

MORPHOMETRICAL STUDIES ON QUEEN BEES REARED FOR HIGH QUALITY AND QUANTITY

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ABSTRACT

The present work was carried to study some morphometrical and biological characters affecting of high quality queens. Highest percentages of acceptance, emerged queens, weight of virgin queens, morphometrics of virgin queens and number of ovarioles as well as highest quality of virgin queen rearing were reared and incubated in colonies fed on dried brewer's yeast cake beside stored pollen in combs and sugar syrup 66.6% Conc..

Double grafting technique was the highest percentages of acceptance and emergence of queens, followed by wet grafting technique and dry grafting method came the latter for the previous parameters. The percentages of accepted larvae of virgin queens in the experimental colonies was affected by the type of cell cups tested. Superior of most morphometrics and biologic characters of virgin queens when bee wax cell cups were used than the other cell cups were tested such as plastic cell cups and cell cups made of wax foundation. The position of bar in holder queen rearing affected of some parameters in produced virgin queen rearing were achieved. Highest percentages of acceptance, emergence queens, weight of virgin queen and number of ovarioles were found in virgin queen reared and situated in center of queen rearing holder, followed by those reared in the top holder and the inferior were those reared in the bottom holder of queen rearing. Highest quality of virgin queens were reared and incubated in colonies contained worker population densities over 9 combs covered with bees on each other sides.

INTRODUCTION

Induced queen rearing of *Apis mellifera* is a very common technique in beekeeping. It has been known since ancient times that a queenless colony produces new queens. Based on this knowledge, Doolittle (1881) developed the basic technique that is still used today, with only small modifications (Morse, 1979). Queen rearing techniques have been nearly universally based on the manual grafting of young worker larvae in to cell cups mad of wax or plastic and transferring these cups to colonies prepared so that the bees sense that they do not have a queen and are induced to rear new queens. The workers feed the larvae with royal jelly and rear new queens (Laidlaw Jr. and Eckert, 1962). The original methodology has several variations, but the principles remain the same. These variations generally involve grafting techniques, which consist of simple or double grafting of larvae, eggs and even cells with larvae (Laidlaw Jr. and Eckert, 1962). In the process of double grafting, where the larvae in an accepted queen cup is replaced after 24h with anew larvae, the queens produced are reported to be heavier and have a higher number of ovariole than queens derived from single grafting (Harp, 1973).

Abd Al-Fattah (1996) found that the highest rate acceptance of grafting larvae was obtained using 48h old followed by 42h. larvae. Others found that the best queens resulted using one day old larvae (Diab, 1986; El-Hanafy, 1991 and Abou El-Enain, 2000). Weiss(1974) found no difference in body weight between the queens that developed from larvae at ages up to 2 days. Taber (1981) recommended with double grafting for queen high production. Abd Al-fattah *et al.* (2003) found that the percentages acceptance and emerging queens were significantly higher when the grafted larvae were 24 h. old followed by 36 h. old. Also insignificant difference were found between double grafting technique and wet grafting methods for body weight of virgin queens and measurement of diameter of queen abdomen, whereas all parameters were in lowest measure when dry grafting methods was followed. So, the present work was aimed to study the following topics:

- 1- Effect of food types on produced virgin queen rearing.
- 2- Effect of grafting methods on produced virgin queens.
- 3- Effect of queen cell cups type on produced virgin queens.
- 4- Effect of level bars containing queen cell cups grafting larvae on produced of virgin queens.
- 5- Effect of colony strength on mean characteristics of produced virgin queens.

MATERIALS AND METHODS

The present work was carried out at Beekeeping Research Department, Dokki, Giza from March during activity 2005 and 2006.

I- Preparation of experimental colonies:-

First hybrid Carniolan colonies relatively equal in population density (7-9 combs covered with bees) were used, the unsealed brood combs and the mother queen were removed from the colony. The queenless colony consisted of 2 honey combs and pollen, 2 sealed brood combs in the center, a suitable space was left to insert a frame holding the wax cups. The queenless colony was provided with 2 sealed brood combs every week. The colonies were examined before and after inserting the grafted cells, and all the natural built queen cells were destroyed. The queenless colonies were fed on 250cc Sugar syrup 66.6% Conc. every day and throughout queen rearing period. The experimental colonies were divided into five groups, each of 3 colonies. Each queenless colony received one grafted frame, with three bars that inserted between two combs of brood and pollen, fifteen artificial wax cups were fixed on each bar before grafting.

