THE EFFECT OF GENETIC ORIGIN OF THE GRAFTED LARVAE AND REARING COLONIES ON THE ACCEPTANCE RATE, WEIGHT AND CELL LENGTH OF *APIS MELLIFERA* QUEENS.

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ABSTRACT

The present work was carried out at Fayoum governorate to study the effect of the genetic origin of breeding colonies (*A.m. carnica*, *A.m. ligustica* and *A.m.bukfast*) and rearing colonies (*A. m. carnica*, and *A. m. ligustica*), queen cell position within the grafted frame (Edge, Near Edge and Center), cell bar level (Upper and Middle) and batches on the acceptance rate of the grafted larvae, queen weights and queen cell length during April, May, July and August. The results indicated that the acceptance rate of the grafted larvae was significantly higher for *ligustica* as breeding (62.90%) or rearing (60.93%) colonies, for related (71.04%) than unrelated larvae (51.86%), for Center than Edge or Near edge positions, for the Middle bar than the Upper bar, for batch 1 than batch 2, and for July or August than April or May. The average weight of queens was significantly heavier for *carnica* (174.37 mg) than *ligustica* (167.58 mg) (as rearing colonies), for the Middle bar than the Upper bar, during April than May, July and August. The average length of queen cells was insignificantly longer for *carnica* (1.94 cm) than *ligustica* (1.90 cm) (as rearing colonies), and was significantly longer for April than May, July and August.

Key words:- Queen rearing, acceptance rate, queen weight, queen cell length, bar, batch. *A.m. carnica*, *A.m.ligustica*, *A.m.bukfast.*

INTRODUCTION

Queen bees are the most important individuals within honey bee colonies for both genetic and social reasons. Thus understanding the reproductive potential of honey bee queens will provide valuable insights for improving queen quality and overall colony fitness **(Winston, 1987).**

The quality of honeybee queen depends on her genotype and the environment where she was reared **(Tarpy *et al.*, 2000).** However, the first step is to find the larvae suitable for queen rearing by the nurse bees. Nepotism is hypnotized to be the underlying reason for the selection of individual larvae to be reared as queens **(Tarpy *et al.*, 2004).** However, the data published so far on this subject are contradictory **(Breed *et al.*, 1984; Page and Erickson, 1984 and Visscher, 1986 a).**

Due to the relation of queen weight with the number of ovarioles, many researchers considered the weight of newly emerged queens as reliable

criterion for the evaluation of queen quality (**Weaver, 1957; Hoopingarner and Farrar, 1959; Marza, 1965; Woyky, 1971; Szabo, 1973; Abd Al-Fattah and El-Shemy, 1996; Zeedan, 2002; and Taha, 2005**).

The effect of the genetic origin of breeding and rearing colonies on the quantity and quality of the resulting queens, was taken into consideration by many researchers (**Mohammedi and Le Conte, 2000; Tarpy *et al.*, 2004; Masry *et al.*, 2013; and Abdelaal and Attia, 2014**). The quality of queens is affected by: the location of a given queen larvae within the queen rearing colony (**Zhu, 1981; Rawash *et al.*, 1983; Sharaf El-Din *et al.*, 2000; and Abd Al-Fattah *et al.*, 2007**), the rearing season (**De Grandi-Hoffman *et al.*, 1993; Abou El-Enain, 2000; Abd Al-Fattah *et al.*, 2003; Hassan and Mazeed, 2003; and Abd Al-Fattah et *al.*, 2011**), months of the year (**Shawer *et al.* 1980; Król 1985; Koç and Karacaoğlu 2004; and Genc *et al.* 2005**), bar level (**Sharaf El-Din *et al.*, 2000; Albarracín *et al.*, 2006; and Abd Al-Fattah *et al.*, 2011**), and batches (**Abd Al-Fattah *et al.*, 2011**).

The production of queens are affected by: the location of a given queen larvae within the queen rearing (**Abd Al-Fattah *et al.*2011**), months of the year (**Shawer *et al.*, 1980; Król, 1985; Koç and Karacaoğlu, 2004; Genc *et al.*, 2005; Guler and Alpay, 2005**), seasons of the year (**El-Mohandes, 1993; Ahmed, 2000; Mohammedi and Le Conte, 2000; Sharaf El-Din *et al.*, 2000; Abd Al-Fattah *et al.*, 2003; El- Enany, 2010; Masry, 2010; and Elsayh, 2012**), and batches (**Abd Al-Fattah *et al.*, 2011**).

