

## **USING THE FOREWING CHARACTERISTICS TO DISTINGUISH THE DIFFERENCES WITHIN THE CARNIOLAN HONEYBEES REARED IN EGYPT.**

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### **ABSTRACT**

Twenty five samples of Carniolan bees were collected from each of two isolated areas used for breeding Carniolan bees in Egypt, Manzala and New Valley. Thirty one characters on the right forewing of collected bees were chosen for differentiation and classification the bees in the two locations. Suitable scanner connected with a computer were used. After displaying the image of the wings on the monitor, and by using a suitable measuring program the characters of the forewing were determined as polar coordinate points. Using discriminant analysis as statistical tool, the two populations could be significantly differentiated from each other, and the bees in each location could be classified correctly to its group with classification rate of 92.84% and 88.84% for bees in New Valley and Manzala, respectively. The results obtained indicated that there was a great differences in forewing characters between the two populations, which could be help in setting a suitable cross breeding program between them for improving honeybees in Egypt.

### **INTRODUCTION**

Carniolan bees were introduced in Egypt since more than 50 years into two isolated areas: Manzala (North Egypt) and New Valley (South Egypt) about 1500 km from each other. Since that time, the two locations were considered as a source for supplying the Carniolan queens for all beekeepers in Egypt.

The consideration of exterior characters in the differentiation between honeybee populations has been of interest since the introduction of carniolan bees in Egypt. Univariate analysis used for analyzing morphometrical data of bees from one or both locations has been established before (El-Banby, 1958, and Mazeed, 1992). Most of the analyses based on univariate provide inconclusive results, since measurements overlapped between the two groups of bees. Vastly improved identification of bees was obtained when modern statistical methods of multivariate analysis were applied simultaneously to multiple measurements (DuPraw, 1964 & Daly and Bailing, 1978). Many morphometrical characters are used for a long time to differentiate between different bee races and populations (Alpatov, 1929 and Goetze, 1964). Morphological characters of the wing are important in classifying different races and strains of bees (Kauhausen Keller and Keller, 94), and it could be measured precisely (Kauhausen and Keller, 1998). Using of wing characteristics only has been proved as a useful tool for detecting the hybridization zone between two honeybee populations in Italy (Nazii, 1992),

and using a large number of wing characters increased the probability of detecting different populations within the subspecies (Kauhausen keller and Keller, 1994).

This raised a question about the possibility of using a large number of wing characteristics to study the similarity between Carniolan bees in the two isolated areas, Manzala and New Valley, and the relationship between the bees in both locations.

## MATERIAL AND METHODS

### 2.1. The origin of bees under study

The Collection of Carniolan bees, on which this study was carried out, were made from 5 colonies at each of 5 localities in Manzala and New Valley.

**In Manzala, the samples were collected from the following regions:**

New Manzala, New Kafr, Beekeeping Section, El-Sero, El-Amra, and from New Valley, the samples were collected from the following regions: Moot, Moshee, Rashda 1, Rashda 2, Instruction Apiary

Having been collected, the samples were preserved in ethanol until its preparation for mounting. In each bee sample, the number of worker bees ranged between 10 to 15.

### 2.2. Wing characteristics

The characters of the forewing were chosen according to Kauhausen (2002). She used the polar coordinate system for describing the locations of 17 junction points between the veins of the fore wing.

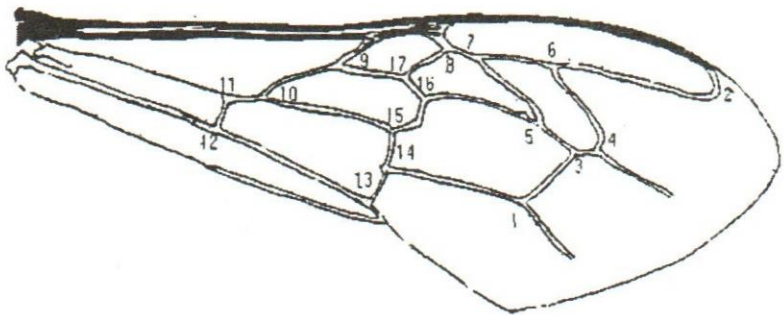


Fig. (1): Coordinates taken on the forewing

The first two intervention junctions were chosen as references to 15 intervention points on the forewing (Fig. 2). From the first two points, the polar coordinate system was established and the characters were determined in terms of distances and angles.

Because the first point has no reference point, it has no values ( $X = 0$  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 total numbers of characters measured were 31 forewing characters.

