

IMPROVING THE LOCAL CARNIOLAN CHARACTERISTICS THROUGH THE ARTIFICIAL INSEMINATION PROCESS

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ABSTRACT

The effects of using artificial insemination for improving important characters of carnica honeybees (*Apis mellifera carnica*) reared in the two isolated areas was studied. Biometrical studies include (body weight, number of ovarioles, size of spermatheca and number of spermatozoa) revealed that parents significantly vary from their crosses. High recorded for these parameters was noticed in queens of (Dakhla x Manzala). Insignificant differences were observed in this biometrical parameter between parents. The important morphological characters (proboscis length, forewing length and width, second wax mirror length and width and sting length) was studied in progenies of carnica and their crosses.

High recorded for proboscis, forewing length, hind tibia and sting length indicated that using artificial insemination can be improved these traits.

INTRODUCTION

The local pure honeybee race *Apis mellifera Carnica* which was reared in both two isolated areas, Dakhla oasis and Manzala region, showed unfavorable characters due to continuous inbreeding occurred among queen bees. Although several trials have been made by many scientists to solve this problem, there still more work required for controlling the mating of these queens. On the other hand for beekeepers, this local pure race are very important, its considered as a main source of pure queens in Egypt. So improving queen characters depend on controlling of parentage. Meixner (1992), mentioned that the morphological investigations play an important role for discriminate the pure race and its hybrids, or the relationships between them, Poklukar and Kezic (1994).

Recently artificial insemination of honeybee queens has become an indispensable process technique in breeding work and inheritance studies. Wilde (1989), found that colonies with queen inseminated instrumentally had more brood in spring and summer compared to colonies with queens naturally mated. He add that this confirm the great use of hybrid queens artificially inseminated in productive apiaries. Metvdenko (1973) stated that it is possible to insure pure line breeding only by using instrumental insemination. Rothenbuhler (1968) mentioned that to obtain genetically uniform worker bees required highly inbred homozygous queens which inseminated artificially. In addition, in cross breeding with artificial insemination some qualities of different races can be used in fixed combination in order to obtain certain useful qualities in the new hybrid generation. For that, many investigators recommended hybridizing between races using artificial insemination, El-Banby (1968); El-Bassiony (2003) and Fath-Allah (2004). Flores *et al.* (1998) mentioned that artificial insemination is a technique used in order to control genetic origin, and open a way to

control those tasks useful for beekeepers, such as honey ,pollen , or royal jelly production or linked with behavior ,for instance ,less aggressive bees with a reduced tendency to swarm and a natural resistance . Fracoy and Oncalves (2004) stated that instrumental insemination of queen honeybees has been very useful for genetics studies , maintenance of bee lines and genetic improvement programs.

The present study aimed to use artificial insemination (to control over mating) of honeybee queens and throw more light on the inheritance and evaluation the morphological characters in pure honeybee races via hybridized between carnica races reared in the two isolated areas of Dakhla and Manzala.

MATERIALS AND METHODS

The present field work was carried out through the period from February (2004) till January (2006), in Dakhla Oases, New Valley Governorate and Manzala region, Dakahlia Governorate, where the local pure Carniolan honeybees race was reared and propagated . Laboratory studies were achieved in Beekeeping Department ,Plant Protection Research Institute, Dokki , Giza.

1: Selection of mother queens:

Carniolan honeybee *Apis mellifera carnica* which was reared in the two isolated area(Dakhla and Manzala), were inspected for selecting certain populated and seminally healthy colonies which were used as mother queens. Selecting these colonies was based on a selection index incorporating the honey production value of the colony itself. Ten colonies were selected from ten apiaries located in every investigated region in Dakhla and Manzala , The tested colonies were transferred to queen rearing station established in every region ready for rearing virgin queens.

