Morphometric Analysis and Population Characteristics of Egyptian New Valley Honeybees

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Abstract

A collection consisted of 1500 worker honeybees from 100 colonies at 10 locations in the New Valley, an isolated area in upper Egypt, were taken and measured . Thirty-eight characters were measured in each worker using image analyzer . The analysis showed that the predicted Group membership ranged between 54% and 82.7% for the ten studied populations in the New Valley. This finding means that within each Group there were some populations belonging to another group.

Results showed significant variation between original *Apis mellifera carnica* and the Egyptian *Apis mellifera carnica* which has been kept as pure race in the New Valley since more than 50 years ago. Therefore, it could be concluded that the honey bee populations in the New Valley have become a new bee population but still morphometrically close to the European A. m. *carnica.*

Key word : Morphometric analysis, New Valley honeybee, *Apis mellifera.*

Introduction

European Alpi, *Apis mellifera carnica* was introduced to Egypt in the early part of the last century. Bee breeding programs within Egypt led to the development of an Egyptian *carnica* better adapted for local conditions. The current population of Egyptian *carnica* in the new valley was found to be free from maternal gene introgression (Kamel, 2001). Egyptian bees are distinguishable from Italian bees by the whitish creamy fuzz, which is traceable in all Egyptian blood (Abu - Shady 1949).In Europe and Africa, the races of *Apis mellifera* are nearly alike in morphology and differ mainly in behaviour and certain quantitative features. Open mating among the subspecies, as well as selective breeding have produced extensive genetic recombination (Daly and Balling 1987). A large number of additional characters were used to establish the basis for multivariate statistical analysis. Classifications of 24 races of *Apis mellifera* were established including African Group (Ruttner *et al.* 1978). The computer calculates the various measurements of different bee samples and then determined by discriminant analysis whether the bees were likely to be European or Africanized (Daly *et al* 1982). Graded variation of a number of quantitative characters was established, strongly correlated to geographic latitude. These observations, which correspond well to general rules of zoogeography, cannot be generalized for other geographic races of honeybees, as demonstrated by various examples (Ruttner1985). The discriminant analysis of morphometric data has become the tool of choice for identifying Africanized honeybees. Cost of analysis led to the development of simple methods to screen large numbers of samples without sacrificing the overall quality of identifications (Rinderer 1998). According to measurements of samples from the New Valley, differences were found in the following characters: no. of hooks, length and width of the fore and hind wings, proboscis length, cubital index, corbicula width and second wax mirror length, while insignificant data existed among the rest of the thirteen qualitative characters (Mabrouk 1999). Two groups of carniolan bees could be considered as two different populations of carniolan honeybee in Egypt (Mazeed 1999). Twenty characters were used to discriminate between the pure Egyptian and Carniolan races of honeybe and their hybrids. When discriminant analysis was applied, three different and well separated groups representing the three honeybee strains (Abd ElAl 2001). The New Valley province in very wide and naturally isolated area along the west of the Nile Valley country. The actual importance of this area in beekeeping industry is referred to its isolated geographical situation (about 200 km. From the Nile valley). This situation succeeded to maintain the honey bee race almost pure wh no contamination for almost 50 years. Unfortunately, due to in breeding queens rearing program through, the last time in the New Valley, certain change

were observed in some characters of the honeybees population. The goal of the present owrk is to characterize as comprehensive as possible the bees in the New Valley through the orphometric analysis, in addition to cormpare and to determine these populations with other well known races of bees. Also, the differences within populations were studied in different locations.

Material and Methods

Sampling: Samples of the present study have been taken from 10 different locations of the New Valley province. These locations were as follow:Elkassar A, Elkassar B, Bedkhelo, Elrashda, Elsoba, Elmagless, Elshekh Wally, M. r Ibrahim, Elhendaw and Elewina.In each location, 10 colonies were selected to be sampled. Thirty workers were taken from every hive. All workers were young as possible to avoid drift bees. All bees were killed in boiling water, for 2 minutes. Bees were passed through different concentration of alcohol 25%, 50%, and 70%. In the last one (70%), bees were kept to be ready for dissecting.

List of characters measured (Table 1)

Technique of measurement and description of the program.

This program supports the measurements of morphometric characters of honeybees according to (Ruttner 1988) by video camera. Some characters can also be measured manually and the data can be entered and edited in the program. This program was developed and modified from software written at the Institute Fur Bienenkunde in Oberursel by Marina Meixner and Alfred Meixner.