The objective of the present work was to evaluate the acceptance rate of the grafted larvae, queen weights and queen cell length reared within queenless honeybee colonies, as affected by the genetic origin and distribution of queen cells within queen rearing colonies.

MATERIALS AND METHODS

The present work was carried out in a private apiary situated at Kafr Abbod village, Abshway district, Fayoum governorate, during the period from April to August, 2015. For this purpose three honey bee hybrids (*A.m.carnica, A.m.ligustica* and *A.m.bukfast*) were used during this study. Pure virgin queens were obtained, open mated at the apiary, and their daughter queens were used as 1st hybrids.

Nine honey bee colonies were used for this study. Three colonies (one from each hybrid) were used as breeder colonies (BC), and six colonies (3 from *A. m. carnica*, and 3 from *A.m. ligustica*) were used as rearing colonies (RC) during April & May (spring) and July & August (summer) of 2015. Each rearing (queenless) colony received 240 larvae, 60 larvae / month throughout two successive batches (30 larvae / batch). Each colony received about ½ liter of 1:1 (w/w) sugar syrup /week for two weeks before and during the period of queen rearing. At the 9th day after grafting, queen cells were

carefully removed from the bars and individually caged in queen cages until emergence.

Cell cups, bars and frames

Experimental queen cell frames were constructed from standard Langstroth brood frames. Each cell frame was constructed of two horizontally removable wooden bars of thirty plastic queen cup cells equally spaced (2.5 cm apart). The Upper (1st) bar was hung 4 cm. apart from the top bar of the rearing frame and the Middle (2nd) bar was hung under the Upper one with 5 cm. and so there is about 10 cm below the middle bar, which was then found nearly in the middle of the rearing frame. Larvae of the three hybrids were grafted into positions on the bars that alternated horizontally to eliminate possible position effects. Regions (zones) used in position effect evaluation are: E=edge, NE=near edge and C=center, as shown in Figure (1).

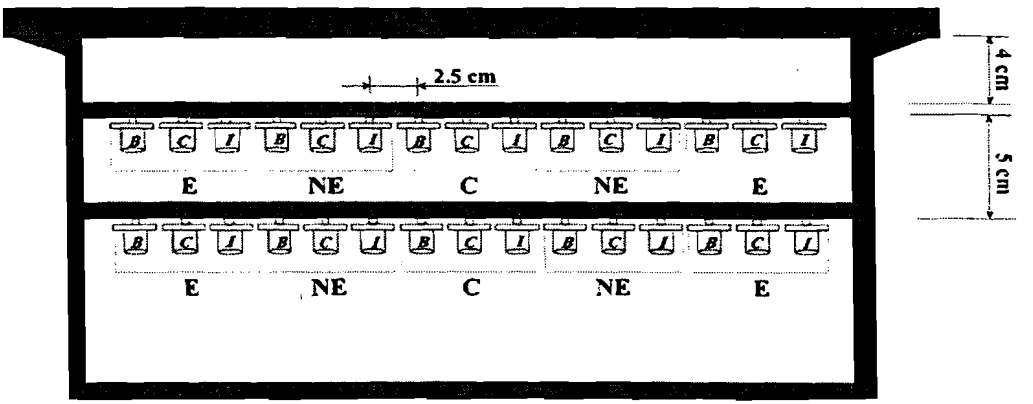

Figure (1): Experimental queen cell frame.
E = edge NE = near edge C = center.
B = A. m. buckfast C = A. m. Carnica I = A .m. ligustica

The following parameters were chosen to evaluate the effect of the previous factors on the quantity and quality of the resulting queens:

- The acceptance rate of the grafted larvae

$$\text{The acceptance rate of grafted larvae} = \frac{\text{No. of accepted larvae}}{\text{No.of grafted larvae}} * 100$$

- Queen weight and queen cell length

After queen emergence (12-24 hours) the following characteristics were measured:

a. Weight of queen (in mg) using electronic balance for 3 decimal numbers.
b. Length of queen cells (in cm) as described by **Skowronek *et al.*, 2004.**

-Statistical analysis:

Data are to be statistically analyzed by using the Statistical Analysis System software package (SPSS 21) and the treatment means are to be compared at 5% probability levels by LSD.