### **2.3. Equipment and measurement procedure**

The morphometrical system used consisted of the following parts:

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

The wing measurements were made by using a 35 mm slide projector to project the images of mounted wings onto a monitor screen. A particular type of slide known as a Gepe consists of two plastic half frames containing thin metal masks and each half frame is separately glazed. For each bee, the right fore wing was pulled out with a fine forceps and mounted on the slide. Each slide had 15 fore wings and each 4 slides were put in a slide mount holder, which was then put inside the Scanner, in order to scan the mounted wings. After setting a suitable displaying program, the wings were displayed on the screen monitor of a computer, and by the computer-mouse, the different intervention points were clicked by the computer mouse.

Morphometrical studies were prepared, measured and the data were analyzed in bee section in Faculty of Agriculture, Cairo University

### **2.4. Statistical analysis:**

In this study, discriminant analysis was applied to differentiate between the bees in Manzala and New Valley. Wilk's lambda test (Jobson, 1992) was used to compare multivariate population means between the two groups. The discrimination between the two groups was supported by reclassifying the bees in each locality into Manzala or New valley. In this study, the average value of each location and the values of the individual bees were used as operational units. The reclassification of the bees in each locality was carried out by using split sample procedure (Jobson, 1992). The bees in the locality to be classified were excluded from discriminant analysis and the resulted coefficients were used for reclassifying the bees in that locality.

Discriminant analysis was used also to determine the biometrical relationship between the bees in the different localities under study, and to evaluate the contribution of the different characters to the discrimination of the two major groups. The data were analyzed using Almo statistical system (Holm, 1997)

## **RESULTS**

The discriminant analysis between Manzala and New Valley was represented by displaying the values of the centroids (mean values) of both locations as in Figure (2 ). A multivariate analysis of variance (MANOVA)

showed significant difference between the means of the two groups ( $\lambda = 0.282$ ,  $df = 31$ ,  $Ch^2 = 702.48$ ,  $P < 0.001$ )

The linear discrimination function was then used to classify the average values of the bees in each locality into two major locations (Manzala and New Valley).

The determination of group membership is based on an interpretation of posterior probability of group membership (Rinderer, et al, 1993). The multivariate discriminant functions assigned correctly all the localities to its Major location, in other words, the bees in each locality was assigned to the major group, from which it has been collected.

Concerning the localities of Manzala, the posterior probabilities of membership were 99%, 98%, 99.5%, 81.4%, and 99.9% for average values of bees in Beekeeping Section, El-Amra, New Kafr, New Manzala and Shehab respectively; and it were 98%, 99.4%, 99.9, 97.7 and 99.8 for Rashda 1, Rashde 2, Instruction Apiary, Moot and Moshee, respectively, for the 5 localities in New Valley.

In each location, the group membership was determined when the posterior probability of membership is  $> 0.75$  (Crewe et al, 1994).

The previous classification was repeated but among the individual bees in each locality in reference to the bees in both Manzala and New Valley. As shown in table (1), most of the bees in each locality in both Manzala and New Valley were correctly assigned to their own major groups with different assigning percentages.

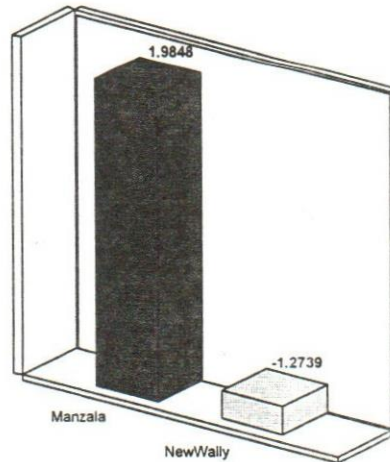


Fig. (2) : Centroides values of Manzala and New Valley

**Table (1): Classification results of the multivariate discriminant analysis**

Actual groups	Predicted groups	
	Manzala	New Valley
Manzala		
Bee section	93.19%	6.81%
El-Amra	91.31%	8.69%
New Kafr	96%	4%
New Manzala	66,7%	33.3%
El-Sero	93,34%	6,66%
New Valley		
El-Rashda 1	8,1 %	91,9%
El-Rashda 2	5.55 %	94,45%
Instruction Apiary	4,68%	95,32
Moot	15,78%	84,22%
Moshee	2.63%	97,37%

Regarding Manzala, the greatest percentage was obtained in New Kafr (96%), while the lowest one was obtained in New Manzala (66.7%). In New Valley, the highest percentage was in Moshee (97.37%), whereas the smallest percentage was in Moot (84.22%). In 11.16% cases, the bees in Manzala were misclassified as bees from New Valley, whereas 7.16% of the bees in the samples collected from New Valley were morphometrically considered as bees from Manzala, as shown in table (2).