Statistical analysis of the data:

Mean & standard deviation of the samples were automatically computed for each character by morphometric program.

Discriminant analysis and Principal component analysis.

Discriminant analysis and Principal component analysis (PCA) were performed using the well known subspecies of honey bees *A. m. mellifera* , *carnica, ligustica, caucasica* and *lamarckii*. They were compared with the samples of the present study to estimate the difference and the distance between each population (this analysis was carried out in Oberursel).

Multivariate discrimininant analysis:

Multivariate discrimininant analysis was conducted to calculate Canonical Discriminant Functions and Group Centroid for the ten groups. Also to estimate the predicted membership and posterior probability of every individual of each group. As well as to find out the differences within the New Valley population itself.

An ANOVA test:

An ANOVA test was preformed to determine the differences and homogenous subsets among the bees of all groups.

Results and Discussion

The measurements of characters of different groups table (2):

Discriminant analysis:

On the basis of the factor analysis a discriminant analysis was performed using the well known subspecies of honey bees *A. m. mellifera, carnica, ligustica, caucasica* and *lamarckii*, were compared together with the samples form the New Valley. To classify all populations, also to estimate the difference and the distance between each population. (using the data of all bee subspecies from data bank of Oberursel). According to the initial factor and principal component analysis (Fig.1), it was observed that the ten groups of bee samples from the New Valley had close relationship with *A.m. carnica* from Europe, but without interaction between both populations. Also clear distinction between the New Valley samples and the other well known subspecies that was observed. The arrangement of different subspecies from the nearest one to the far one from the New Valley population were as follows: *A.m.carnica, A.m.ligustica, A.m.mellifera, A.m.caucasica* and *A. m. lamarckii* , respectively.

On the other hand, when discriminant analysis was performed (Fig.2), close relationship was observed between the hundred samples of the New valley and *A.m. carnica* from Europe with few interactions between both populations. A clear distinction with *A.m.ligustica* and *A.m. lamarckii* was observed too. This result strongly means that the New Valley population although descending from the European *A.m. carnica* but in the present time it shows some differences. This fact could be due to the inbreeding since these bees were kept in this isolated area for more than forty years ago. This may limit out any good selection program for queen rearing.

Multivariate discrimininant analysis: .

Multivariate discrimininant analysis was conducted to the data of all measurements to calculate Canonical Discriminant Functions and Group Centroid for the ten groups. Also to estimate the predicted membership and posterior probability of every individual of each Group , beside finding out the differences within the New Valley population.

Canonical Discriminant Functions and Group *Centroid:*

The canonical discriminant Functions (the distance of Function 1 and Function 2) and Group Centroid of the all ten groups and the total are

shown in. table (2) & fig.(3) , fig. (4)

From the previous Canonical discriminant Functions (the distance of Function 1 and Function 2) and Group Centroid of the all ten groups, it has been concluded that, there were a clear distance and significant difference within the groups. Some groups were very close to each other and the others showed large distance in between Also the influence of inbreeding within the New Valley population was obviously clear .

Predicted Group *Membership:*

Predicted Group membership (posterior probability of every individual of each group) of the all ten groups of samples were as follows:

Group 1 : 121 workers belong to Group 1, 9 workers belong to Group 2, 5 workers belong to Group 3 , 5 workers belong to Group 4. One worker belong to Group 7 and 9 workers belong to Group 10. Fig. (4) and Table (3)

Group 2 : 98workers belong to Group 2, 7 workers belong to Group 1, 3 workers belong to Group 3, 7 workers belong to Group 4. 4 worker belong to Group 5, one worker belong to Group (6,8 and 9) 9 workers belong to Group 7 and 19 workers belong to Group 10. Fig. (4) and Table (3)

Group 3 : 124 workers belong to Group 3, 3 workers belong to Group 2, 5 workers belong to Group 4, 7 workers belong to Group 5 and Group 6. one worker to to Group 7, 3 workers belong to Group 9. Table (3)

Group 4 : 97 workers belong to Group 4, 7 one worker belong to Group 1, 8 workers belong to Group 2, 6 workers belong to Group 3. 10 worker belong to Group 5, 7 workers belong to Group 6, 11 workers belong to Group 7, 4 workers belong to Group 9 and 6 workers belong to Group 10. Table (3)

