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MICROTAXONOMY OF HONEYBEES (*Apis mellifera* L .) IN EGYPT USING WING VENATION PATTERN

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ABSTRACT

From 16 Governorates in Egypt, many samples of workers honeybee were collected in order to classify them and determine the relationship between the bees morphometrically. From these samples two represent Egyptian and local Carniolan bees, while the remaining samples represent natural hybrid bees. For this purpose, a Slide-Scan connected with a computer was used to display the forewing of worker honeybee on the monitor and then 17 wing intervenation points were chosen for establishing a coordinate system representing 17 coordinate points. Performing discriminant and cluster analysis on the resulted coordinates, it could be concluded that the local Carniolan bees have a great effect on the hybrid bees, whereas the Egyptian bees were clearly separated from both Carniolan and hybrid bees in the biometrical space. Classifying the hybrid bees in reference to both Carniolan and Egyptian ones, the results indicated that the percentage of the hybrid bees classified as Carniolan bees was significantly higher in northern Governorates than the Egyptian bees, and *vice versa* in the Governorates located in the South. The biometrical relationship between the bees in the different Governorates showed that in most cases clusters of samples in the morphometrical space were close to each other geographically.

Key words: *Apis mellifera carnica, Apis mellifera lamarckii, biometry, cluster analysis, coordinate system , discriminant analysis, forewing, wing venation.*

1. INTRODUCTION

The diversity of honey bee populations is reflected in different patterns. One of these patterns is the external view of the body. The external view is registered through different measurements being done on specific parts of the body.

There are two kinds of morphometrical characters used for discrimination between the different honeybee populations: the first one being qualitative and the second one being quantitative characters. Using qualitative characters is restricted mainly in the differentiation between honeybee species; these characters show little variation within the species.

On the level of subspecies, however, purely descriptive methods are usually insufficient at this taxonomic level, and only quantitative differences (morphometric measurements) are found.

Many morphometrical characters are used for a long time to differentiate between different bee races and populations (Alpatov, 1929 and Goetze, 1964). A decisive progress in morphometric taxonomy of *Apis mellifera* was attained by introducing multivariate analysis (DuPraw, 1964). By representing the species as a set of selected samples, and by performing a principle component analysis on its morphometric characters, previous work resulted in the identification of 24 well-defined subspecies.

In Egypt, 2 subspecies of *Apis mellifera* come into contact: *A. m. carnica* and *A. m. lamarckii*. Hybridization between the 2 races occurs in all regions along the Nile Valley from North to South, and the hybrid bees in Egypt, which resulted from different cross mating between the two races, constitute the majority of the honeybee population. For beekeeper, it is too hard to distinguish between the different hybrids forms by their appearance, thus it is very important to search for a fast, precise method to differentiate these hybrids forms.

Morphological characters of wings are important in classifying different races and strains of honeybee (Ruttner *et al*, 1978; Mattu and

Verma, 1984).

The present study was carried out in order to explore the significance of new established characters of the wing for classifying honeybee population in Egypt .

2.MATERIAL AND METHODS

2.1. The origin of bees under study

The bees, on which this study was made, belong to the two subspecies established in Egypt; the local Carniolan bees (*A.m. carnica*) and the Egyptian bees (*A. m. lamarckii*), as well as the hybrid colonies, which are spreading from north to south Egypt.

The Egyptian bees were collected from 4 mud hives in Manfalot (Assiut) (Fig.1, No.13), where a high number of Egyptian bee colonies are still reared.

Concerning the local Carniolan bees, 3 samples were collected from the New Vally and 4 samples from Manzala (Dakahlia), (Fig. 1, No. 16).

With regard to the hybrid bees, there are 15 locations, from which the samples were collected (Fig. 1)

4 samples from Dakahlia (No.1)
6 samples from Kafr El-Sheik (No.2)
5 samples from El-Beheira (No. 3)
4 samples from Gharbia (No.4)
3 samples from Sharkia (No.5)
2 samples from Ismailia (No.6)
4 samples from Menofia (No. 7)
3 samples from Qaliobia (No. 8)
3 samples from Giza (No.9)
4 samples from Faium (No.10)
4 samples from Benisuif (No.11)
6 samples from Minia (No. 12)
7 samples from Assiut (No. 13)
4 samples from Sohag (No.14)
3 samples from Qena (No.15)

In each bee sample, the number of individuals ranged between 10 to 20 .

