apis mellifera mellifera* (1.89- 2.00) in El-Bosrat, 36.67% were *Apis mellifera yemenitica or syrinca* (2.1 – 2.3) in Met- Silsil, 28.00% were *Apis mellifera lamarkii* (2.3 – 2.5) in Baziia, 17.33% were *Apis mellifera ligustica or carnica* (2.5 – 2.7) and 18.67% were *Apis mellifra Cypria* (2.7 – 2.9) in Azbit El-Ltifa. There were differing percentages of other strains in different apiaries at the EL-Manzla region.
Keywords: *Apis mellifera*, Races, Morphometric analysis, cubital index, El-Manzla.

INTRODUCTION

The honey bee is one of the most important social insects known in the world. The importance of this insect is obvious and it plays a major role in the field of agriculture (pollination) and medicine (production of important substances like: honey, royal jelly, bee wax, propolis, pollen grains and bee venom). Egypt had ancient Egyptian bees, *Apis mellifera lamarkii*, was kept 5000 years ago. This *A. m. lamarkii* is lived in the local circumstances of the region. Then it was prevalent out Egypt. Many honey bee breeding projects have been performed to improve the expectation of household honey bees (Mortiz, 2004). *A. m. ligustica* has indicated to increase economic makings to trade beekeeping for its productivity (Dall,Olio *et al* 2004). Therefore *A. m. ligustica* queen bees have been imported. Also, a large population of *A. m.* carnica was imported over 30 year period and maintained in Egypt (Kamel *et al.*, 2003). Also, other races were imported to improve honey production and other economic characters in Egypt (Page *et al.,* 1981). Estimating honey bee morphometric characters in different time is very important for races (Abou- Shaara and Ahmed, 2015). Nowadays, the Egyptian geographic honey bee race, *A. m.* lamarkii is being bred in a limited region of Assiout in Upper Egypt. By the time, honey bees in other regions of Egypt have gained some morphological, physiological and behavioral characters from different genetic resources (Eid *et al.*, 2010)

The discrimination among honey bee subspecies is important for beekeeping and preserving honey bee biodiversity. Using morphometric analysis was very good for indicating the bee species and subspecies (Francoy *et al.*, 2006). Honey bee races determination is important to save it (Oleksa and Tofilski, 2015). Morphometric analysis is the most used official methodology for identifying the honey bee races, its highly practicability and low costs (Francoy *et al.,* 2008 & Uniyal *et al.*, 2017). Angels and distances are landmark in different shapes can be measured and analyzed by statistical methods (Zelditch *et al.* 2004).

Beekeeping practices such as migratory beekeeping might induce high levels of integration within populations (Drazic *et al.,* 2004; Rortais *et al.,* 2004). Also, 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). *Apis mellifera subspecies* can be distinguished by using molecular methods (Witfield *et al.*, 2006)

Here is the morphometric analysis (cubital index) used for identifying the types of the honey bee races *Apis mellifera subspecies* founded in the isolated region (El-Manzla region) in El- Dakhlia Governorate, Egypt. The honey bee workers samples were collected from different seven apiaries locations in El-Manzla. Samples measured by using the cubital index methods as one of importance morphometric characters to determine races and their hyprids, through the forewings for thirty samples from different colonies in previous apiaries regions by easy and precise way in computer program for measure the cubital index (Tofilski, 2007), used for comparing the diversity of the honey bee strains and to distinguish *Apis mellifera subspecies* then compared with the original honey bee races *(Apis mellifera carnica, Apis mellifera ligustica, Apis mellifera lamarkii and others subspecies).*

MATERIALS AND METHODS

El-Manzla region was the isolation place in El-Dakhlia Governorate, Egypt; it was the first place used the imported original honey bee craniolan race (*Apis mellifera carnica*) in Egypt from long time ago. Today it is most difficult in all Egypt to find an original honey bee race because imported another race like Italian race (*Apis mellifera ligustica*) beside the cariniolan one. Then the Egyptian beekeepers leave the both of previous races hybrid, through inbreed system, with the Egyptian honey bee race (*Apis mellifera lamarkii*). Now beekeepers are not sure for types of races are being in Egypt. Because that, this research was started by collecting different honey bee workers samples from seven different locations at El- Manzla region, these

samples collected in Autumn 2013 from El-Bosrat, Al-Kfer El-Gaded, Azbat El-Latifa, Bazlla, El-Satayta, El-Manzla research station and Met-Selseel. From each location collected five samples with 30 new emergences of honey bee workers. Then saved them in the -80°c and after one day collected the fore right wings in the transparent sheet for morphometric analysis CI (Cubital Index).