RESULTS

1- Effect of BC and RC on the acceptance rate of grafted larvae.

Data presented in Table (1) indicate that the general mean of acceptance rate of grafted larvae was 58.23%. The means of the acceptance rate were 55.53% & 60.93% for *A.m. carnica* and *A.m. ligustica* (as rearing colonies) respectively. On the other hand, they were 59.95%, 62.90% and 51.85% for *A.m. carnica, A.m. ligustica* and *A.m. bukfast* (as breeding colonies) respectively. This means that the acceptance rate is higher for *ligustica* as BC or RC.

The acceptance rate of grafted larvae, of *A.m. carnica* reared in *A.m. carnica* or *A.m .ligustica* were 66.2% and 53.7%, respectively. The corresponding rates of *ligustica* larvae reared in *A.m. carnica* or *A.m. ligustica* were 50.0 %, and 75.8%, respectively. For *A.m. bukfast* the rates were 50.41% and 53.3%, respectively.

Table (1): The acceptance rate of grafted larvae from *carnica, ligustica,* and *bukfast* reared in *carnica* or *ligustica* colonies.

R C	Grafted larvae	Breeding colonies (BC)			Mean
		A.m. carnica	*A.m. ligustica*	*A.m. bukfast*	
A.m. carnica	Mean of grafted larvae	10	10	10	10
	Mean of accepted larvae	6.62^{b}	5^{c}	5.04^{c}	5.553^{b}
	Acceptance rate	66.2%	50.0%	50.41%	55.53%
A.m. ligustica	Mean of grafted larvae	10	10	10	10
	Mean of accepted larvae	5.37^{c}	7.58^{a}	5.33^{c}	6.093^{a}
	Acceptance rate	53.7%	75.8%	53.3%	60.93%
Mean	Mean of grafted larvae	10	10	10	10
	Mean of accepted larvae	5.995^{b}	6.29^{a}	5.185^{c}	5.823
	Acceptance rate	59.95%	62.90%	51.85%	58.23%

LSD for B C = 0.55 LSD for R C = 0.45 LSD for interaction = 0.790
Total No. of grafted larvae = 1440 Total No. of accepted larvae =839
Means designated with the same letter do not differ significantly at 0.05 level probabilities

Data presented in Table (2) indicate that the acceptance rate of related and unrelated grafted larvae were 71.04% and 51.86%, respectively, with significant differences between the two groups.

Table (2): The acceptance rate of related and unrelated grafted larvae from *carnica, ligustica,* and *bukfast,* reared in *carnica* or *ligustica* colonies.

Genetic origin of grafted larvae	Related larvae	Unrelated larvae
Mean of grafted larvae	20	40
Mean of accepted larvae	14.208^{a}	20.744^{b}
Acceptance rate	71.04	51.86

Related larvae = larvae from the same genetic group and different colonies
Unrelated larvae = larvae from different genetic groups LSD = 1.04
No. of related grafted larvae = 480 No. of unrelated grafted larvae = 960

1-1- Effect of BC, RC and positions of the grafted larvae on the acceptance rate

Data in Table 3 indicate that the position of the grafted larvae from breeding colonies (*A.m. carnica, A.m.ligustica* and *A.m. bukfast*) reared in *carnica,* colonies greatly affects the acceptance rate. The central position of grafted larvae gave an acceptance rate higher than those of the other two positions (68.33, 44.04, and 52.75 % for Center, Edge and Near Edge, respectively). Statistical analysis proved that there were significant differences between the acceptance rate of the grafted larvae in the center position and other ones. For *ligustica* colonies when used for rearing the larvae of the same breeding colonies, the acceptance rate for the center position (70.08%), was significantly higher than those of the other two positions (47.54, and 48.21% for Edge and Near Edge, respectively).

Concerning the interaction between BC, RC and positions, the acceptance rate of grafted larvae for Center position reached its peak (91.50%) when the larvae from *carinca* were reared in *carnica* colonies, while it was (79.00%) for larvae from *ligustica* reared in *ligustica* colonies. For the two hybrids, the lowest acceptance rate occurred for the larvae grafted in the Edge position within the graft frame. This means that the acceptance rate of the grafted larvae does not only depend on their position, but also on their relatedness and hybrids.

Table (3):The effect of breeding, rearing colonies and grafted larval positions on the acceptance rate of larvae from *carnica*, *ligustica,* and *bukfast* reared in *carnica* or *ligustica* colonies.