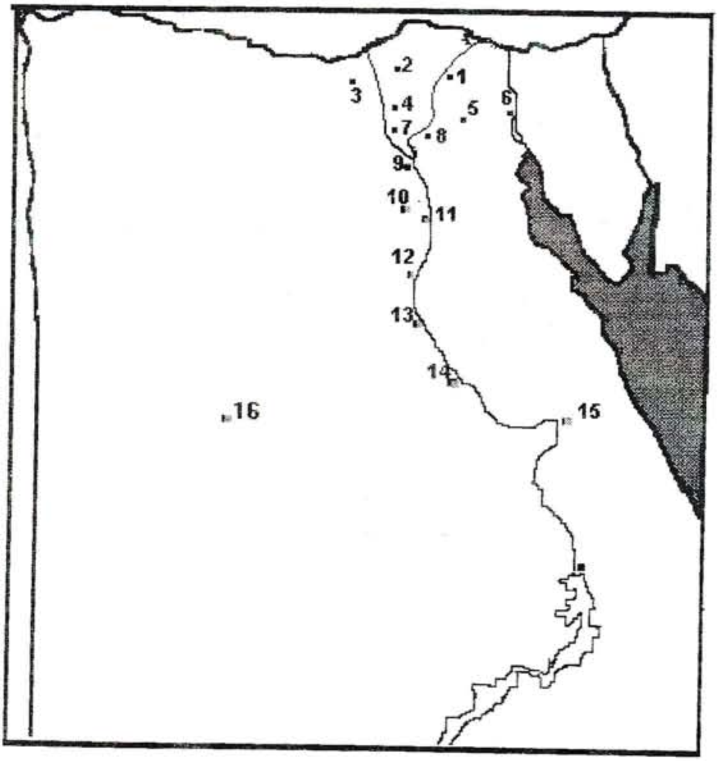

Fig. (1): Map of Egypt showing the locations (the numbers from 1 to 16) from which the bee samples were collected.

2.2. Morphometrical characters

The characters measured are chosen according to Kauhausen-keller (2002), being sensitive to any changes, which may take place in the wing venation of worker honeybees. Firstly, 2 intervenation points were chosen as reference for the 16 intervenation points on the forewing (Fig. 2). From the first two points, a coordinate system (X and Y) was established and the characters were determined as coordinate points relative to the established coordinate system.

Because the first point is chosen randomly, it has not any points (X= 0 and Y= 0) on the coordinate system. The second point, however, was represented by one value on the first reference point. All the remaining points are represented by two values. The number of points chosen were 17 (Fig. 2), that is, 34 points. Considering the first two points, the actual points would be 34-3= 31 points, which represent the measured characters.

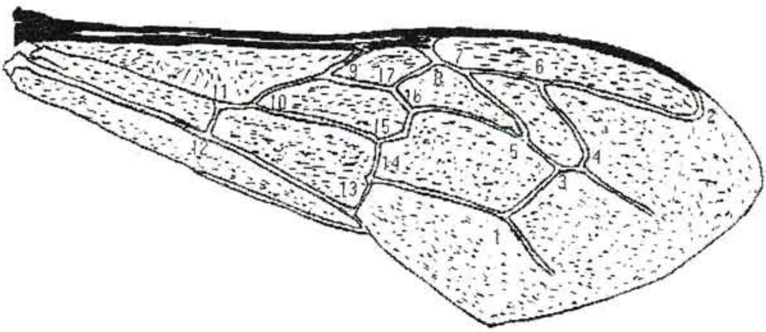

Fig. (2): The forewing of the honeybee showing the coordinates (from 1 to 17) points.