1-Cubital index determination:

Thirty of workers samples collected from each 5 colonies from previous locations at El-Manzla region in El- Dakhlia Governorate, Egypt, and then the right forewing were taken from each worker. The forewings were transferred to transparent paper, scanned them with computer, scanner, then transferred them to Quanutum GIS computer-measuring program as a photo to measure the part A (C-B) and part B (B-A). After that, the measurements putted in Excel sheet to calculate the value of cubital index (a/b) in each wing for each worker sample (figure, 1). The data were in Excel sheet divided into special categories of cubital index ($\geq$ or $\leq$ 2.00 to 4.5) for *Apis mellifera sp.* (Ruttner, 1986) to indicate the frequency and variety of the (cubital index) in different experimented colonies.
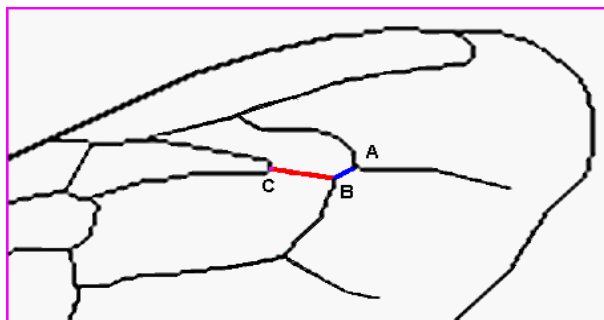

Figure 1. cubital index (CI=a/b) in the forewing of worker

2- Statistical analysis:

The program was used in the statistical analysis was SPSS, 22. The obtained data were statistically analyzed by calculating the means. Analysis of variance "ANOVA" was carried out to check the significant differences among the different treatments. The least significant difference (L.S.D) was calculated and determined in 0.05 levels. The percentages of the multiplied measured in the samples were calculated.

RESULTS AND DISCUSSION

1. Morphometric analysis of Cubital index in forewings of the honeybee workers samples:

The results in table, 1 indicated that the means of cubital index (C.I.) for the five experiments colonies in El-Bosrat were (1.9563, 2.5346, 2.0469, 1.9350 and 2.1382) with total mean 2.1222. Statistical analysis for El-Bosrat location illustrated that *F. value* was 12.094 then calculated L.S.D was 0.199 in 0.05, that's mean there was a significant difference between the five colonies in El-Bosrat, and cubital index ranged within (1.94 – 2.5). In El-Kafer El-Geded location the means of cubital index for the five colonies were (2.1447, 2.6145, 2.5783, 2.6339 and 2.2178) with general mean 2.4379 also *F. Value* was 9.517 which indicated the significant

differences between the five colonies at 0.05 with L.S.D 0.217. The third experiment's location was Azbat El-Lataifa and the means of C. I for samples collected from five colonies were (2.7579, 2.6398, 2.5783, 2.6462 and 2.6684) with total mean 2.6581. The results of Azbat El-Lataifa showed that there was no significant difference at level 0.05 and 0.01 between the samples collected from the five colonies with *F. value* (0.777). In the fourth location Bazlla the means of C. I for the each experiment colony were (2.5114, 2.5856, 2.5907, 2.1462 and 2.3177) with total mean 2.4303, statistical analysis showed significant differences between the five colonies when *F. value* was 12.006 in 0.05 levels and L.S.D was 0.158. El- Manzla research station was the fifth location for collecting honey bee workers samples from five colonies, and the means of C. I for each colony were (2.2110, 2.4634, 1.8991, 2.0775 and 2.5156) with generally mean 2.2333, in addition *F.* value was 21.954 and calculated L.S.D was 0.157 at level 0.05 that's mean a significant difference between the five colonies at previous region. El-Stayta as the sixth location for collecting experiment's samples, the means of C.I for the five colonies were (2.3421, 2.4949, 2.5558, 2.2075 and 2.2150) with general mean of 2.3631, there was a significant difference between the colonies and *F.* value = 5.607, L.S.D. = 0.190 at 0.05 levels. The last location was Met-Silsil, the means of C.I were (2.1370, 2.3012, 2.6028, 2.2388 and 2.7192) with general mean 2.3998 and *F. value* was 13.904 with calculated L.S.D. = 0.189 at 0.05 levels, which illustrated a significant difference between the five colonies in Met-Silsil.

The results presented in Table, 1 showed that there was a significant difference between the samples collected from the five colonies from the six lcations (El-Bosrat, El-Kafer El-Geded, Bazlla, El-Manzla Research Station, El-Satayta and Met-Silsil). Such as, there was no significant difference between the samples collected from the five colonies in Azbat El-Ltaifa region. The morphometric analysis of cubital index for the fore wings indicated that there is a variation not only between the eight regions were collected the sample from but also inside each region.