R C	Larval position		Breeding colonies (BC)			Mean
			A. m. carnica	*A. m. Ligustica*	*A. m. bukfast*	
A.m.carnica		Mean of grafted larvae	8	8	8	8
	Edge	Mean of accepted larvae	3.83	3.33	3.41	3.523
		Acceptance%	47.87%^{e}	41.62%^{f}	42.62%^{f}	44.04^{c}
		Mean of grafted larvae	8	8	8	8
	N.Edge	Mean of accepted larvae	4.41	4.50	3.75	4.22
		Acceptance%	55.12%^{d}	56.25%^{de}	46.87%^{de}	52.75^{b}
		Mean of grafted larvae	4	4	4	4
	Center	Mean of accepted larvae	3.66	2.16	2.38	2.73
		Acceptance%	91.50%^{a}	54.00%^{de}	59.50%^{bc}	68.33^{a}
A.m.ligustica		Mean of grafted larvae	8	8	8	8
	Edge	Mean of accepted larvae	3.83	4.25	3.33	3.803
		Acceptance%	47.87^{e}	53.12%^{de}	41.62%^{df}	47.54^{bc}
		Mean of grafted larvae	8	8	8	8
	N.Edge	Mean of accepted larvae	3.91	4.16	3.50	3.857
		Acceptance%	48.87%^{e}	52.00%^{e}	43.75%^{e}	48.21^{bc}
		Mean of grafted larvae	4	4	4	4
	Center	Mean of accepted larvae	2.67	3.16	2.58	2.803
		Acceptance%	66.75%^{c}	79.00%^{b}	64.50%^{cd}	70.08^{a}

LSD for interaction = 11.8 LSD for Mean =6.86

No. of grafted larvae for Edge, N.edge and Center = 96, 96, and 48

1-2- Effect of BC, RC and bar level of the grafted larvae on acceptance rate.

Data in Table (4) indicate that, the acceptance rate of larvae from *A. m. carnica, A m. ligustica,* and *A. m.bukfast* (breeding colonies), reared in *A. m.carnica* colonies, were significantly higher (59.73 %) for the 2nd bar than those of the 1st bar (50. 80%). Also for the larvae from the same breeding colonies reared in *ligustica* colonies, the acceptance rates were significantly higher (65.23%) for 2nd bar than that of 1st bar (51.10%).

Concerning the interaction between BC, RC and bars, the acceptance rate of grafted larvae reached its peak (67.5%) for the larvae from *carinca* reared in *carnica* colonies, while it was (68.3%) for larvae from *ligustica* reared in *ligustica* colonies. This means that the acceptance rate does not only depend on the different bars, but also on the relatedness and hybrids.

Table (4):The effect of breeding, rearing colonies and bars on the acceptance rate of larvae from *carnica, ligustica,* and *bukfast* reared in *carnica* or *ligustica* colonies.

RC			Breeding colonies			
	Bars		*A. m. carnica*	*A. m. ligustica*	*A. m. bukfast*	Mean
A.m.carnica	1st	Mean of grafted larvae	10	10	10	10
		Mean of accepted larvae	5.08^{c}	5.25^{bc}	4.91^{c}	5.080^{b}
		Acceptance%	50.8%	52.5%	49.1%	50.80
	2nd	Mean of grafted larvae	10	10	10	10
		Mean of accepted larvae	6.75^{a}	5.67^{abc}	5.50^{bc}	5.973^{a}
		Acceptance%	67.5%	56.7%	55%	59.73
A.m.ligustica	1st	Mean of grafted larvae	10	10	10	10
		Mean of accepted larvae	5.33^{bc}	5.33^{bc}	4.67^{c}	5.11^{b}
		Acceptance%	53.3%	53.3%	46.7%	51.10
	2nd	Mean of grafted larvae	10	10	10	10
		Mean of accepted larvae	6.33^{ab}	6.83^{a}	6.41^{ab}	6.523a
		Acceptance%	63.3%	68.3%	64.1%	65.23

LSD= 1.24 LSD for Mean=0.71 No. of grafted larvae = 120 larvae for each bar

1-3- Effect of BC, RC and batches on acceptance rate

Data in Table (5) indicate that the acceptance rates of grafted larvae from breeding colonies (*A.m. carnica, A.m.ligustica* and *A.m. bukfast*) reared in *carnica;* colonies were significantly higher (66.57%) for batch 1 than batch 2 (44.41%). Also for *ligustica* colonies when used to rear the larvae of the same breeding colonies, the acceptance rate was significantly higher for batch1 (67.73%) than for batch 2 (54.13).