2: Virgin queen rearing :

To obtain equalized larvae for breeding new virgin queens , the selected queens were caged in the hive on a comb covered with an isolator during six hours. 72 hours after releasing out the queen, the rearing of virgin queens from these larvae started using double grafting method to give excess amount of natural royal jelly to the grafted larvae of the forthcoming virgin queens, (Doolittle, 1940). The emerged virgin queens were introduced to small nuclei (two combs covered with younger workers) for ten days, (Woyke, 1978). A piece of queen excluder was fixed in the entrance of every nuclei to prevent virgin queens from natural mating . The nuclei were fed daily with sugar syrup (1:1).

3: Drone rearing:

Several empty drone combs were provided to the colony of appropriate colonies. After the eggs were laid, and drone brood were completely sealed, the drone combs were placed in an queen excluder and transported to queenless colonies. The emerging drone were maintained in special cages and placed under the feeders of the queenless colonies. Ten days aftermergence the drones were ready for collecting the semen. This

procedure was carried out for rearing drones in the two tested region used in this study.

4: Inseminating the queens:

Twelve virgin queens from each tested region were used for the insemination process. These virgin queens were divided into three groups (four queens each). The first group from each region was dissected after 3 days of insemination and the dimension of the spermatheca was measured and the number of the spermatozoon's inside were counted using spectrophotometer, (Harbo, 1975). While the second group of Dakhla, queens were instrumentally inseminated with semen collected from Dakhla drones, while the third group were inseminated with semen from Manzala drones. The same process was done with Manzala virgin queens, the second group inseminated with semen from Manzala drones, while the third group inseminated with Dakhla drone semen. After insemination process, queens were left undisturbed for about ten days in nuclei (two combs full of honey and pollen, covered with bees, and the third comb was empty), until they began egg laying. The successfully inseminated queens from each of the investigated areas and their crosses were kept protection till the sealed worker brood appeared. A wire cage (10x10 cm.) were fixed over an area of sealed worker brood till the adult workers emerged.

5: Morphometric studies :

Standard samples of ten workers (Ruttner *et al.* 1978), were collected from the tested nuclei represented the two tested region and its crosses. These samples were slightly preserved in 70 % alcohol. The following parameters were measured: length of proboscis, right and left forewing length and width, number of hooks, corbicula length and width, wax mirror length and width, length of sting apparatus.

RESULTS AND DISCUSSION

1- Weight of virgin queens:

Data presented in table (1) showed that the average weight of local carnica honeybees varied from 157 ± 1.0 up to 192 ± 2.0 mg. This variation in weight within particular race of Dakhla and Manzala were smaller, but these differences between the pure races and their crosses were higher. As average these weight were 168 ± 2.082 , 157 ± 1.0 , 192 ± 2.0 , 185 ± 1.0 mg in Dakhla, Manzala and their crosses ($D \text{♀} \times M \text{♂}$), ($M \text{♀} \times D \text{♂}$) respectively, the percentage of change of weight between Dakhla and Dakhla x Manzala) was +14.29 %, while it was -17.83 % between Manzala and (Manzala x Dakhla). The highest weight of virgin queen were obtained from cross of ($D \text{♀} \times M \text{♂}$). Regards the present data in table (1) statistical analyses shows a highly significant differences in weight among the investigated virgin queens. It can be also noticed from table (1) that using double grafting method in rearing these virgin queens increased their weight. these results confirmed those obtained by Volsovich (1954), who stated that double grafting gave the best queens. Several investigators such as Weaver (1957), Woyke (1971) concluded that the factors which influence the fecundity of queen are numerous and closely associated with each other, i.e, the weight of virgin queens depend on the age of grafted larvae, the amount and quality of royal

jelly received by the larvae during its developmental period . These food factor in turn dependent of the number of bees of the right age. Woyke (1978) mentioned that double grafting technique was put under investigation. This results also go in line with Eid, *et al.* (1980) who stated that queens weight were light on emergence mated later than those which were heavy. Rawash and El-Gayar (1985) found that the heaviest queens (mean 175 mg) were obtained by using one day larvae . He also found a significant higher mean in number of ovarioles and in size of spermatheca of one day old grafted larvae than two or three days. Furthermore, Zhi and Haung (1987) suggested that the weight at emergence of queen might be useful index for selecting rich oviposition queens . In contrast it could be concluded that the virgin queen weight could be used as an indicator for the number of ovarioles, spermatheca diameter, and may gave some indication of here stamina.