2. *Apis mellifera subspecies* percentage % in the seven different locations at El-Manzla region

In El-Bosrat region, the cubital index varied (1.94 - 2.53) with a mean of 2.12. When it's compared with cubital index for *Apis* subspecies found the category of El-Bosrat belong to the category of *Apis mellifera* (1.65 - 2.95) according to Ruttner (1986). In addition, the C.I in colonies one, three and four (1.94, 2.05 and 1.93) nearly belonged in the category of the western European bees *Apis mellifere mellifera* (1.89 - 2.00). On the other hand, colony no. 2 was (2.53) nearly to *Apis mellifera scutellata* (2.52) as African races. While colony no. 5 was (2.14) may be its nearly *Apis mellifera yemenitica* (2.22).

According to table, 2 in El-Bosrat, 49.33% of all the samples collected were belonged in *Apis mellifere mellifera* category (1.89 - 2.00) Ruttner (1986). Nevertheless, 20.67% of samples belonged in the category (2.1 - 2.3) almost to *Apis mellifera yemenitica* or

syrinca, Ruttner (1986). 12.73% of El-Bosrat samples within (2.3 - 2.5) nearly to Egyptian bees *Apis mellifera lamarkii*. 7.33% of El-Bosrat samples located in the category (2.5 - 2.7) almost to *Apis mellifera ligustica* or *carnica* as European Races, otherwise most African bees easy to distinguish morphologically from European bees (Becu *et al.* 1987, Rinderer *et al* 1993). 3.93% of Albosrat samples were inside (2.7 - 2.9) nearly to *Apis mellifera cypria* (Pollmann, 1875).

In the second location El-Kafer El-Geded roughly 15.33% of the samples collected belonged in the category of *Apis mellifera mellifera* (1.89 - 2.00). Other samples almost 29.33% found within (2.1 - 2.3) and were nearly to *Apis mellifera yemenitica* or *syrinca*. Furthermore, 16.67% of the samples were in the category (2.3 - 2.5) and roughly to *Apis mellifera lamarkii*. 12.67% of samples belonged in (2.5 - 2.7) and nearly to *Apis mellifera ligustica* or *carnica* as (European Races). Also, 10.67% of samples found in category of (2.7 – 2.9) and almost to *Apis mellifera cypria*.

Azbit El-Latifa has just 2% of samples in category (1.89 – 2.00) and nearly to *Apis mellifera mellifera*. On the other hand, 21.33% of samples belonged in (2.1 – 2.3) and roughly to *Apis mellifera yemenitica* or *syrinca* (Eastern Races). 15.33% of samples found in category of (2.3 – 2.5) and they were nearly to *Apis mellifera lamarkii*. 17.33% of samples were inside the category of (2.5 – 2.7) nearly to *Apis mellifera ligustica* or *carnica* as (European Races).

Furthermore, 18.67% of samples belonged in (2.7 – 2.9) and they were nearly to *Apis mellifera cypria*. In Bazlla 10.67% of samples found inside (189 – 2.00) the category of *Apis mellifera mellifera*. 24.67% of samples belonged in (2.1 – 2.3) the category of *Apis mellifera yemenitica*. 28.00% of samples found in the category of *Apis mellifera lamarkii*, (2.3 – 2.5). 16.00% of the samples were in the category (2.5 – 2.7) and roughly of *Apis mellifera ligustica* or *carnica* as (European Races). 12.00% found in the category (2.7 – 2.9) and nearly to *Apis mellifera cypria*.

Through the three other locations El- Manzla Research station, El-Satayta, and Met-Silsil the percentages of the samples collected from each region and belonged in the categories of the different subspecies were (32.00%, 28.00% and 13.33%) respectively belonged in (1.89 – 2.00) and nearly to *Apis mellifera mellifera*. Other samples from the previous three locations belonged in (2.1 – 2.3) and nearly to *Apis mellifera yemenitica* or *litorea* (African Races) by percentage of (28.00%, 17.34% and 36.67%) respectively. Furthermore, 20.67%, 23.33% and 17.33% sequentially, belonged in (2.3 – 2.5) the category of *Apis mellifera lamarkii*. In addition, 8.67%, 14.00% and 16.00% sequentially, were found within (2.5 – 2.7) the category of *Apis mellifera ligustica* or *carnica* as (European Races). Other samples from the four regions located in the category (2.7 – 2.9) and nearly to *Apis mellifera cypria* with a percentage of (7.33%, 8.00% and 7.33%) respectively.