Concerning the interaction between BC, RC and batches, the acceptance rate of grafted larvae for batch 1 reached its peak (81.60%) for the larvae taken from *A.m. carnica,* and reared in *A.m.carnica,* and also for the larvae taken from *A.m.ligustica* and reared in *A.m. ligustica* (85.83 %) with

significant differences with those of batch 2 and other hybrids. This means that the acceptance rate does not only depend on the different batches, but also on the relatedness and hybrids.

Table (5):The effect of breeding, rearing colonies and batches on the acceptance rate of larvae from *carnica*, *ligustica*, and *bukfast* reared in *carnica* or *ligustica* colonies.

R C			Breeding colonies			
	Batches		*A. m. carnica*	*A. m. ligustica*	*A. m. bukfast*	Mean
A.m. carnica		Mean of grafted larvae	10	10	10	10
	Batch	Mean of accepted larvae	8.16^{a}	5.90^{b}	5.91^{b}	6.657^{a}
		Acceptance%	81.60%	59.00%	59.17%	66.57
		Mean of grafted larvae	10	10	10	10
	Batch	Mean of accepted larvae	5.083^{c}	4.08^{c}	4.16^{c}	4.441^{c}
		Acceptance%	50.83%	40.83%	41.67%	44.41
A.m. ligustica		Mean of grafted larvae	10	10	10	10
	Batch	Mean of accepted larvae	6.16^{b}	8.58^{a}	5.58^{b}	6.773^{a}
		Acceptance%	61.67%	85.83%	55.83%	67.73
		Mean of grafted larvae	10	10	10	10
	Batch	Mean of accepted larvae	4.58^{c}	6.58^{b}	5.08^{c}	5.413^{b}
		Acceptance%	45.87%	65.83%	50.83%	54.13

LSD = 1.11 LSD for Mean=0.64 No. of grafted larvae for each BC within each batch = 240

1-4- Effect of BC, RC and months on the acceptance rate

Data in Table (6) indicate that, the acceptance rate of larvae of BC reared in *carnica* was significantly higher throughout July than other months, while for *ligustica* the acceptance rate was significantly higher throughout August than other months.

Table (6): The effect of breeding, rearing colonies and months on the acceptance rate of larvae from *carnica*, *ligustica*, and *bukfast* reared in *carnica* or *ligustica* colonies.

R C	Months		Breeding colonies			
			A. m. carnica	*A. m. ligustica*	*A. m. bukfast*	Mean
A.m.carnica	April	Mean of grafted larvae	10	10	10	10
		Mean of accepted larvae	6.83^{ab}	4.83^{cd}	4.83^{cd}	5.497
		Acceptance%	68.3%	48.3%	48.3%	54.97
	May	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	5^{b}	3.83^{b}	3.67^{b}	4.167
		Acceptance%	50%	38.3%	36.7%	41.67
	July	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	7.67^{a}	6^{b}	6^{b}	6.557
		Acceptance%	76.7%	60.0%	60.0%	65.57
	August	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	7^{ab}	5.33^{c}	5.67^{bc}	6.000
		Acceptance%	70%	53.3%	56.7%	60.0
A.m. ligustica	April	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	5.33^{bc}	7.50^{a}	4.67^{cd}	5.833
		Acceptance%	53.3%	75%	46.7%	58.33
	May	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	4^{b}	7.33^{a}	3^{c}	4.777
		Acceptance%	40.0%	73.3%	30%	47.77
	July	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	5.67^{c}	7.33^{a}	5.16^{c}	6.053
		Acceptance%	56.7%	73.3%	51.6%	60.53
	August	Mean of grafted larvae	10	10	10	
		Mean of accepted larvae	6.50^{bc}	8.16a	8.50^{a}	7.72
		Acceptance%	65.0%	81.6%	85.0%	77.20

LSD = 1.5 No. of grafted larvae = 360 larvae for each month LSD for Mean=0.91

The acceptance rates of larvae from *A. m. carnica*, *A m. ligustica*, and *A. m.bukfast* (breeding colonies) reared in *A. m.carnica* reached their peaks during July (76.7, 60.0 and 60.0% respectively). For *ligustica* colonies when used to rear the larvae of the same breeding colonies, the acceptance rates reached their peaks during August with percentages of 65.0, 81.6 and 85.0, respectively. For the two hybrids, the acceptance rates of larvae reached their minimum during May.