2: Number of ovarioles:

Data in table (1) indicated that the number of ovarioles as well as the dimension of spermatheca are feature which have substantial influence on the number of fertilized eggs produced by the queens . the presented data in table (1) show that the number of ovarioles in virgin queens varied from 175 ± 2.646 up to 205 ± 2.0 as a total of both ovaries. The variation in ovarioles number within particular races of Dakhla and Manzala were smaller but there was a great differences in this number between the pure races and their reciprocal crosses . it can be also noticed from the present data in table (1) that heavy queens may result in a big number of ovarioles and this in turn lead to a great colony population and a big honey crop . Furthermore there was a positive correlation between weight of virgin queen in their number of ovarioles .These results confirm there obtained by Mabrouk, (2000). Statistical analyses shows a highly differences in ovariole numbers of investigated queens . It was also noticed that the percentage of increase in number of ovarioles was 13.25 % and 8.57 % in crosses of (D x M and M x D), respectively .

3: Spermathica diameter :

the present data in table one shows that spermatheca diameter differed among geographical carnica races and their crosses used in this investigation. Therefore virgin queens of cross between (D x M) had the highest spermatheca diameter ($1.43 \text{ mm}^3 \pm 0.036$) while these diameter was 1.12 ± 0.152 , 1.10 ± 0.1 , 1.43 ± 0.036 and 1.20 ± 0.152 in Dakhla, Manzala ,and their reciprocal crosses respectively. A statistically significant differences however was proved her only in Spermathica diameter between the two crosses (D x M and M x D), while these size of Spermathica was very closely similar between Dakhla and Manzala . the percentage change in spermatheca diameter was 27.68 % and 7.14 % in (D x M and M x D) respectively. So its possible to mention that hybridization between local Carniolan honeybees resulted in improving biological characteristics , such as heavy queens may result in a large number of ovarioles and a big size of Spermathica. These results agreed with that obtained by Mazeed (1992) who founded that reciprocal insemination improve all morphometrical and biometrical characters.

Table (1): Mean of biometrical parameter of certain organs in local Carnica queens from Dakhla and Manzala region

Characters	Strains	Dakhla	Manzala	DXM	MXD	L.S.D
Weight(mg)/Virgin. Queen.		168.33±2.082	157.0±1.0	192.0±2.0	185.0±1.0	3.026
No. of ovarioles		181.0±1.0	175.0±2.646	205.0±2.0	190.0±1.732	3.646
Size of spermatheca(mm ³)		1.12±0.152	1.10±0.1	1.43±0.036	1.20±0.152	0.102
No. of spermatozoa		3.543±0.11	3.077±0.067	4.106±0.093	3.52±0.044	0.152

D: Dakhla

M: Manzala

Table (2): Mean of morphometrical measurements of the progeny Carniolan honeybees in Dakhla and Manzala and their crosses

Characters	Strains	D	DxD	DxM	M	MxM	MxD	L.S.D
Proboscis L.		6.164±0.183	6.294±0.215	6.631±0.106	6.177±0.107	6.273±0.121	6.515±0.136	0.135
Forewing L.		9.243±0.067	9.295±0.056	9.29±0.147	9.05±0.108	9.246±0.080	9.101±.157	N.S
Forewing W.		2.211±0.0562	2.295±0.066	2.326±0.072	2.139±0.060	2.272±0.109	2.309±0.42	0.067
No. of hooks		24.9±0.994	24.9±0.738	24.3±0.823	24.1±0.994	24.6±1.074	24.0±0.942	3.424
1 st basitarsus of hind leg L.		3.306±0.739	3.34±0.055	3.535±0.199	3.196±0.119	3.126±0.040	3.241±0.027	0.078
1 st basitarsus of hind leg W.		1.36±0.388	1.434±0.171	1.455±0.222	1.189±0.79	1.297±0.07	1.433±0.023	0.0499
Second Wax mirror L.		1.446±0.007	1.515±0.009	1.439±0.165	1.342±0.021	1.409±0.017	1.350±0.019	0.154
Second Wax mirror W.		2.266±0.018	2.446±0.009	2.326±0.132	2.014±0.025	2.013±0.015	2.021±0.0317	0.016
Sting L.		3.357±0.045	2.359±0.015	2.426±0.019	2.215±0.036	2.262±0.024	2.233±0.019	0.076