1- Food types (Diets):

Nine queenless honeybee colonies of first hybrid Carniolan bees were prepared as previously mentioned. These colonies have been divided into three groups and each group has three colonies as replicates.

Preparation of Brewer's Yeast cake: The Brewer's yeast cake was composed of dry yeast, bee honey and warm water at the ratio 4:3:1, respectively. It was prepared by weighing 1500g honey diluted with 500g warm water to form 2000g honey solution, 2000g of Dried brewer's yeast was added gradually to

2000g honey solution to form homogeneous cake. This cake was left in container for 72 hours before use.

Preparation of Wheat Germ: The Preparation of Wheat Germ as previously mentioned in Brewer's yeast cake was achieved.

Diets of group A: Dried brewer's Yeast + 2 combs (pollen and honey) + Sugar syrup 66.6%. Diets of group B: Wheat Germ + 2 combs (pollen and honey) + Sugar syrup 66.6%. Diets of group C (Control): 2 combs (pollen and honey) + Sugar syrup 66.6%.

The diets were offered to the colonies in cake form and placed over the combs (100g/colony/ weekly) and the sugar solution was offered to the bees in Dummy feeder (250cc./colony /day).

2- Grafting methods:

The preparation of queen right colonies for obtaining larvae defined age was according El-Banby and El-Sherif (1982). Worker larvae aged 24 hours old were used and three types of grafting were carried out as follows: 1- Dry grafting. 2- Wet grafting (royal jelly grafting). 3- Double grafting. In the wet grafting, where a small droplet of royal jelly solution was contained of one part of royal jelly and one part of water (w/w) was putted in each queen cup before grafting the larvae. The double grafting as the previous (wet grafting with worker larvae 24 hours old and the cup grafted frame was removed and the accepted larvae were replaced by another young worker larvae aged 24 hour old).

3- Types of queen cell cups:

Three prepared queenless colonies of each group used as starter and finisher. **The 1st group** received queen cell cups manufacture of foundation wax. **The 2nd group** received queen cell cups manufacture of plastic. **The 3rd group** received queen cell cups manufacture of bee wax. Each colony prepared as previously mentioned contained 45 cell cups (3bars × 15cups) grafted with worker larvae of 24 hour old were inserted in each prepared colony.

4- Level of bars containing queen cell cups:

Nine honeybee colonies having 7-8 combs covered with bees were prepared as previously mentioned. The colonies were divided into 3 groups, each consisted of 3 colonies. A frame holding 3 bars with 15 cell cups each. That means one colony has 45 cell cups. The mentioned frame was inserted in the middle of combs of the colony. Each colony prepared as previously mentioned and cell cups were grafted larvae aged 24 hours.

5- Colony strength:

Nine honeybee colonies F1 Carniolan bee was divided into 3 groups according to their population density in queenless colonies:

The 1st group: Moderate colonies, each colony containing 5-7 combs covered with bees. **The 2nd group:** Strong colonies, each colony containing 7-9 combs covered with bees. **The 3rd group:** over strong colonies, each colony containing over 10 combs covered with bees.

II- Preparation technique of experiments:

The preparation of queenright colonies for obtaining larvae of defined age was according El-Banby and El-Sherif (1982). The Doolittle method (1888) was used for queen rearing by larvae aged 24 hour from pure

Carniolan. The frame containing 45 grafted cups was introduced to experimental colonies, all cell cups was equal in depth and diameter 8×9mm. the queen cells started and finished in the same colony. After nine days ripe queen cells were carefully removed from the bars, and each cell was placed in a screened cage unit emergence. The number of accepted larvae and the emerged of queen cells were counted and weighed. About 30 virgin queens from each treatment were taken for measuring the morphological characters as the length and width of right forewing and the length of abdomen according to Alpatov (1929). Fifteen virgin queens from each treatment were taken for measuring the length of right ovaries and the number of ovarioles according to Woyke (1971).