Concerning the interaction between BC, RC and months, the acceptance rate of grafted larvae reached its peak (76.7 %) for the larvae from *carnica*, reared in *carnica* colonies in July. When larvae from *ligustica* reared in *ligustica* colonies the acceptance rate reached 81.6% in August, and reached 85.00% for larvae *bukfast* reared in *ligustica* colonies. This means that the acceptance rate of the grafted larvae

does not only depend on the months of the year, but also on their relatedness and hybrids.

2- Effect of BC and RC on queen weights.

Data in Table (7) indicate that the average weight of queens produced by *carnica* or *ligustica* as rearing colonies were 174.37 mg and 167.58 mg, respectively. For breeding colonies, the average weights of queens were 170.66 mg, 170.40 and 171.87 mg for *carnica, ligustica* and *bukfast*, respectively. Queens from *ligustica* produced by *carnica* colonies were heavier (175.55 mg) without significant difference from the queens of the other two hybrids. On the other hand, queens from *carnica* produced by *ligustica* were lighter (166.74 mg). Queens reared from *carnica* larvae and nursed by *carnica* workers were significantly heavier (174.58mg) than those from *ligustica* produced by *ligustica* (165.25mg). Queens reared from *bukfast* larvae and nursed by *carnica* or *ligustica* workers were of moderate weight (172.97 and 170.77 mg, respectively), without significant difference.

Table (7): The effect of breeding and rearing colonies on queen weights of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica* colonies.

R C	B C			Mean
	A. m. carnica	*A. m. ligustica*	*A. m. bukfast*	
A. m. carnica	174.58^{a}	175.55^{a}	172.97^{a}	174.37^{a}
A. m. ligustica	166.74^{b}	165.25^{c}	170.77^{ab}	167.58^{b}
Mean	170.66^{a}	170.40^{a}	171.87^{a}	170.97

LSD for breeding colonies = 3.56 LSD for rearing colonies = 3.54 LSD for interaction = 5.04

2-1- Effect of BC, RC and positions of the grafted larvae on queen weights

Data in Table (8) indicate that the average weight of queens from breeding colonies (*A.m. carnica, A.m. ligustica* and *A.m. bukfast*) reared in *carnica* colonies for Center position was insignificantly heavier (172.52 mg) than those of Edge (171.59 mg) and N. edge (171.97 mg) positions. For *ligustica,* the average weights of queens were 163.97, 164.44 and 163.88 mg, for Center, Edge, and N. edge positions, respectively.

Table (8): The effect of breeding, rearing colonies and positions of grafted larvae on queen weights of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica* colonies.

R C		Breeding colonies (BC)			
positions		*A.m.Carnica*	*A.m.ligustica*	*A.m.bukfast*	Mean
A.m.Carnica	E	172.30^{a}	169.34^{a}	173.12^{a}	171.59a
	N.E	170.45^{a}	172.51^{a}	172.96^{a}	171.97a
	C	172.38^{a}	173.61^{a}	171.57^{a}	172.52a
A.m.ligustica	E	163.70^{b}	164.02^{b}	163.92^{b}	163.88b
	N.E	166.27	163.82^{b}	163.23^{b}	164.44b
	C	164.98^{b}	166.60	160.34^{b}	163.97b

LSD for interaction = 5.4 LSD for Mean = 3.13

2-2- Effect of BC, RC and bars on queen weights

Data in Table (9) indicate that the average weight of queens for BC reared in *carnica* colonies was significantly heavier (173.62 mg) for the 2nd bar than that of the 1st bar (170.43 mg). When the larvae of the same previous BC were reared in *ligustica* colonies, the average weight of queens was insignificantly heavier (165.22 mg) for the 2nd bar than that of the 1st bar (162.97 mg).

For the breeding colonies *A. m. carnica*, *A.m. ligustica*, and *A. m. bukfast*, reared in *A. m. carnica* colonies, the average weights of queens were heavier (173.81, 172.78 and 174.29 mg) for the 2nd bar than the 1st bar (169.62, 170.86, and 170.81 mg). Also when the larvae from the same breeding colonies were reared in *ligustica* colonies, the average weights of queens were heavier (167.24, 166.14 and 162.28 mg) for the 2nd bar compared with those of 1st bar (162.73, 163.49, and 162.71 mg).