Group 5 : 81 workers belong to Group 5, one worker belong to Group 1,2 workers belong to Group 2 and Group 10 , 3 workers belong to Group 3. 13 workers belong to Group 4, 32 workers belong to Group 6, 5 workers belong to Group 7 and 11 workers belong to Group 9. Table (3)

Group 6 : 100 workers belong to Group 6, one worker belong to Group (2,4 and 10), 29 workers belong to Group 5 , 10 workers belong to Group 7. 2 workers belong to Group 8 and one worker belong to Group 10. Table (3)

Group 7: 101 workers to be belong to Group 7, 3 workers to be belong to Group 2, 7 workers to be belong to Group 4, and 10 workers to be belong to Group 5. 16 worker to be belong to Group 6, 6 workers to be belong to Group 9 and 7 workers to be belong to group10. Table (3)

Group 8 : 90 workers belong to Group 8, 13 workers belong to Group 2, 2 workers belong to Group 4, 3 workers belong to Group 5. 8 worker belong to Group 6 , 11 workers belong to Group 7 and 23 workers belong to Group 10. Table (3).

Group 9 : 100 workers belong to Group 9, one worker belong to Group 1, 3 workers belong to Group 4, 18 workers belong to Group 5. 7 workers belong to Group 6, 13 workers belong to Group 7 and 7 workers belong to group10. Table (3)

Group 10 : 123workers belong to Group 10, 2 workers belong to Group 1, 4 workers belong to Group 2, 3 workers belong to Group 4, one worker belong to Group (5 ,6 and 8), 6 workers belong to Group 7 and 9 workers belong to Group 9. Table (3).

According to Table (3), it has been observed that the highest Group with the large similar number of worker was Group 3 (124 workers).The smallest number of similar workers was in Group 5 (81 Workers). Bees in-Group 2 were divided to the all ten of groups but bees from Group 1 were divided to only 6 groups.

From the previous data it could be concluded that, there were a great differences between the all ten groups, also there were differences within groups themselves.

ANOVA Analysis:

An ANOVA test was preformed to determine the differences and homogenous subsets among the groups (Fq = 18.9), there were significant difference between all groups in all the forty five characters. Also the previous table showed the interaction of different groups of different subsets. Two characters had only one subset, three characters had two subsets and two characters had three subsets with no interaction between groups*

Moreover, it was observed that 11 characters were divided to four subsets and six characters were divided to five subsets with interaction between groups. It could be concluded from ANOVA analysis that low values of homogenity in worker bees were found, also high value of heterogenity were recorded. These conclusions support the previous two analyses, that the population of honeybees has become new bee population and morphometricaly was close to the European *A.m. carnica.* From the results of the previous morphometric analysis, it could be concluded that the honey bee populations in the New Valley are not similar to any of the tested five standard races of honey bee i.e. *A.m. carnica, A.m.ligustica, A.m.millefera , A.m .caucasica* , and *A.m. lamarckii* . When comparing the New Valley population by means of the discriminant analysis, some similarity to A. m. *carnica* was recorded.

* Interaction means that the number of different groups which has been devided into more than one set

This finding was expected to be more evident since this population was originally started from pure *A.m. carnica* and was kept isolated for almost 40 years in the New Valley area. This period of inbreeding without any good selection program for queen rearing revealed the present situation that; the race still has close relation to *A.m. carnica* and at the same time has no interaction with *A.m. ligustica* and *A.m.* lamarkii.

When applying the multivariate discriminant analysis after calculate the Canonical discriminant functions and Group Centroid, to compare all the measurements. It was found that there were distinct differences between the tested 10 groups and at the same time there were also some differences within each group. This analysis also showed that the predicted Group membership ranged between 54% and 82.7% for the ten studied populations in the New Valley. This finding means that within each Group there were some populations belonging to another group.

ANOVA analysis when applied further, confirmed this finding that homogeneous was not common in the tested populations and on the other hand, heterogeneous was distinct among studied populations in the New Valley.

Therefore, it could be concluded that the honey bee populations in the New Valley has become a new bee population having its own characteristics but still morphometrically close to the European *A. m. carnica*.

This results of the present study could be of practically applied importance through the following points:

(1) - A certain relation could be established between some of the morphometric measurements used in the present study and some of commercial characters of the honey bee races e.g. broad area , size of workerís honey stomach , longevity, nectar collection , ... etc.

(2) - In such relation, measurements could be carried out with workers only and queen rearing programs could be conducted within these selected population in certain chosen areas.