RESULTS AND DISCUSSION

1- Effect of food types in bee colonies on produced virgin queen rearing:

a- The percentages of accepted larvae and number of emerged virgin queens:

Table (1) show that, the highest percentage of accepted larvae and emergence of virgin queens were 82.22% and 95.5% respectively, when feeding queenless colony rearing on diets of group **(A)**. On the contrast, the lower value percentage of accepted larvae and emergence of virgin queens were 61.48% and 87.95% respectively, when feeding queenless colony rearing on diets of group **(C)**.

b- Weight of virgin queen:

The highest rate of body weight of the virgin queens was obtained when virgin queen reared and incubated in colonies feeding diets of group **(A)**, followed by that of virgin queens which was reared in colonies feeding diets of group **(B)**, and the latter that of queens were that reared in queenless colonies feeding diets of group **(C)**.

c- External characters of virgin queens:

The virgin queens which were reared and incubated in queenless colonies fed on diets of group **(A)** had the maximum measurements of the length and width of right forewing and length of abdomen followed by those reared and incubated in queenless colonies fed on diets of group **(B)**, and these reared and incubated in queenless colonies fed on diets of group **(C)**. These results coincide with Shower, (1980) who stated that the length of abdomen of the queen proved to be a good guide to its fertility.

d- Length and number of ovarioles in the right ovary:

The highest length and number of ovarioles were found in virgin queen reared and incubated in queenless colonies fed on diets of group **(A)**, followed by those reared and incubated in queenless colonies fed on diets of group **(B)**, and the inferior were those reared and incubated in queenless colonies fed on diets of group **(C)**. These results coincide with Shower, (1980) who found that a good queen could be determined by the length and number of ovarioles. And Abou El-Enain, (1997) who found that a correlation between the number of ovarioles and brood rearing activity.

It can be concluded that to obtain virgin queen with a high quality, it is advisable by using queenless rearing colonies feeding on (Dried brewer's Yeast cake or Wheat Germ) beside stored pollen in the combs and Sugar syrup 66.6% Conc. at the all queen rearing period in the colonies.

Table (1): Effect of different types of food on the mean characteristics of produced virgin queen.

Mean of characters on virgin queens	Food types			L.S.D at 5%
	A	B	C	
Number of accepted cell cups	37±1.73	35.67±1.86	27.67±2.73	4.42
% Number of accepted cell cups	82.22%	79.26%	61.48%	
Number of emergence virgin queens	35.33±1.33	32±1.15	24.33±2.85	6.37
% Number of emerging virgin queens	95.5%	89.92%	87.95%	
Weights of virgin queens (mg)	185.4±1.09	182.33±1.67	176.87±1.43	5.23
Length of right forewing (mm.)	9.554±0.16	9.516±0.19	9.492±0.22	0.071
Width of right forewing (mm)	3.34±0.26	3.31±0.16	3.29±0.11	0.053
Length of abdomen (mm)	12.61±0.52	12.59±0.49	12.45±0.58	0.046
Length of the right ovary (mm.)	4.28±0.14	4.11±0.07	4.06±0.09	0.057
Number ovariole in the right ovary	172.63±1.84	170.6±2.85	162.23±2.52	7.14

A: Dried brewer's Yeast + 2 combs (pollen and honey) + Sugar syrup 66.6%.

B: Wheat Germ + 2 combs (pollen and honey) + Sugar syrup 66.6%.

C: 2 combs (pollen and honey) + Sugar syrup 66.6%.

2- Effect of grafting method on characteristics of the resultant virgin queens:

a- The percentages of accepted larvae and number of emerged virgin queens:

Data in table (2): show the effect on methods of grafting on some characters of virgin queens. The highest percentage of accepted larvae and emergence of virgin queens were 82.96% and 87.5% respectively, obtained by double grafting method. On the other hand, the queens were reared by dry grafting methods caused a general decrease in characters of resultant of virgin queens.

b- Weight of virgin queen:

The highest rate of body weight of virgin queens was obtained when using double grafting technique followed by wet grafting and dry grafting technique. These rates of body weight were 175.78, 171.51 and 170.29 mg, respectively.

c- External characters of virgin queens:

Data recorded in Table (2) appeared that the length and width of forewing and length of abdomen were significantly when the queen rearing program occurred by double, wet and dry grafting. Means of those characters were (9.45, 3.19 and 12.31mm), (9.42, 3.19 and 12.19mm) and (9.41, 3.18 and 12.15) for the previous grafting techniques, respectively.

d- Length and number of ovarioles in the right ovary:

The highest length and number of ovarioles were obtained in virgin queens rearing program occurred by double grafting, followed by those reared from wet grafting method and the inferior were those reared by dry grafting method. These results agree with Diab, (1986), Ibrahim, (1997) and Abd Al-Fattah *et al.* (2003). It can be concluded that, double grafting

technique produced better quality of virgin queens in their some biological characters than those obtained by wet or dry grafting technique.

Table (2): Effect of grafting methods on the mean characteristics of produced virgin queen.

Mean of characters on virgin queens	Methods of grafting			L.S.D at 5%
	Dry grafting	Wet grafting	Double grafting	
Number of accepted cell cups	26.33±2.19	32.67±1.73	37.33±1.21	9.16
% Number of accepted cell cups	58.52%	72.59%	82.96%	
Number of emergence virgin queens	21±0.58	28.7±3.38	32.7±1.29	8.13
% Number of emerging virgin queens	79.75%	87.75%	87.5%	
Weights of virgin queens (mg)	170.29±1.44	171.51±1.26	175.78±2.45	3.19
Length of right forewing (mm.)	9.411±0.22	9.416±0.18	9.451±0.36	0.032
Width of right forewing (mm)	3.181±0.11	3.186±0.26	3.189±0.13	0.011
Length of abdomen (mm)	12.15±0.47	12.19±0.59	12.31±0.62	0.031
Length of the right ovary (mm.)	3.71±0.04	3.87±0.08	4.14±0.25	0.012
Number ovariole in the right ovary	159.91±2.11	163.82±3.17	172.45±3.96	7.19

3- Effect of queen cell cups type on produced virgin queen:

a- The percentages of accepted larvae and number of emerged virgin queens:

Data in table (3) show, the percentages of accepted larvae of virgin queens in the experimental colonies was affected by the type of cell cups tested. The highest number of accepted larvae was recorded when bee wax cell cups were used and the lowest was obtained when tested cell cups from foundation wax. Plastic cell cups gave results an intermediate of accepted larvae in queen cell cups. The percentages of accepted larvae in queen cell cups could be arranged descendingly orders as follows: bee wax cell cups 69.63%, plastic cell cups 53.33% and cell cups from foundation wax 42.2%. On the other hand, the highest percentage emergence of virgin queen was recorded 76.39% when plastic of cell cups tested and the lowest was obtained when cell cups from foundation were used.

Table (3): Effect of queen cell cups type on the mean characteristics of produced virgin queen.

Mean of characters on virgin queens	Type of cell cups			L.S.D at 5%
	Cell cups from foundation wax	Plastic cell cups	Bee wax cell cups	
Number of accepted cell cups	19±1.53	24±3.21	31.33±1.86	8.86
% Number of accepted cell cups	42.22%	53.33%	69.63%	
Number of emergence virgin queens	13.67±2.11	18.33±1.86	23.3±2.13	6.54
% Number of emerging virgin queens	71.92%	76.39%	74.47%	
Weights of virgin queens (mg)	151.51±2.26	152.45±1.83	158.62±1.67	5.96
Length of right forewing (mm.)	9.341±0.51	9.344±0.47	9.411±0.39	0.089
Width of right forewing (mm)	3.140±0.17	3.146±0.22	3.156±0.15	0.051
Length of abdomen (mm)	11.89±0.51	11.91±0.46	12.12±0.35	0.79
Length of the right ovary (mm.)	3.45±0.11	3.67±0.15	3.75±0.19	0.042
Number ovariole in the right ovary	157.51±6.18	161.7±5.72	162.42±4.15	4.01

b- Weight of virgin queen:

Highest weight of virgin queens was obtained when bee wax cell cups were used and the lowest when cell cups from foundation were tested.

c- External and internal characters of virgin queens:

The superior of morphometric and biologic characters of virgin queens when bee wax cell cups were used than the other cell cups were tested. The results coincide with Ebadi and Gary (1980).

It can be concluded that, to obtain a good quality of virgin queen, it is advisable by using bee wax cell cups on artificial queen rearing.