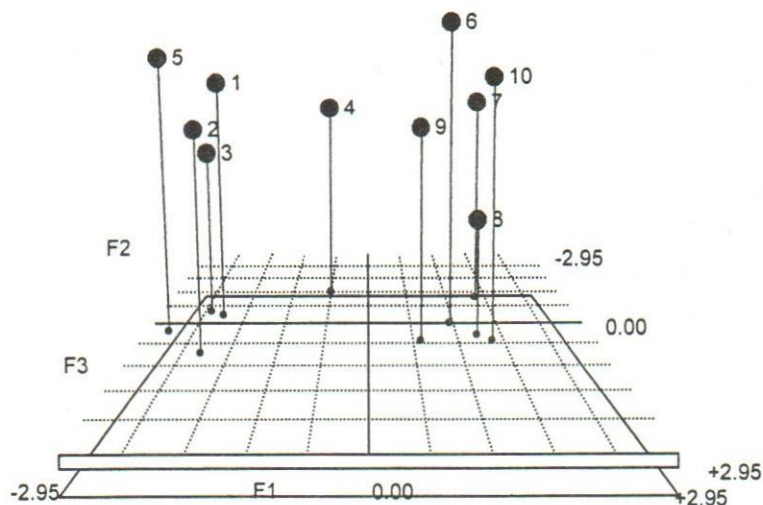
According to Ruttner (1992), a sample is considered belongs to a population, when > 75% of the individuals are be assigned to that population.

**Table (2): Classification results of discriminant analysis between Manzala and New Valley.**

Collected as	Classified as	
	Manzala	New Valley
Manzala	224 (88.84%)	25 ( 11.16% )
New Vally	25 (7.16%)	349 (92.84%)

Discriminant analysis was carried out among the ten localities of both Manzala and New Valley to show the relationship between them, and extract the powerful characters for the discrimination between them.

Using Bartlett test (jobson, 1992), it was established that only the first two factors show significant effect. The first two discriminant factors could, therefore, discriminate between two or more groups ( $P < 0.001$ ). The correlation between coefficients between canonical discriminant functions and original characters were calculated in order to determine the effective characters for discriminating the two groups from each other (table 3).



**Fig. (3): 3-Dimensional plot of the discriminant scores the ten localities in both Manzala and New Valley (1 = Beekeeping Section , 2 = El-Amra, 3 = New Kafr, 4 = New Manzala, 5 = Shehab (El-Sero), 6 = Rashda 1, 7 = Rashda 2, 8 = Instruction apiary, 9 = Moot, 10 = Moshee)**

The relationship between the ten localities of both Manzala and New Valley is shown in Figure (3). It shows the graph of the first three discriminant functions derived from the discriminant analysis. As shown in figure (3), the first function (F1) separates two groups which comprise the localities in the two locations, Manzala and New Valley. On that function, and as shown in table (3), the angles characters A9, A10, A11, A12 and A13 are very potent tool in discrimination the two groups from each other. These characters have the highest positive correlation values with the first discriminant function (table 3). Across the first function, moving from left to right resulted in moving from bees with low values to high values of the angle characters. On the contrary, the second function, although having significant effect, don't participate in any meaningful separation between both locations.

In that figure, there are mainly two groups which represent Manzala and New Valley. In Manzala, one locality is located outside the others and takes an intermediate location between the two groups of bees. As shown in table (3), the characters contributing most to the discrimination of the bees in the two locations are Angle 11 and 13

The using of wing venation pattern of the honeybees as discriminating characters supports the classification of Manzala and New Valley as two groups. According to Ruttner (1992) the bees are considered belonging to a specific population, when 75% or more of the concerning bees were assigned to that population. Therefore, and as the results indicated, the two populations in Manzala and New Valley could be considered as two different genotypes. The differences between honeybee populations within the same race may result from different environmental factors prevail in the two localities.

**Table (3): Correlation coefficients between canonical discriminant functions and the original variables (AP = distance, A = Angle).**

Characters	Function1	Function2	Function3
AP1	0.2260	-0.0013	0.3682
AP2	0.0729	-0.1100	0.4332
A2	0.2614	0.2286	-0.1384
AP3	0.1405	-0.1035	0.3555
A3	0.1856	0.0936	-0.1758
AP4	0.0572	-0.3070	0.4071
A4	0.2152	-0.1602	0.0859
AP5	0.0954	-0.2158	0.2194
A5	0.1844	-0.0176	-0.0841
AP6	-0.0257	-0.1860	0.3625
A6	0.0308	0.2530	0.1077
AP7	0.1145	-0.2858	0.3971
A7	0.1348	0.3122	0.0658
AP8	0.0425	-0.1373	0.2634
A8	0.1799	0.2125	0.0180
AP9	0.1008	0.0094	0.2956
A9	0.3127	0.2147	0.0072
AP10	0.0822	0.1184	0.2117
A10	0.3456	0.2845	-0.0254
AP11	0.1714	0.1285	0.2435
A11	0.4143	0.2300	0.0257
AP12	0.0764	0.0605	0.1476
A12	0.3287	0.0964	0.0446
AP13	0.1129	0.0379	0.3502
A13	0.3768	0.2427	-0.0500
AP14	0.0309	-0.1202	0.3805
A14	0.2956	0.2812	0.0236
AP15	-0.0089	-0.0916	0.3749
A15	0.1556	0.3330	0.0272
AP16	0.1018	-0.2922	0.1976
A16	0.1944	0.2264	-0.0360

