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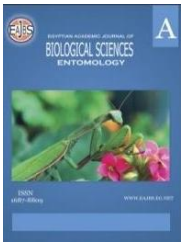

Improvement of Morphometrical Characteristics of *Apis mellifera jemenitica* Workers by Crossing with Carniolan Drones Using Instrumental Insemination

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ABSTRACT

Honeybees (*Apis mellifera* L.) live in a wide geographic range preoccupy various climatic regions. *Apis mellifera jemenitica* Ruttner is variable morphometrically in size, hair length, color, and ecological adaptions. The present study was conducted through 2024 season, in the Apiary of Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. The main target to improving honeybees of Yemeni, *Apis mellifera jemenitica* by crossing with a drone of Carniolan race and the morphological characters were measured. The instrumental insemination was according to the mentioned target of the present work. Data were subjected to analysis of variance using "SPSS" computer statistical program. The data obtained for the mean values of thirteen morphometric characters were significant. The increasing percentage change in the morphometrical parameters in hybrid after crossing inheritance between Yemeni and Carniolan races were higher, 9.21% for the length of proboscis, 8.53 for the width of the first wax mirrors, 7.70% for the tibial length, 6.83% for the length of hindwing, 6.06% for width of the hindwing,5.53 for width of the third sternum, 5.08% for length of the first wax mirror, 4.67%for the width of basitarsus, 4.26% for the length of basitarsus, and 4.04% for the tibial width. The least value of increasing was 2.48% for the length of the third sternum. The F1 generation exhibit a positive heterosis for proboscis length, forewing length, length and width of hindwing, length and width of hind tibiae, basitarsus length and width, length of the first wax mirrors, and length of the third sternum.

INTRODUCTION

Honeybees (*Apis mellifera* L.) live in a wide geographic range preoccupy various climatic regions and facing different challenges in different parts of the world and times of the year (Ruttner 1988): Moreover, bees are important pollinators of many ornamental and other crops, proffering to about one-third of the human diet and providing unlimited ecosystem services.The Western honeybee, has been included a variety of geographical races or subspecies. *A. mellifera jemenitica* Ruttner, not *A. mellifera yemenitica* Ruttner (Engel, 1999 revised these names and brought them into compliance (as far as possible) with rules of zoological nomenclature) is variable morphometrically in size, hair length, color, and ecological adaptions with *A. mellifera*, and completely overlapping with *A. cerana* in this respect (Ruttner, 1988).Yemeni race is found in areas of the highest seasonal temperature

and the zone of lowest and most irregular rain-fall where other honeybee subspecies are unable to persist (Amssalu *et al*., 2004; Taha and Al-Kahtani 2019). *A. mellifera jemenitica* is widely distributed through Africa and Asia from east to west. In Africa, *A. m. jemenitica* is mainly distributed in the Sahel, a dry tropical ecological-climatic zone south of the Sahara, north of the wetter, and tropical Africa (Ruttner, 1988; Hepburn and Radloff, 1998).The Arabian or Nubian honeybees scientifically known as *Apis mellifera jemenitica* is a distinct subspecies of the western honeybee(Nowar *et al.,* 2023). *A. mellifera jemenitica* is indigenous to several tropical locations, including Saudi Arabia, Sudan, and Somalia (Horth *et al.,* 2017; Al-Ghamdi and Nuru, 2013 and Alabdali *et al.,* 2021).The Asian populations occupy the Arabian Peninsula (Al-Ghamdi *et al*., 2013).The visual observation hasn't validate to knowing the beginning of the appearance of Yemeni bees *A. mellifera jemenitica*, that has not been recorded before in Egypt. Meanwhile, fourteen morphometric measures and two molecular markers PCR based technique (Simple Sequence Repeats; SSRs and 16s-rDNA) were used to establish morphological and genetic identity among *A. mellifera jemenitica* samples collected in Egypt and those acquired in Saudi Arabia as a reference which revealed close similarities between the studied bees, which confirm our hypothesis about the presence of Yemeni bees in Egypt(Nowar *et al.,* 2023).Rearing pure Carniolan queens (*Apis mellifera carnica*) in Egypt was conducted in two isolated regions. The first one in the north, called Manzala region, the second was established in the New Valley, west of Egypt. The two regions produce thousands of virgin and mated queens every year, and these queens are usually used for the beekeeping developing (Mazeed, 1992). The instrumental insemination is reasoned sufficient method to maximize effectiveness of the colony, and improve characteristics of both honeybee races and their offspring. Moreover, an artificial insemination and breeding program of bees are selected as critical for developmental breeding of honey bee races (Özkök, and Selçuk 2020). The improvement of honeybee characters is the first step in establishing beekeeping. On the other hand, the queen bee's mate in an open area can produce a race with a variable characteristic, so the instrumental insemination is used as the sole method to control mating (Nafaa *et al*., 2023). Such insemination is presently crucial for rearing and inheritance research (Cobey, 2007). The artificial insemination is subbing statute method to isolating mating. So, this procedure is requires less space, allows to control of mating at the same location without geographical isolated areas that necessary to natural insemination, as well as allows the breeder to use different type of drones in the same place depending on the choosing of behavioral, and reproduction characters at the colony level (Mattila and Seeley, 2007;Richard *et al*., 2007, and Seeley &Tarpy, 2007).The present investigation is aimed to improving morphometrical characteristics of *Apis mellifera jemenitica* workers by crossing with a drone of Carniolan race using instrumental insemination.