4- Effect of level bars containing queen cell cups grafting larvae on produced virgin queens:

a- The percentages of accepted larvae and number of emerged virgin queens:

Table (4) show that, the highest percentages of accepted larvae and number emergence of virgin queens were 84.55% and 95.47% when this bar was situated in the center holder queen followed by were 80.42% and 86.57% when this bar was situated on the top holder queen and the lowest were 71.88% and 77.27% when this bar was situated on the bottom holder queen. It can be concluded that, the position of bar in holder queen rearing affected the number and emergence of virgin queen from queen cell cups.

b- Weight of virgin queen:

The highest weight of virgin queens were obtained when this bar was situated in the center of holder queen followed by was situated on the top of holder and the least was situated on the bottom holder queen rearing.

c- External characters of virgin queens:

The queen cell cups were situated in the center of holder queen rearing had the maximum measurement of the length and width of forewing and length of abdomen followed by those situated in the top holder queen and the least those situated on the bottom holder queen rearing.

d- Length and number of ovarioles in the right ovary:

The highest length and number of ovarioles were found in virgin queen reared and situated in the center of queen rearing holder followed by these reared and situated in the top holder and the inferior were those reared and situated in the bottom holder of queen rearing.

Table (4): Effect of level bars on the mean characteristics of produced virgin queen.

Mean of characters on virgin queens	Level of bars			L.S.D at 5%
	Top bar	Center bar	Bottom bar	
Number of accepted cell cups	36.19±2.13	38.05±0.58	32.35±2.71	6.69
% Number of accepted cell cups	80.42%	84.55%	71.88%	
Number of emergence virgin queens	31.33±2.62	36.33±1.67	25±1.08	9.46
% Number of emerging virgin queens	86.57%	95.47%	77.27%	
Weights of virgin queens (mg)	178.04±0.79	181.67±1.42	174.82±1.05	7.02
Length of right forewing (mm.)	9.491±0.26	9.511±0.19	9.47±0.21	0.061
Width of right forewing (mm)	3.207±0.07	3.215±0.09	3.191±0.08	0.043
Length of abdomen (mm)	12.39±0.49	12.42±0.61	12.36±0.51	0.081
Length of the right ovary (mm.)	3.78±0.03	3.91±0.02	3.71±0.056	0.041
Number ovariole in the right ovary	169.79±2.74	171.89±1.69	169.03±1.49	5.18

5- Effect of colony strength on the mean characteristics of produced virgin queen.

The data summarized in Table (5) show that, the highest percentages number of accepted queen cells and emergence of virgin queen from queen cell cups were obtained in over strong colony containing over 10 combs covered with bees, followed by strong colony containing 7-9 combs covered with bees and the lowest moderate colony containing 5-7 combs covered with bees. The morphometrics and biologic characters in the offspring of virgin queen were superiority in the over strong queen rearing colonies which contained over 10 combs covered with bees than those colonies contained 7-9 combs covered with bees (strong colonies) or the colonies contained 5-7 combs covered with bees (moderate colonies). It can be concluded that, high quality of virgin queens could be reared successfully in queen rearing colonies contained over 9 combs covered with bees. These results coincide with Abd Al-Fattah *et al.* (2003).

Table (5): Effect of Colony strength on the mean characteristics of produced virgin queen.

Mean of characters on virgin queens	Colony strength			L.S.D at 5%
	Moderate colony	Strong colony	Over strong colony	
Number of accepted cell cups	29.35±1.21	36.67±0.88	38.33±1.26	4.71
% Number of accepted cell cups	65.19%	81.48%	85.19%	
Number of emergence virgin queens	25.67±1.24	33±0.58	36.33±0.67	7.14
% Number of emerging virgin queens	87.5%	90%	94.78%	
Weights of virgin queens (mg)	180.85±1.13	181.93±0.88	185.38±1.51	5.39
Length of right forewing (mm.)	9.51±0.28	9.512±0.26	9.522±0.21	0.061
Width of right forewing (mm)	3.252±0.17	3.291±0.15	3.311±0.11	0.041
Length of abdomen (mm)	12.34±0.51	12.34±0.42	12.37±0.61	0.021
Length of the right ovary (mm.)	3.89±0.11	4.12±0.12	4.22±0.07	0.062
Number ovariole in the right ovary	171.65±2.8	171.5±3.11	172.91±2.26	3.47