2.3. Equipment and measurement procedure

As shown in Fig. (2), the morphometrical system used consisted of the following parts:

- Computer unit with a suitable analysing program developed by Kauhausen-Keller (2002).
- A Slide-Scanner (Dimâge Scan Dual II).

For every bee, the right forewing was pulled out with a fine forceps and mounted on a glass-slide. Each slide had 40 forewings. Each 4 slides were put in a slide mount holder, which was then put inside the Scanner, in order to scann the mounted wings. After setting a suitable display-program, the wings were displayed on the monitor of the computer, and by the computer-mouse, the different intervenation points were clicked. The measuring-program converted these coordinate points into actual lengths of the different intervenation points relative to the first two points. The converted measurements are then stored in a new file.

For every bee sample, the converted data and the informations about its origin, the type of bee, to which it belongs and the date of collection are registered in a data-bank.

The measuring programme contains another programme, which can filter the desired groups to be compared to each other.

2.4. Statistical analysis used

This study every individual bee was used as an operational unit. The groups to be compared are then entered into a suitable statistical program (Holm, 1997). Discriminant analysis was used to determine the group-membership of the bees and cluster analysis was used to clarify the biometrical relationship between the hybrid bees in the different Governorates (Jobson, 1992).

3. RESULTS AND DISCUSSION

The results of the discriminant analysis between Egyptian, hybrid and local Carniolan bees indicated that there are considerable overlappings of the hybrid and Carniolan bees, which are both separated clearly from Egyptian bees. Using Barttelts test (Jobson, 1992), the significance of the first 2 Canonical functions were established (Table 1). The multivariate analysis of variance (MANOVA) of the measurements of the 31 characteristics showed that significant differences existed among the 3 groups.

Table (1): Bartellt statistical test of the two canonical functions extracted from discriminant analysis between Egyptian, Carniolan and Hybrid bees.

Canonical factors	Wilks λ	Chi Square	Significance
F1	0.53	679.6	$P< 0.01$
F2	0.9	106.49	$P< 0.01$

Table (2) shows the results of the classification of the discriminant analysis for the three groups. Using the individual bees as operational units, the bees were classified on the basis of their greatest membership. The lowest misclassification rate was related to the Egyptian bees; hence (2 %) of the individual bees were misclassified.

as hybrid bees, while the Carniolan bees were misclassified by 17 %. As shown in Figure (3) , there is a great influence of the Carniolan bees on the hybrids` genotype in Egypt. This result is in agreement with Kamel (1991). Using both mtDNA and morphometrical analysis, he found that there is a great overlapping between hybrid and Carniolan bees in Egypt.

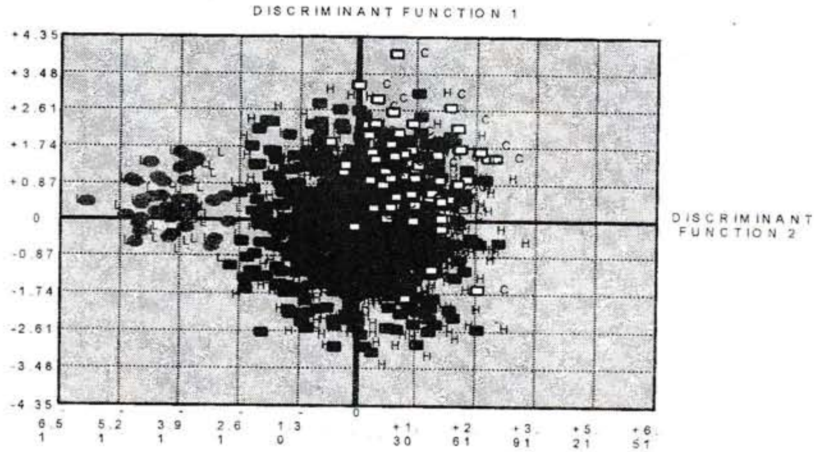

Fig. (3): Scatterplot of the two canonical axes extracted from discriminant analysis of Egyptian (L), Carniolan (C) and Hybrid bees (H).

Table (2): Classification of Egyptian, Carniolan and Hybrid bees.