Table (1)

List of characters measured for the study of morphometry

	No	*Character*
Hair	1	Length of cover hair on tergite 5
	2	Width of tomentum on tergite 4
	3	Width of stripe posterior of tomentum
Size	4	Proboscis
	5	Femur
	6	Tibia
	7	Metatarsus length
	8	Metatarsus width
	12	Tergite 3, longitudinal
	13	Tergite 4, longitudinal
	14	Sternite 3, longitudinal
	15	Wax plate of sternite 3, longitudinal
	16	Wax plate of sternite 3, transversal
	17	Distance between wax plates, st. 3
	18	Sternite 6, longitudinal
	19	Sternite 6, transversal
Fore wing	20	Fore wing, long
	21	Fore wing, transversal
	24	Cubital vein, distance a
	25	Cubital vein, distance b
	-26 36	Angles of wing venation (NoA4,No.27-B4,No.28=D7,No.29=E9,No.30=G 18,No.31=J10,No.32=I 16,No.33=K19,No.34=L13,No.35= N23 and No.36=O26
Color	9	Pigmentation of tergite 2
	10	Pigmentation of tergite 3
	11	Pigmentation of tergite 4
	22	Pigmentation of scutellum (Sc)
	23	Pigmentation of scutellum (B,K)

Table (2)

The status of group centriod of the ten groups of samples.

No. of Group	*Group Centriod*	
	Function 1	Function 2
1	+3.3	-0.8
2	+1.2	-0.3
3	-0.5	-2.6
4	-0.2	-0.6
5	-1.6	-0.3
6	-1.5	+0.1
7	-0.9	+0.4
8	+0.6	+1.8
9	-1.5	+0.5
10	+1.2	+1.6
Total	+3	-0.4

Table (3)

Discrininant analysis, predicted group membership of the ten group of samples (posterior probability of membership in each group)

Group		Predicted Group Membership										Total
		1	2	3	4	5	6	7	8	9	10	
Original Count	1	121	9	5	5	0	0	1	0	0	9	150
	2	7	98	3	7	4	1	9	1	1	19	150
	3	0	3	124	5	7	7	1	0	3	0	150
	4	1	8	6	97	10	7	11	0	4	6	150
	5	1	2	3	13	81	32	5	0	11	2	150
	6	0	1	0	1	29	100	10	2	6	1	150
	7	0	3	0	7	10	16	101	0	6	7	150
	8	0	13	0	2	3	8	11	90	0	23	150
	9	1	0	0	3	18	7	13	0	100	7	149
	10	2	4	0	3	1	1	6	1	9	123	150
Percentage %	1	80.7	6	3.3	303	0	0	0.7	0	0	6	100.0
	2	4.7	65.3	2	4.7	2.7	0.7	6	0.7	0.7	12.7	100.0
	3	0.0	2	82.7	3.3	4.7	4.7	0.7	0	2	0	100.0
	4	0.7	5.3	4	64.7	6.7	4.7	7.3	0	2.7	4	100.0
	5	0.7	1.3	2	8.7	54	21.3	3.3	0	7.3	1.3	100.0
	6	0.0	.7	0	0.7	19.3	66.7	6.7	1.3	4	0.7	100.0
	7	0.0	2	0	4.7	6.7	10.7	67.3	0	4	4.7	100.0
	8	0.0	8.7	0	1.3	2	5.3	7.3	60	0	15.3	100.0
	9	0.7	0	0	2	12.1	4.7	8.7	0	67.1	4.7	100.0
	10	1.3	2.7	0	2	0.7	0.7	4	0.7	6	82	100.0