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دراسات مورفو مترية لتربية ملكات نحل العسل بمواصفات عالية الإنتاج
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تم إجراء هذا البحث بهدف دراسة بعض الصفات المورفومترية والبيولوجية علي الملكات العذاري الناتجة بهدف الحصول علي ملكات نحل عالية الجودة من حيث النسبة المئوية لعدد الكؤوس الملكية المطعومة و كذلك النسبة المئوية لعدد الملكات العذاري التي خرجت من البيوت الملكية و اوزان الملكات العذاري الناتجة وكذلك الصفات المورفومترية والبيولوجية للعذاري الناتجة مثل طول و عرض الجناح و طول البطن و طول الانابيب المبيضية و كذلك عدد اللانابيب المبيضية فكانت النتائج كالاتي:

- ١- تفوقت هذه الصفات في العذاري التي ربيت و حضنت في طوائف التربية التي تغذت علي خميرة البيرة الجافة و التي تغذت علي جنين القمح هذا بجانب حبوب اللقاح المخزونة في الاقراص في الحالتين بجانب المحلول السكري، بينما جاءت الملكات التي ربيت و حضنت في طوائف تغذت علي حبوب لقاح فقط مخزنة في الاقراص في المرتبة الاخيرة.
 - ٢- الملكات العذاري الناتجة من استخدام التطعيم المزدوج تفوقت بمعنوية عالية في معظم الصفات المورفومترية والبيولوجية عن مثيلاتها الناتجة من استخدام التطعيم المبتل او الجاف.
 - ٣- تأثرت الملكات العذاري الناتجة تأثيرا ملحوظا باختلاف نوع الكؤوس المستخدمة حيث تفوقت الملكات العذاري الناتجة من استخدام الكؤوس المصنوعة من شمع النحل و جاءت الملكات العذاري المرياة في كؤوس بلاستيكية في المرتبة الثانية بينما احتلت الملكات العذاري الناتجة من كؤوس مصنوعة من شمع الأساس المرتبة الاخيرة.
 - ٤- تأثرت كذلك معظم الصفات للملكات العذاري الناتجة باختلاف اوضاع السدائب التي تحمل الكؤوس الشمعية في الخلية حيث تفوقت الملكات العذاري التي ربيت و حضنت في السدابة الوسطي في معظم الصفات ثم جاءت السدابة العليا في المرتبة الثانية بينما جاءت السدابة السفلي في المرتبة الأخيرة.
 - ٥- تأثرت كذلك الملكات العذاري الناتجة التي ربيت و حضنت في طوائف تتميز بكثافة عددية من النحل الحاضن حيث تفوقت الطوائف التي زاد عدد الاقراص المغطاة بالنحل فيها عن ١٠ اقراص يليها الطوائف التي كثافتها العددية تغطي ٧-٩ اقراص، بينما جاءت الطوائف التي يغطي النحل فيها ٥-٧ اقراص في المرتبة الأخيرة.
- ومن النتائج المتحصل عليها في البحث يمكن التوصية : باستخدام طوائف نحل قوية لتربية الملكات بحيث لا يقل عدد الأقراص المغطاة بالنحل عن ٩ أقراص من الجانبين، مع الاهتمام بوجود حبوب لقاح طبيعية مخزنة في الأقراص مع الاهتمام بتغذية الطوائف بخميرة بيرة جافة معجونة بالعسل بنسبة ٤ خميرة : ٣ عسل : ١ ماء دافئ (بالوزن) أو التغذية بجنين القمح بنفس النسب السابقة مع تغذية الطوائف بالبدايل البروتينية بجانب المحلول السكري يوميا و طول فترة التربية.
- استخدام الكؤوس الشمعية المصنوعة من شمع النحل الخام أفضل من الكؤوس البلاستيكية أو المصنوعة من شمع الأساس.
 - استخدام التطعيم المزدوج أفضل من استخدام التطعيم المبتل أو الجاف مع الاهتمام بنقل يرقات صغيرة في العمر لا تزيد عن ٢٤ ساعة.