MATERIALS AND METHODS

The present study was conducted through 2024 season, in the Apiary of Department of Plant Protection, Faculty of Agriculture, Al-Azhar University. The main target to improving honeybees of Yemeni, *Apis mellifera jemenitica*(from the private apiary in El-Menofyia governorate) by crossing with a drone of Carniolan race (from El-Manzala isolated region), and the morphological characters were measured. The instrumental insemination was according to the mentioned target of the present work was categorized as follows:

A-Selecting the Source of Yemeni and Carniolan Races:

Three colonies from each Carniolan race and Yemeni race were brought to continue this study in the apiary of the Faculty of Agriculture, Al-Azhar University.

B-Preparation of Samples:

Samples of not less than twenty young workers were taken from the middle of brood nest (young bees) in each colony. Workers were dissected to separate (tongue, right forewing, right hindwing and right hind-leg).

C-Morphological Characters:

The tested morphological characters were forewing length and width, hindwing length and width, proboscis length, corbicula length and width, basitarsus length and width, first wax gland length and width, third sternum length and width. Measurements of the above-mentioned characters were taken according to Ruttner (1988). All specimens are photographed by using Tucsen 2.0MP, attached to a dissecting microscope Olympus SZ61 Stereomicroscope and measured in millimeters after calibration.

D- Artificial Insemination:

1-Instrumental Insemination Materials:

Good insemination is dependent on the quality of apparatus and how much it cost, especially the fine adjustment, wide range of movements, type of hooks, type of syringe, shape and size of tips.

The Basic Equipments:

1- Stereomicroscope. 2- Cold light source. 3- Co_2 anesthetic apparatus.
4- Insemination stand 5- Hooks. 6- Micro syringe

2-Drones Collecting:

Drones were collected for semen production from the nurse colony of the drones imported from the isolated region in Manzala (El-Dakhlia Governorates), Department of Apicultural Research, Ministry of Agriculture.

3-Semen Collecting:

Drone spermatozoa were collected under the stereomicroscope using Schley's. By slightly moving the drone to the syringe tip, the mucus could be skim of its semen covering. Semen was taken from drones as necessary, and when the syringe glass tip was filled to the desired point, a small amount of tested physiological saline solution was withdrawn into prevent sealing the end of the syringe tip by drying semen. This physiological saline solution could inject with the semen without harm.

4-Instrumental Insemination of Honey Bee Queens:

The virgin F1 Yemeni queens of 8 days old were inseminated with 8µl of semen from Carniolan (*Apis mellifera carnica*) drones by using the artificial insemination apparatus.

E-Program of Improving Morphometric Characters:

1-The Hybridization Among Two Races Yemeni and Carniolan:

Ja q. (Yemeni queen) × Ca d. (Carniolan drone)

2-Increasing Percentage:

According to the results obtained from the morphometric characters the increasing percentage of morphometric measurement were calculated by the following formula:

$$\text{Increasing } (\%) = \frac{\text{After} - \text{before}}{\text{Before}} x100$$

3 - **Estimate of Heterosis**:

Heterosis was estimated in the generation as described by Pederson, 1980.

F-Statistical Analysis:

Data were subjected to analysis of variance (ANOVA) using “SPSS” computer statistical program. Mean values were compared by using Duncan’s multiple range test, and $p \leq 0.05$ was considered statistically significant **(**Duncan, 1955).

RESULTS

1-Morphological Characteristics:

Apis mellifera jemenitica or Arabian honeybees have a smaller than Carniolan honey bee, with a hair length, resemble the Egyptian honey bee in color, and have adapted to ecological environments. The morphometrical measurements of thirteen characters are made for Yemeni, Carniolan and their hybrids ecotypes which are important for bee breeding of honeybee. The present study is compared biometrics of various important structures of Yemeni race, Carniolan race, and their hybrids.

2-Characteristic of Honeybee Workers:

Morphometrical parameters conclude the length of proboscis, length and width of the forewing, length and width of the hindwing, length and width of tibia, length and width of basitarsus, length and width of the first wax mirror, length and width of the third abdominal sternum. The results were investigated in Tables (1, 2, and 3), and the mean with Standard error values for each group of studied bees are presented. In a one-way variance analysis, it was determined were significantly different from each other in terms of fourteen morphometrical features, ($P < 0.05$).