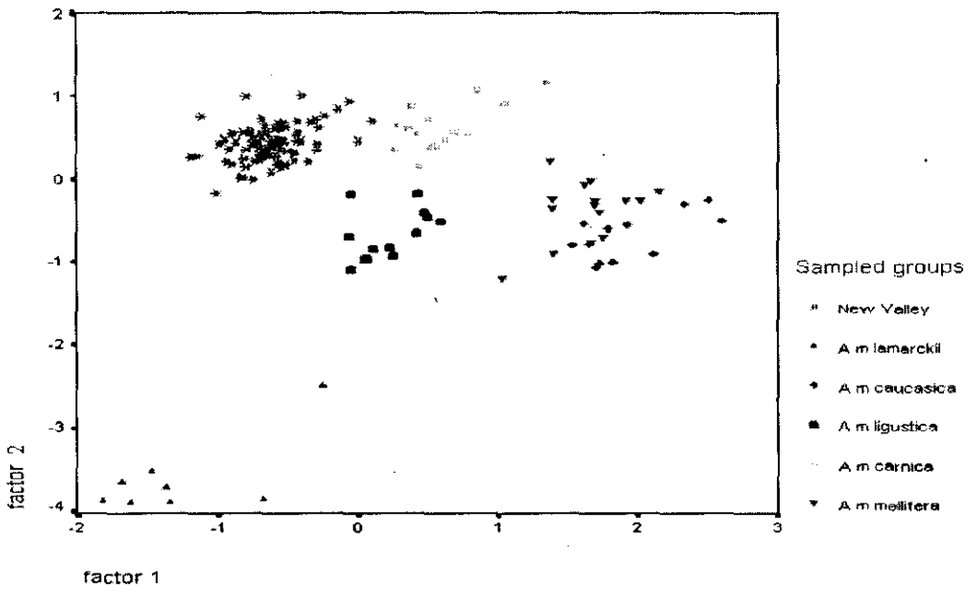

Fig. (1): Shows an initial factor (principal component) analysis that was performed using samples of *A., m. mellifera* , A. m. *carnica* , A. m. *ligustica* , *A. m. caucasica* and *A. m. lamarckii* together with the samples from the New Valley. factor 1 is plotted against factor 2 .

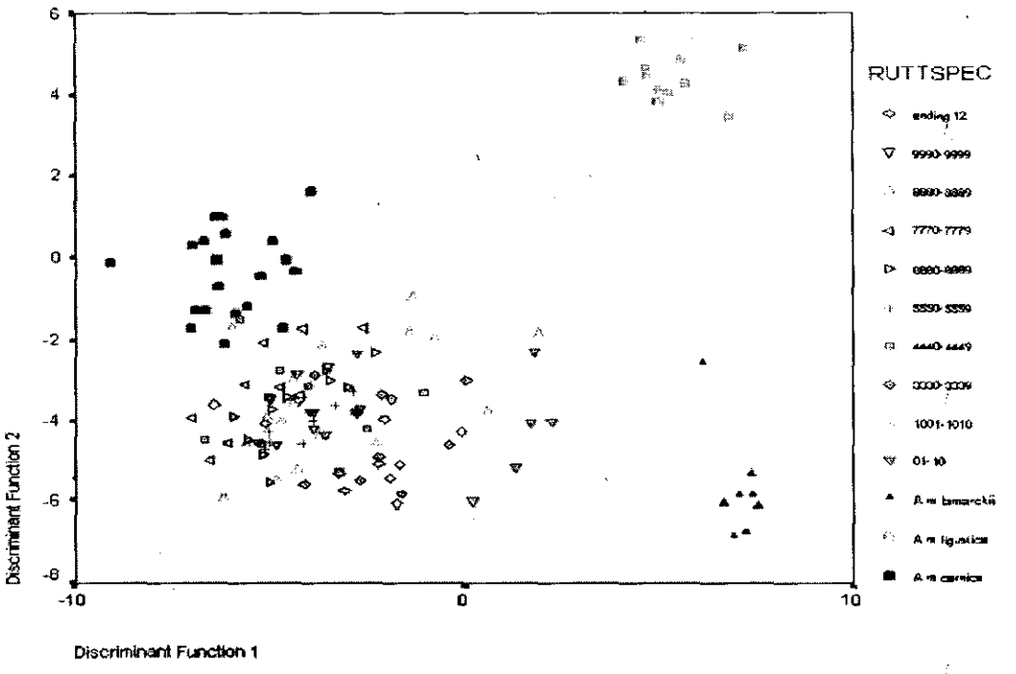

Fig. (2): Shows a discriminant analysis that was performed including samples of A. m. *carnica*, A. m. *ligustica* , *A. m. lamarckii* , and the ten groups of the New Valley samples.