Table 1. Mean of cubital index for the honey bee workers forewings

Regions No. of colonies	Samples	El-Bosrat	El-kafer El-Geded	Azbat El-Ltaifa	Bazlla	El-Manzla Research Station	El-Satayta	Met-Silsil
1	30	1.9563	2.1447	2.7579	2.5114	2.2110	2.3421	2.1370
2	30	2.5346	2.6145	2.6398	2.5856	2.4634	2.4949	2.3012
3	30	2.0469	2.5783	2.5783	2.5907	1.8991	2.5558	2.6028
4	30	1.9350	2.6339	2.6462	2.1462	2.0775	2.2075	2.2388
5	30	2.1382	2.2178	2.6684	2.3177	2.5156	2.2150	2.7192
General mean	-	2.1222	2.4379	2.6581	2.4303	2.2333	2.3631	2.3998
F value	-	12.094	9.517	.777	12.006	21.954	5.607	13.904
L.S.D. 0.05	-	0.199	0.217	-	0.158	0.157	0.190	0.189

Table 2. The percentage of cubital index for *Apis mellifera subspecies* according to (Rinderer, 1986) with different locations at El-Manzalla region, Dakhlia Government (2013)

Cubital Index for *Apis mellifera subspecies*	El-Bosrat %	El-Kfer El-Gded%	Azbit El-ltifa %	Bazlla %	El-Manzla Research Station %	El-Satayta %	Met-Silsil %	General %
Apis mellifera mellifera (1.89- 2.00)	49.33	15.33	2.00	10.67	32.00	28.00	13.33	23.25
Apis mellifera yemenitica or syrinca (2.1 – 2.3)	20.67	29.33	21.33	24.67	28.00	17.34	36.67	25.92
Apis mellifera lamarkii (2.3 – 2.5)	12.73	16.67	15.33	28.00	20.67	23.33	17.33	19.26
Apis mellifera ligustica or carnica (2.5 – 2.7)	7.33	12.67	17.33	16.00	8.67	14.00	16.00	12.66
Apis mellifra cypria (2.7 – 2.9)	3.93	10.67	18.67	12.00	7.33	8.00	7.33	9.07
Apis mellifera spp. (2.9 – 3.1)	2.67	4.67	10.67	4.66	0.66	6.00	2.00	4.00

As a discussion of the previous results in table 1& 2 the seven experiments location in El- Manzla region. The results indicated that, the highest percentage (49.33%) of the category of *Apis mellifera mellifera* was in El-Bosrat location. In addition the 36.67% belonged in the category of *Apis mellifera yemenitica* were in the

Met-Silsil region. Furthermore, 28.00%, the highest percentage of category for *Apis mellifera lamarkii* were located in Bazlla location. 17.33%, the highest percentage for the category of *Apis mellifera ligustica* or *carnica* was found in Azbat El-Ltiafa. *Apis mellifera cypria* was found in highest percentage 18.67% in Azbat El-Ltiafa. In addition, Azbat El-Ltiafa region has 10.67% of samples belonged (2.9 – 3.1) unknown subspecies, those samples may be hybrids from different races and they cannot be identified according to (Rinderer *et al.* 1993, Guzmán- Novao *et al.* 1994).

In general percentage for all different locations through Figure (2), the highest percentage was 25.92% for the group of *Apis mellifera yemenitica or syrinca*, followed by 23.25% for *Apis mellifera mellifera*. In addition, the group of (*Apis mellifera lamarkii* or *adonsonii* or *monticola* or *intermissa*) coexisted by the percentage of 19.26%. Moreover, the group of *Apis mellifera ligustica* or *carnica*, multiplied by the percentage of 12.66%. The percentage of 9.07% for the group of *Apis mellifera cypria* found in the all seven locations also for the hybrid strain (unknown*) Apis mellifera spp* the percentage was 4.00%. The previous results disagreed with (Mazeed, 2011), who was indicated that the percentage of A.m. carnica was higher than A.m. lamarkii in Egypt.