## DISCUSSION AND CONCLUSION

Also, the results showed that, the bees in New Valley are more homogeneous than those in Manzala. The misclassification rate of the bees in New Manzala was more than 25%. Accordingly, the samples from New Manzala are considered to be another genotype, which is different from that of Manzala bees. Accordingly, the samples from New Manzala (with classification ratio of 66.7%) may be considered hybrid bees, or may be extreme samples from Manzala. Therefore, more samples are needed from that locality to verify this statement.

Crossing between queens and drones belonging to different genotype or different bred lines (cross breeding) has been proved to be as a powerful tool in honeybee breeding programs (Ruttner, 1996). The heterosis

which appears on this occasion leads to a much higher production than that obtained from the parents.

Mazeed (1992) used instrumental insemination to achieve reciprocal crossings between the bees in Manzala and New Valley and obtained a higher parameter values in the hybrid progeny than in the original genotypes. This could be a useful indicator to carry out crossing within and between the bees in both locations. As shown in Figure (3), the cross breeding could be achieved between the bees in both Manzala and New Valley in order to testing the hybrid vigor of the resulted progeny. As well, and concerning the bees in each location, it is advisable to conduct cross breeding between the bees in the localities having the greatest distance across one or more of the canonical factors extracted as shown in figure (3). For example, it is advisable for the breeding in Rashda1 to have their queens mating with drones of Instruction Apiary or vice versa, and between the bees in El-Sero and New Kafr in Manzala.

The value of using morphometrical characters is probably affected by the number and types of characters measured (Ruttner, 1992 and Moritz, 1991). As the results indicated, only the angles of the forewing are responsible for the most separation of the two groups than using distances between the coordinates of the forewing. Angles are characters, which have to do with the shape of the venation in comparison with the distances between coordinates which relate more with the size. So the difference between the bees in both Manzala and New Valley is restricted to the difference in the pattern of the venation.

According to Keller and Kauhausen (1998), the aim of the biometrical analysis is to identify the racial type, so that if a colony did not fulfill the racial requirements it was eliminated from the breeding program, irrespective of its honey production or any other behavioral characteristics.

Therefore, using classification by applying discriminant analysis may help in identifying the strange bees from a specific population and exclude it from the breeding programs or in verifying the purity of any race or testing the progeny of breeding queens in order to retain the desired ones in the following generations.

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### استخدام صفات الجناح الأمامي لإظهار الاختلافات داخل النحل الكرنيولي المربى في مصر

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في هذا البحث تم دراسة امكانية فصل وتمييز عشيرتي النحل في كل من محافظة الوادي الجديد ومركز المنزلة التابع لمحافظة الدقهلية وذلك باستخدام نموذج الاحداثيات القطبي كوسيلة لتحديد عدد 31 احدائية على سطح الجناح الامامي في مناطق النقاء العروق بعضها ببعض. هذا وقد تم تحديد مواقع هذه النقاط واجراء القياسات المختلفة باستخدام جهاز مكون من ماسح الكتروني متصل بجهاز كمبيوتر مزود ببرنامج قياس مناسب، وبعد الحصول على قيم الصفات المختلفة تم معاملة البيانات احصائيا باستخدام بعض الطرق الاحصائية الحديثة والتي تقوم بتحليل أكثر من متغير في ان واحد، وقد اظهرت نتائج التحليل امكانية فصل عشيرتي النحل في كلا من الوادي والمنزلة عن بعضهم البعض احصائيا بصورة معنوية، كما تم تصنيف نحل كل منطقة الى منطقتيه الأصلية بنسبة نجاح 92.84% بالنسبة لنحل الوادي الجديد و 88.84% لنحل المنزلة. وعلى ذلك وفي ضوء النتائج المتحصل عليها يمكن استنتاج عدم تجانس عشيرتي النحل في كلا من الوادي والمنزلة كما أن صفات الجناح المختارة كافية لفصل وتمييز نحل المنطقتين عن بعضهم. هذه النتائج تعطى مؤشر لامكانية نجاح برامج التهجين بين نحل المنطقتين وذلك املا في ظهور خاصية قوة الهجين في الأجيال الناتجة..