W= Width

L= Length

4: Number of spermatozoon's:

Data presented in table (1) shows that the number of spermatozoa in millions succeeded to pass to the spermatheca of virgin queens of the local pure race, Dakhla and Manzala and their crosses (DxM & MxD) used in this study inseminated with equal dose of 8 μ of semen was 3.543 ± 0.11 , 3.077 ± 0.067 , 4.106 ± 0.093 and 3.52 ± 0.044 respectively. It was also noticed that queens of (DxM) had the highest number of spermatozoa, while Manzala queens had the lowest number of spermatozoon's. There was a very large range of spermatozoa number reaching nearly 1.878 million among the investigated queens. On the other hand it was noticed from data in table (1) that the number of spermatozoa in spermatheca of Dakhla and (Manzala x Dakhla) queens not differing significantly.

Its is interest to mention that the results in table (1) in case of diameter of spermatheca go in line with results of spermatozoon's number, There was a positive correlation between size of spermatheca and number of spermatozoon's. These results obtained confirmed those of Woyke (1971) who mentioned that the number of spermatozoa passed to spermatheca depend to a great extent on its volume.

5: Morphometric studies:

Table (2) presents the average measurements for different organs of the local pure parents either Dakhla or Manzala carnica pure race and their two crosses (D x M and M x D) during 2005 season. The data clearly indicated that there are non significant differences between parents Dakhla and Manzala in measured morphological characters i.e. Proboscis length, forewing length and width , number of hooks and sting apparatus length. These measurements were 6.164 ± 0.183 , 9.243 ± 0.067 , 2.211 ± 0.0562 , 24.9 ± 0.994 and 2.357 ± 0.045 in Dakhla progeny, while it was 6.294 ± 0.215 , 9.295 ± 0.056 , 2.295 ± 0.0562 , 24.9 ± 0.738 and 2.359 ± 0.015 in (D x D). A significant increase of these parameters was observed in reciprocal crosses. Meanwhile, the maximum length of proboscis was obtained in cross of (Dx M). The percentage of increase in this parameter was 7.58% . Table (2) also showed the flight morphological characters (forewing length and width and the number of hooks), differ among the investigated workers was non significant ,the highest main number of hooks were observed in Dakhla progeny, while the lowest hook numbers was in Manzala worker. A significant increase of the value of pollen basket area was detected in the case of (Dx M). The percentage of increase was 6.93 % . The results in table (2) also clearly showed that there were increase in wax mirror length and width , the percentage of increase was 2.63% in progeny of D x M. A significant differences in sting length parameter was clearly noticed among the investigated worker the percentage increase of this parameter was 2.93%, this result confirmed those of Eshbah, *et al* (1994) ,who advised that queen rearing procedure should be processed in sites very far from their original sites , in order to avoid inbreeding .In contrast Mabrouk (2000) ,found that using artificial insemination improve the quantitative and qualitative characters of the New Valley. In addition Mazeed ,(1992) mentioned that the hybridization between the New Valley carnica and Manzala through artificial insemination increase the age of hybrid workers . these results go in line with

Kobernicky (1987) who applied queen rearing and mating program and tested the productivity of progenies.