The total mean of cubital index ranged between 2.12 to 2.67 in all regions located at El- Manzla region in El- Dakhlia Governorate. On the other hand, (Eid *et al.*, 2010) illustrated that cubital index ranged between 2.45 to 3.38 at the Damanhor region in El- Beharra Governorate in Egypt.
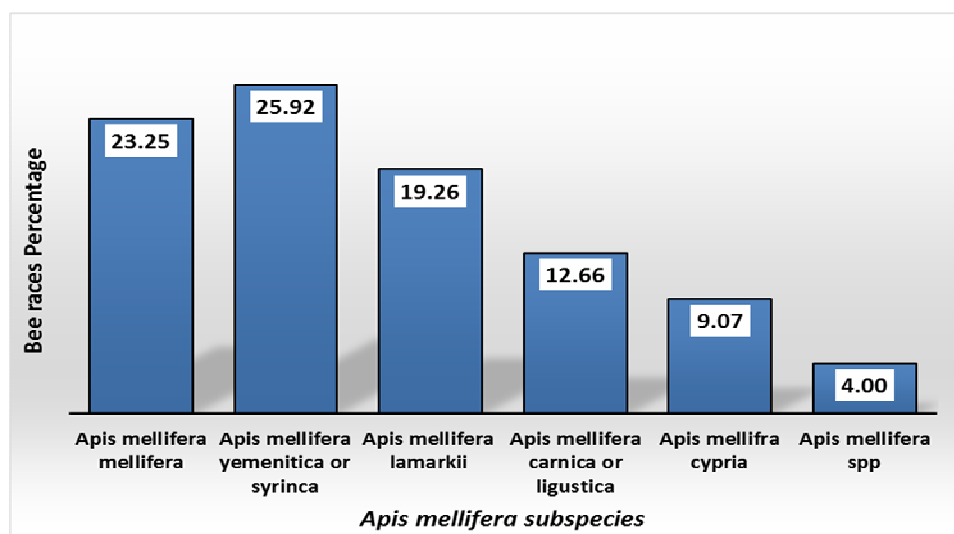

Figure 2. Percentage of *Apis mellifera subspecies* in different locations at El- Manzla region, Dakhlia Government (2013)

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تحديد Cubital index لبعض أنواع نحل العسل العالمى فى منطقة المنزلة بمحافظة الدقهلية بجمهورية مصر العربية

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تمتلك مصر سلالات مختلفة من نحل العسل وذلك يرجع الى استيراد الكثير من السلالات المختلفة فى السنوات السابقة. فى هذا البحث تم أختيار سبع مواقع مختلفة من منطقة المنزلة المعزولة بمحافظة الدقهلية (حيث أنها من أكثر الأماكن المعزولة بجمهورية مصر العربية والمتوقع ان يوجد بها سلالة مهجنة واحدة مثل النحل الكرنيولى) وذلك لإختبار السلالات المهجنة والنقية بأستخدام cubital index للأجنحة الأمامية لشغالات نحل العسل المجمعة من السبعة مواقع مختلفة حيث بلغت المتوسطات العامة للـ cubital index (2.22) مع (12.094 =F value، 0.199 =L.S.D) فى البصراط، وكانت (2.44) مع (9.517 =F value، 0.217 =L.S.D) فى الكفر الجديد، (2.66) فى عذبة اللطيفة، (2.43) مع (12.006 = value F، 0.158 =L.S.D) فى بزلة، وكانت (2.23) مع (F value= 21.954، 0.157=L.S.D) فى محطة بحوث المنزلة، و(2.36) مع (5.607 = F value، 0.190 = L.S.D) فى الستايتة، وكانت (2.40) مع (13.904 = F value، 0.189 = L.S.D) فى ميت سلسيل. وأوضحت النتائج السابقة للسبع مواقع أن 49.33% من العينات المجمعة من منطقة البصراط كان (*Apis mellifera mellifera*)، و36.67% (*mellifera yemenitica or syrinca*) فى منطقة ميت سلسيل. أما فى بزلة فكانت النسبة 28.00% للسلالة المصرى (*Apis mellifera lamarkii a*). وأما بعذبة اللطيفة وجد بنسبة 17.33% للسلالتين (*Apis mellifera ligustica or carnica*) وأيضا يوجد بها السلالة (*Apis mellifra Cypria*) بنسبة 18.67%. ويوجد أنواع أخرى غير متعارف عليها فى بعض المناطق الأخرى. مما سبق يتضح أن العينات المجمعة من السبع مواقع العشوائية من منطقة المنزلة لا يوجد بها سلالة محددة بعينها مع وجود تنوع كبير للسلالات وان المنطقة لم تعد معزولة ولم تحافظ على السلالة الكرنيولى الخاصة بها وذلك يرجع الى دخول سلالات غير معروف مصدرها الحقيقى عن طريق النحالين بشكل عشوائى ولكن بالرغم من ذلك فأنه من المحتمل وجود سلالة النحل الكرنيولى فى منطقة مثل عذبة اللطيفة بنسبة 17.33%، وأيضا وجود السلالة المصرى بنسبة 28.00% بمنطقة بزلة.