The results obtained from Table (1), there are Significant differences in means values of proboscis lengths; forewing length and width, and the hindwing length and width for Carniolan race followed by F1, then the least values were for Yemeni race were as follows; 6.10 ± 0.021 a, 5.81± 0.0403 b, and 5.32 ± 0.070 c;9.11± 0.025 a, 8.71 ± 0.022 b, and 8.30 ± 0.023 c; 3.13 ± 0.013 a, 2.91 ± 0.011 b, and 2.76 ± 0.012 c; 6.40 ± 0.029 a, 6.26 ± 0.0211 b, and 5.90 ± 0.019 c; 1.85± 0.017 a, 1.75 ±0.005 b, and 1.68 ± 0.008 c. As well as, the tibial length; basitarsus length; width of the first wax mirrors, and the third sternum lengths, for Carniolan race followed by F1, then the least values were for Yemeni race were; 3.04 ±0.013 a, 2.96 ± 0.0142 b, and 2.75± 0.0111 c; 2.03 ± 0.0108 a, 1.96 ± 0.0078 b, and 1.88 ± 0.008 c ; 2.18 ± 0.0136a, 2.07 ± 0.0189 b, and 2.02 ± 0.0154 c; 2.60 ± 0.011 a , 2.48 ± 0.0162 b, and 2.35 ± 0.0104 c.

Table 1: The morphometrical characters of Yemeni and Carniolan honeybee workers and their hybrids.

Characters	Yemeni	Carniolan	F1	L S D
	Mean	Mean	Mean	0.05
L. of proboscis	5.32 ± 0.070 **c**	6.10 ± 0.021 **a**	5.81± 0.0403 **b**	0.142
L. of forewing	8.30 ± 0.023 **c**	9.11± 0.025 **a**	8.71 ± 0.022 **b**	0.0682
W. of forewing	2.76 ± 0.012 **c**	3.13 ± 0.013 **a**	2.91 ± 0.011 **b**	0.0372
L. of hindwing	5.90 ± 0.019 **c**	6.40 ± 0.029 **a**	6.26 ± 0.0211 **b**	0.064
W. of hindwing	1.68 ± 0.008 **c**	1.85± 0.017 **a**	1.75 ±0.005 **b**	0.032
L. of Tibia	2.75± 0.0111 **c**	3.04 ±0.013 **a**	2.96 ± 0.0142 **b**	0.04
W. of Tibia	0.99 ± 0.006 **b**	1.02± 0.008 **a**	1.03 ± 0.005 **a**	0.0204
L. of basitarsus	1.88 ± 0.008 c	2.03 ± 0.0108 **a**	1.96 ± 0.0078 **b**	0.0251
W. of basitarsus	1.07 ± 0.0076 **b**	1.13 ± 0.0072 **a**	1.12 ± 0.0081 **a**	0.0229
L. of first wax mirror	1.29 ± 0.0121 **b**	1.45 ± 0.0131 **a**	1.40 ± 0.0166 **b**	0.0423
W. of first wax mirror	2.02 ± 0.0154 **c**	2.18 ± 0.0136 **a**	2.07 ± 0.0189 **b**	0.0442
L. of third sternum	2.35 ± 0.0104 **c**	2.60 ± 0.011 **a**	2.48 ± 0.0162 **b**	0.0373
W. of third sternum	4.89 ± 0.050 **b**	5.67 ± 0.024 **a**	4.94 ± 0.070 **b**	0.155

L.=Length, W.=Width, and No.=Number.

Mean in each row followed by different letters are significant different from each other at < 0.05 (Duncan's test).

While, there isn't a significant in means values of the tibial and basitarsus width between Carniolan race and F1, and a significant between these and Yemeni race. Moreover, there is a significant in mean values of the first wax mirror lengths, and the third sternum

width between Carniolan race from one side and Yemeni race with F1 from other side.

3-The Increasing Percent of Worker Characteristics:

The increasing percentage change in morphometrical parameters in hybrid after crossing inheritance between Yemeni and Carniolan races shown in Table (2) were; 9.21% for the length of proboscis, followed by 8.53 for the width of the first wax mirrors, then 7.70% for the tibial length followed by 6.83% for the length of hindwing, then 6.06% for width of the hindwing. The increasing after hybridization were; 5.53, 5.08, 4.67, 4.26, and 4.04% for the width of the third sternum, length of the first wax mirror, width of basitarsus, length of basitarsus, and tibial width respectively. The least value of increasing was 2.48% for the length of the third sternum.

Table 2: Increasing percentages of worker characteristics of Yemeni honeybee and F1 hybrids.