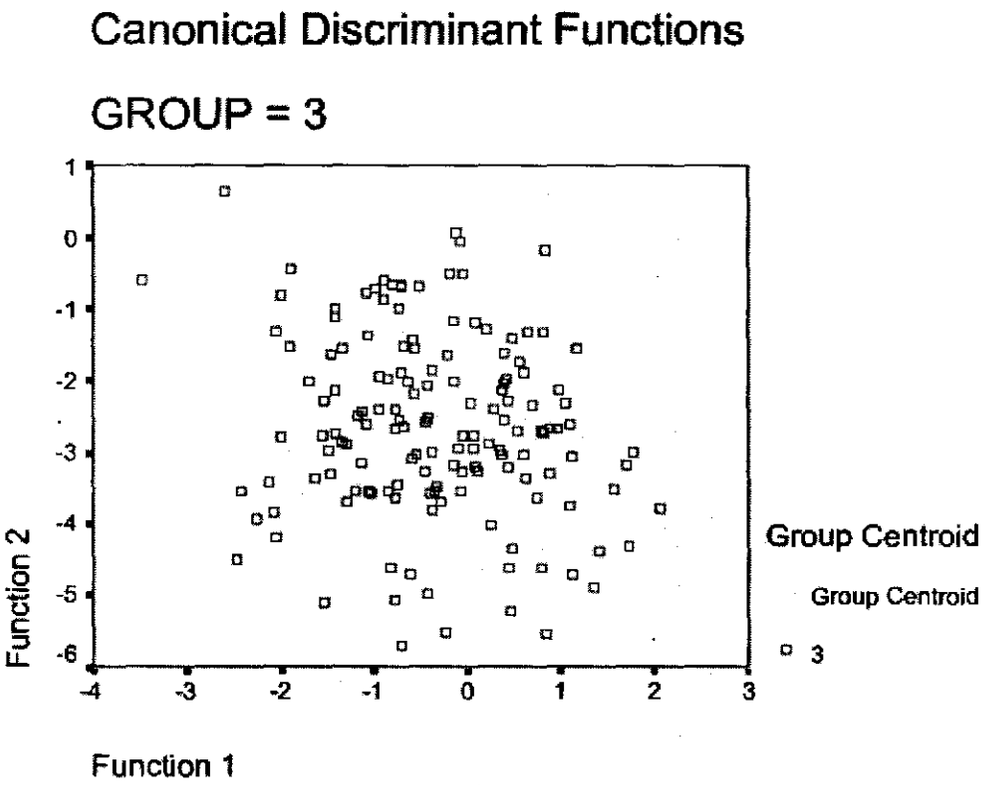

Fig. (3): Shows canonical discriminant functions and Group centriod of Group 3 (Bedkhelo)