Predicted group Actual group	Egyptian bees	Hybrid bees	Carniolan bees
Egyptian bees	**98%**	**2%**	**0%**
Hybrid bees	**3%**	**77%**	**20%**
Carniolan bees	**0%**	**17%**	**83%**

In many breeding programs, it is important to control the purity of the progeny obtained in every generation with respect to a specific subspieces or genotype within subspieces. Using morphological characters for doing a primary selection was already used by Adam (1982). The breeder can make a primary selection and decide easily which queens should remain, before the basic selection would be carried out, and thus one can save time and material by working with a few number of colonies.

By using discriminant analysis as one of the most important tool for the classification and the individual bees as operational units, each bee could be assigned with a defined probability to a specific genotype. This is also very important when the genotype of a colony would be determined. The mating system of the honeybee may be accompanied with the appearing of some problems concerning the classification of the honeybees. This problem may face the honeybee breeders, when two or more races are being bred in the same area, so that various genotypes would originate, and it would be very difficult to determine the genotype in each colony.

Aiming the verification of the purity of any honey bee colony, it is necessary to use individual bees as operational units in the discriminant analysis, to use many morphometrical characters, and to calculate how many bees are assigned to a defined genotype within a defined confident level (Kauhausen and Keller, 1994) or calculate the probability of membership to reference groups (Crewe *et al.*, 1994 and Daly *et al.* 1982).

3.1. Classification of the hybrid bees to both Carniolan and Egyptian bees

The two subspecies of honeybees in Egypt (local Carniolan and Egyptian bees) were used to classify the hybrid colonies from the 16 locations representing the hybrid bees in Egypt.

Table (3) shows the percentage of the bees being classified as Carniolan and Egyptian bees in each governorate. The governorates in South Egypt have relatively higher values of bees being classified as Egyptian bees than those located in northern Egypt.

To explain the previous differences, all governorates are comboind as north and south regions. The governorates in North were represented by Dakahlia, Qaliobia, Menofia, Kafr-El-Sheik, Beheira, Gharbia, Sharkia, Ismailia and Dameta, while the remaining were grouped as Southern governorates. As shown in Table (4), the percentage of the bees assigned as Egyptian bees in Southern governorates was significantly higher than those in northern ones, and *vice versa* with respect to the Carniolan bees ($P < 0.05$).

Table (3): Number of bees resulted from the classification of the Hybrid Bees from 16 Governorates in Egypt in reference to Carniolan and Egyptian bees.

	Egyptian bees	Carniolan bees	Total	Egyptian bees %
Qena	9	70	79	11
Sohag	14	85	99	14
Assiut	24	60	84	28
Minia	18	48	66	27
Benisuif	26	69	95	27
Giza	12	50	62	19
Faium	24	51	75	32
Qaliobia	9	50	59	15
Gahrbia	3	53	56	5
Kafr El-Sheik	16	55	71	22
Sharkia	5	27	32	16
Menofia	9	67	76	12
Beheira	2	32	34	6
Dakahlia	1	27	28	4
Ismalia	2	19	21	10
Dameta	1	36	37	3

Table (4): Number of hybrid bees in North and South Egypt being classified as Egyptian and Carniolan bees.

	Egyptian bees	Carniolan bees
South	127	433
North	48	366

According to the results obtained, the hybrid bees in north and south could be partly considered as two different populations. These results are attributed to the different percentages of Carniolan and Egyptian bees in both groups as the results showed. For the locations being in Northern Egypt, 89 % of the hybrids were assigned as Carniolan bees against 78 % in Southern Egypt.

3.2. The biometrical relationship between the 16 Governorates in Egypt

Three major biometrical clusters emerged, when cluster analysis was carried out on the cenroids of the first three canonical discriminant axes (Fig. 4). While the samples from Ismailia, Dameta, Sharkia, Dakahlia and Menofia formed the first group, the second group was formed by Qena, Sohag, Assiut, Minia, Benisuif, Faium and Giza and the third group was formed by four governorates: Kafr-El-Sheik, Beheira, Gharbia and Qaliobia.