Characters	Yemeni	h1 Ja × Ca	%
	Mean	Mean	Increasing
L. of proboscis	5.32	5.81	9.21
L. of forewing	8.3	8.71	4.94
W. of forewing	2.76	2.91	5.43
L. of hindwing	5.9	6.26	6.83
W. of hindwing	1.68	1.75	6.06
L. of Tibia	2.75	2.96	7.70
W. of Tibia	0.99	1.03	4.04
L. of basitarsus	1.88	1.96	4.26
W. of basitarsus	1.07	1.12	4.67
L. of first wax mirror	1.29	1.4	5.08
W. of first wax mirror	2.02	2.07	8.53
L. of third sternum	2.35	2.48	2.48
W. of third sternum	4.89	4.94	5.53
T_{X1-X2}	4.03*		

L.=Length, and W.=Width. ***=** Significant at the 0.05 probability.

Ja *Apis mellifera jemenitica* ; Ca *Apis mellifera carnica*& h hybrid**;** $T_{x}1-T_{x}2=$ was 4.03*between Yemeni and h1 of Ja x Ca honeybees**.**

Table 3: Heterosis percentage of worker characteristics in F1 hybrid between Carniolan and Yemeni honeybees**.**

Characters	F1%
L. of proboscis (mm.)	1.721
L. of forewing (mm)	0.057
W. of forewing (mm	-0.856
L. of hindwing (mm.)	2.077
W. of hindwing (mm.)	0.312
L. of Tibia (mm.)	2.256
W. of Tibia (mm.)	2.427
L. of basitarsus (mm.)	0.255
W. of basitarsus (mm.)	1.786
L. of first wax mirror (mm.)	2.143
W. of first wax mirror (mm.)	-1.449
L. of third sternum (mm.)	0.202
W. of third sternum (mm.)	-6.883

L.=Length, and W.=Width.

4- Heterosis of F1 (hybrid) Worker Characteristics:

The heterosis is a distinctive feature for the whole colonies of honeybees not for queens, workers or drones. The F1 generation exhibits a positive heterosis for proboscis length, forewing length, length and width of hindwing, length and width of hind tibiae, basitarsus length and width, length of the first wax mirrors, and length of the third sternum. Meanwhile, the heterosis of F1 generation showed a negative value for width of forewing, width of the first wax mirrors, and width of the third sternum (Table 3): The existence of heterosisis evidence to a viablility and resistance of honeybee colonies, and doesn't establish by utility of an external and internal indicators for worker bees, queen bees and drones.

DISCUSSION

As mentioned by Nowar *et al*., (2023), who documented the beginning of the appearance of *Apis mellifera jementica* Ruttner, or Yemeni race in Egypt, because the climatic changes between countries of Asia and Africa, which became more suitable for living this race and breeding it within the Egyptian country. The hybridization by using an artificial insemination of Yemeni race with Carniolan race, which improved the morphometric characters and consequently, the reproductive characters as found by authors of the present study.

The main goal of this investigation is improvement of Yemeni race by crossing with Carniolan race to increase the behavioral and reproductive characters of honeybee queens by rooting of inheritance these characters with utility of an artificial insemination, which are crucial adjectives for colonies and for beekeepers (Amiri *et al*., 2017).Also, the challenge imposed by the introducing of foreign honeybee subspecies that may hybridize with the native population (De la Rúa *et al*., 2009, and Meixner *et al*., 2010):

The present investigations are consistent with Woyke (1985), who mentioned that the first stage to improving a honeybee race is to shift bee colonies from primitive box hives to modern beehives. Hybridization is the second stage in honeybees improving, and the artificial insemination is the only way to keep honeybees from mating.

This study improved that the most important characters with the mean values of F1 hybrid in general were higher than the mean values of Yemeni race after hybridization by using an artificial insemination. Our results were in accordance with the results of Elbassiouny (2003), who found that after hybridization between the Egyptian and Carniolan races, there were a higher value for proboscis length, forewing length, and hindwing length.

Also, El-Banby (1968), found that the mean values of tongue length and the length and width of forewing 6.38, 9.00 and 3.08 mm. for the Carniolan race, respectively and the basitarsus length 2.02 mm. He studied the inheritance of some quantitative characters in the Carnio-Egyptian honeybee hybrid and he found that the progeny of open mated Carniolan queens near an Egyptian native apiary, produced workers whose structures were approximately half-way between those of Carniolan and Egyptian workers, with values closer to the mother race.

In addition,Nafea *et al,* (2023**),** studied the inheritance of some morphological characters in the Egyptian Carniolan hybrid and he found that the offspring after using instrumental insemination between Egyptian queen and Carniolan drone showed increasing the percentage of honeybee workers with improved morphological characteristics. Moreovre, he found that the F1 generation also showed positive heterosis for length and width of the forewing, tongue length, first wax mirror length, hind tibia length and length and width of the basitarsus.

Declarations:

Ethical Approval: Not applicable.

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