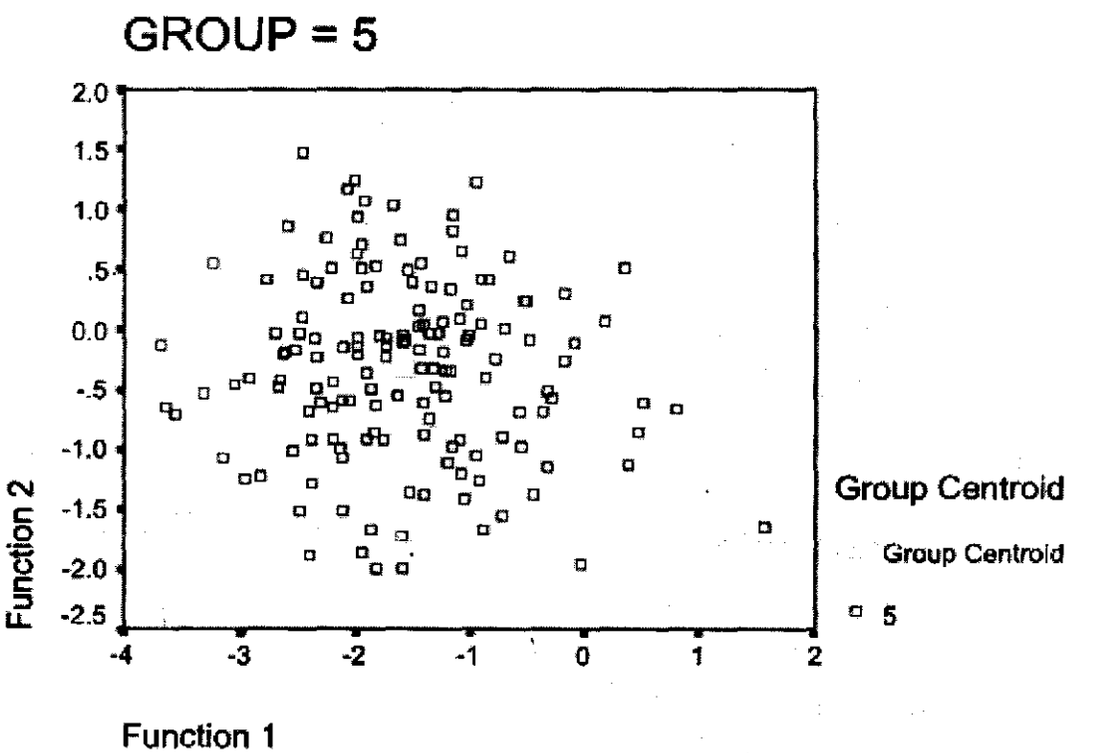

Fig (4): Shows canonical discriminant functions and Group centriod of Group 5 (Elsoba).

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References

1. ABD ELAAL. M.A. (2001): Morphometric and biochemical studies of The honeybee strains and Associated Mite (Varroa Jacobson).M.SC. Thesis Faculty of Science Ain Shams University.
2. ABOUÑSHADY (1949): races of bees ìThe hive and the honey bee Dadant & Sons. Hamilton, III. (Cited of Kamel 1991).
3. Daly, H.V. and Balling, S.S. (1978): Identification of Africanized Honeybees in the Western Hemisphere by discniminant analysis. Journal of the Kansas Entomological society. 51(4), PP. 857ñ 869. (Cited of Kamel 1991)
4. DALY, H.V.; HOELMER, K.; NORMAN, P. and ALLEN, T. (1982): Computer - assisted measurement and identification of honeybees (Hymenoptera: Apidae). Annals of the Entomological society of America, Vol. 75, No.6. (Cited of Kamel 1991).
5. EL-HANAFY.R.E.M. (1998): Studies on some honey bee races in Egypt Ph.D. thesis, faculty of agriculture, Moshtohor, Zagazic university (BenhaBranch).
6. KAMEL, S, M. (1991): Physiological studies on activities in certain honey bee strains. Ph.D. thesis, Suez canal university, Ismailia Egypt.
7. KAMEL, S. M. (2001): Characterization of Egyptian A. m. *carnica* Populations from the new valley of Egypt. Annals of Agric., Moshtohor, Vol. 39 (3): 1793-1799.
8. KAUHAUSEN .D., KELLER. R. (1994): Morphometrical control of pure race breeding in the honeybee (*Apis mellifera* L.). Apidologie 25, 133-143.
9. MABROUK. M. S o (1999): Studies on rearing honeybee queens (*Apis mellifera carnica*) for improving its quantitative and qualitative Characters in New valley. Egypt. Ph.D. Thesis Faculty of Agriculture, MiniaUniversity.
10. MAUL.V.HAHNLE.A. (1994): Morphometric studies with purebred stock of *Apis mellifera carnica* pollmann from Hessen. Apidologie 25, 119, 132.
11. MAZEED. A. (1999): Biometrical differentiation of honeybees *Apis mellifera* L. in Egypt. Ph.D. thesis fachbereichs biologie chemie pharmazie der freien universitat Berlin vorgelegt.
12. POKLUKER.J, KEZIC.N. (1994): Estimation of heritability of some Characteristics of hind legs and wings of honey bee worker (*Apis mellifera carnica* polm) using the halfsibs method. Apidologie 25, 3-11.
13. RINDERER.T.E,SYLVESTER.H.A,UCO.S,M.,LA MCASTER.V.A.,HERBERT.E.W.COLLINS.*A.m.*, HELLMICH.R.L.(1987): Improved simple techniques for Identifying Africanized and uropean honeybees. Apidologie 18 (2), 179196.
14. RINDERER, T, E. (1998): The identification of Africanized homey bees : An assessment of Morphometric, biochemical, and molecular Approaches.Acta-zoologica-Academiae-Scientiarum Hungaricae.1998; 44 (1 - 2) 177 ñ 194. (In Biological Abstracts 1999/01-1999/06)
15. RUTTNER, F.; LUCIENE, T. and louVEAUX. J. (1978): Biometrical statistical Analysis of the geographic variability of *Apis mellifera* L. Apidologie, 1978, (9) (4), 363-381. (Cited of Kamel 1991).
16. RUTTENER.F. (1985): Graded geographic variability in honey bees and Environment. Institiut fur Bienekunde (Polytechnische' Gesellschaft). Interaction, mean the number of different groups which has been divided into more than one subset