Within each biometrical group, clusters of samples in morphometrical space were often, but not always, close geographically to each other (Fig.1). Most of the governorates within the first group are closed geographically, and the same situation concerning the second group. This result is confirmed with the results of Verma et al (1994), who found that clusters of samples in morphometric space were often, but not always, close geographically to each other. Because the hybrids between the Egyptian and local Carniolan bees prevail in most of the apiaries in Egypt, the previous work aimed to determine the relation between these hybrids and the two mentioned races to determine the best way to improve our stock of bees.

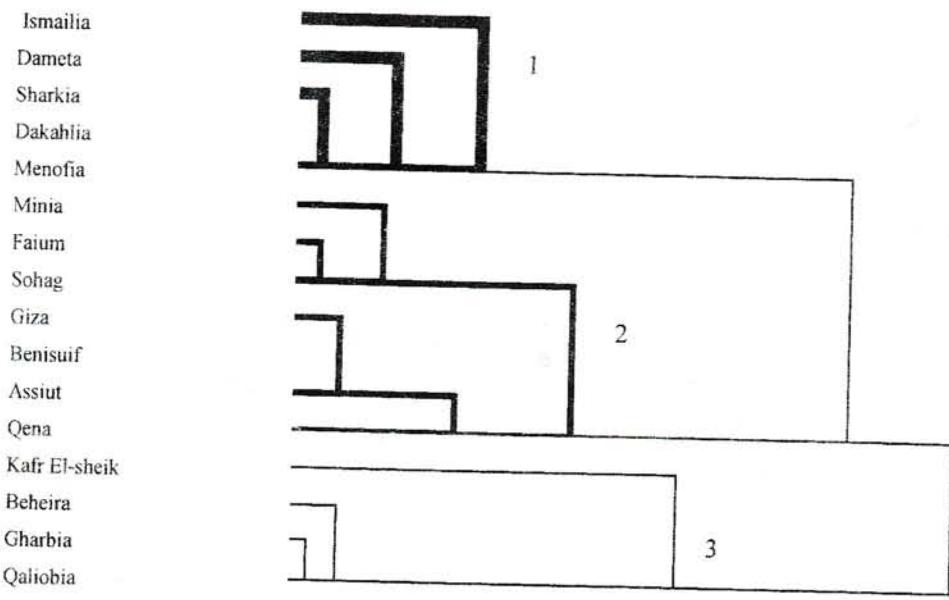

Fig. (4): Hierarichic cluster analysis of group centroids in canonical function space (the numbers 1,2 and 3 are the number of the resulted clusters)

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التصنيف الدقيق لنحل العسل في مصر من خلال مظهر تعريق الجناح

عادل محمود محمد مزيد

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ملخـــص

في هذا البحث تم استعمال الجناح الأمامي لشغالات نحل العسل لتحديد العلاقة بين التراكيب الوراثية الثلاثة السائدة في مصر وهى سلالتي النحل الكرنيولى والبلدي بالإضافة إلي الهجين الطبيعي من ناحية ولتحديد العلاقة بين الهجن المختلفة السائدة في محافظات مصر من ناحية اخرى، وقد اتضح من نتائج البحث سيادة الخلفية الوراثية للكرنيولى فى الهجن المختلفة في الوقت الذي أظهرت فيه السلالة البلدى عدم تاثرها بالسلالة الكرنيولى.

وعند القيام بتصنيف نحل الهجن المختلفة بالنسبة لسلالتى النحل الكرنيولى والبلدى اظهرت النتائج زيادة نسبة النحل المصنف كنحل كرنيولى فى محافظات الوجه البحرى بصورة معنوية عن الوجه القبلى والعكس بالنسبة للنحل البلدى مما يدل على تأثر هجن محافظات الوجه البحرى بالنحل الكرنيولى بصورة أكبر عن مثيله فى محافظات الوجه القبلى.

واخيرا أظهرت نتائج التحليل وجود ارتباط بين التوزيع الجغرافى لمحافظات مصر ومدى قربها او بعدها مورفومتريًا.

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