Table (3): Percentage change in morphometric parameter in Carnica of Dakhia and Manzala and their reciprocal crosses

Characters	Strains	DxD	DxM	MxM	MxD
Proboscis L.		2.11%	7.58%	1.554%	5.47%
Forewing L.		0.56%	0.50%	2.17%	0.563%
Forewing W.		3.799%	0.115%	6.22%	7.947%
No. of hooks		0.0%	0. %	2.1%	0.414%
1 st pasitarsus of hind leg L..		1.03%	6.93%	2.19%	1.408%
1 st pasitarsus of hind leg W.		5.44%	8.41%	9.1%	20.52%
Second Wax mirror L.		4.77%	0.48%	4.99%	0.227%
Second Wax mirror W.		7.94%	2.63%	4.602%	0.348%
Sting L.		0.08%	2.93%	2.12%	5.19%

L= Length

W= Width

Regards, the obtained results it could be advised that using artificial insemination procedure between honeybee pure races reared in Egypt will help for improving biological and morph metrical characteristics of their progeny as will as to avoid inbreeding.

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تحسين بعض صفات سلالة النحل الكرنولي باستخدام التلقيح الصناعي
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يهدف هذا البحث إلى استخدام التلقيح الصناعي للتهجين بين النحل الكرنولي الموجود في منطقتي الداخلة والمنزلة لتحسين بعض الصفات المورفولوجية والبيولوجية للنسل الناتج وتم في هذه الدراسة ما يلي :

- ١- انتخاب أفضل الطوائف الموجودة في الداخلة والمنزلة وذلك لاستخدامها كأمهات لتربية الملكات العذارى وذلك بناء على مواصفات قياسية.
- ٢- تم اختيار ١٠ طائفة تحمل الصفات الجيدة في كلا المنطقتين وتم نقلها إلى محطة تربية الملكات بكل منطقة استعدادا لتربية الملكات العذارى
- ٣- تم تربية ١٢ ملكة عذراء من كل منطقة وقسمت إلى ثلاث مجموعات بكل مجموعة أربعة ملكات ، المجموعة الأولى من كل منطقة تم تشريحها لقيس بعض الصفات مثل وزن العذارى بعد الخروج ، ثم تم تشريحها لمعرفة عدد أنابيب المبيض ، حجم القابلة المنوية ، عدد الحيوانات المنوية التي نجحت صفي الدخول إلى القابلة المنوية بعد التلقيح الآلي، المجموعة الثانية لقحت عذارى الداخلة بسائل منوي مجمع من ذكور مختلطة من طوائف الداخلة المختارة والمجموعة الثالثة لقحت عذارى الداخلة بسائل منوي مجمع من ذكور كرنولي المنزلة وتم عمل نفس البرنامج مع العذارى المرباة في منطقة المنزلة ، حيث لقحت المجموعة الثانية بذكور من المنزلة أما المجموعة الثالثة لقحت بذكور من الداخلة وبعد نجاح عملية التلقيح والإنخال تم إجراء القياسات المورفولوجية على النسل الناتج (طول اللسان - طول وعرض الجناح الأمامي - طول وعرض مرآة غدة الشمع - طول وعرض العقلة القاعدية من رسغ الرجل الخلفية وطول آلة اللسع.

أوضحت الدراسة ما يلي :

- * عدم وجود فروق معنوية واضحة بين الصفات المورفولوجية والبيولوجية بين دربي النحل المربي في كل من الوادي الجديد والمنزلة.
- * أظهر التهجين التبادلي بين دربي السلالة الكرنولي تفوقا في معظم الصفات المورفولوجية والبيولوجية المدروسة عن الأباء الأصلية.
- * أظهر التهجين بين (الوادي والمنزلة) قوة هجين في النسل الناتج في وزن العذارى وعدد فروع المبيض وكذا حجم القابلة المنوية وطول غدة السم ، حيث تلاحظ وجود فروق معنوية بين الهجن والأباء وعدم وجود فروق معنوية بين الأباء (الوادي والمنزلة) .
- * أظهرت قوة الهجين فروق معنوية عالية في القياسات المورفولوجية خاصة في حالة ملكات الوادي الملحة بذكور من المنزلة.