Table (9): The effect of breeding, rearing colonies and bars on queen weights of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica* colonies.

R C		Breeding colonies			
	Bars	*A.m. carnica*	*A.m. ligustica*	*A.m.bukfast*	Mean
A.m. carnica	1st	169.62[b]	170.86[a]	170.81[a]	170.43[b]
	2nd	173.81[a]	172.78[a]	174.29[a]	173.62[a]
A.m. ligustica	1st	162.73c	163.49c	162.71c	162.97[c]
	2nd	167.24b	166.14b	162.28c	165.22[c]

LSD for interaction= 4.40 ***LSD = for Mean = 2.54***

2-3- Effect of BC, RC and batches, on queen weights

Data in Table (10) indicate that the average weight of queens for breeding colonies (*A.m. carnica, A.m.ligustica* and *A.m. bukfast*) reared in *carnica* colonies was insignificantly heavier (172.76 mg) for batch 1 than that of batch 2 (171.30 mg). For *ligustica* colonies when used to rear the larvae of the same breeding colonies, the average weight of queens was significantly heavier for batch 2 (165.98 mg) than that of batch 1 (162.22mg).

Table (10): The effect of breeding, rearing colonies and batches on queen weights (mg) of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica* colonies

(R C)	Batches	Breeding colonies (BC)			Mean
		A.m. carnica	*A.m. ligustica*	*A.m.bukfast*	
A.m.carnica	Batch1	173.01[a]	171.78[a]	173.49[a]	172.76[a]
	Batch2	170.42[a]	171.86[a]	171.61[a]	171.30[a]
A.m.ligustica	Batch1	161.38c	163.85[b]	161.44[c]	162.22c
	Batch2	168.59[b]	165.78[b]	163.56[c]	165.98[b]

LSD for interaction = 4.4 ***LSD for Mean = 2.54***

2-4-Effect of BC, RC and months on queen weights

Data in Table (11) indicate that, the average weight of queens from breeding colonies (*A. m. carnica, A m. ligustica,* and *A. m.bukfast*) reared in *A. m.carnica* were significantly heavier (184.35 mg) during April than other months (168.51, 163.48 and 171.77 mg for May, July and August, respectively).

For *ligustica* colonies when used to rear the larvae of the same breeding colonies, the average weights of queens were also significantly heavier (176.44 mg) during April than other months (156.69, 157.44 and 165.56 mg for May, July and August, respectively).

During April, the average weight of queens from BC reared in *carnica* colonies were 185.35, 180.73 and 186.97 mg for *A. m. carnica, A m. ligustica,* and *A. m.bukfast*, respectively. For *ligustica* colonies, when used to rear the larvae of the same BC, the average weights of queens were 178.25, 176.26 and 174.81 mg, respectively. Statistical analysis proved that the average weights of queens during April were significantly heavier than other months.

The average weight of queens reached its maximum (186.97mg) during April, for larvae of *bukfast* reared in *carnica* colonies, while it reached its minimum (155.88 mg) during July for *ligustica* larvae reared in *ligustica* colonies.

Table (11): The effect of breeding & rearing colonies and months on queen weight of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica* colonies

R C		Breeding colonies			
Months		*A.m. carnica*	*A.m. ligustica*	*A.m.bukfast*	Mean
A.m. carnica	April	185.35a	180.73b	186.97a	184.35[a]
	May	168.02	169.27	168.23	168.51[d]
	July	161.20	163.55	165.69	163.48e
	August	172.27	173.72	169.31	171.77[c]
A.m. ligustica	April	178.25b	176.26b	174.81b	176.44[b]
	May	157.12	157.00	156.76	156.96f
	July	159.00	155.88	157.44	157.44f
	August	165.57	170.12b	160.98	165.56[d]

LSD for interaction = 5.47 ***LSD for Mean = 3.15***

3- Effect of BC and RC on queen cell length.

Data presented in Table (12) indicate that the general mean of queen cells length was 1.92 cm. The mean length was 1.94 and 1.90 cm for *A.m. carnica* and *A.m. ligustica* (as rearing colonies) respectively. On the other hand, it was 1.89, 1.93 and 1.94 cm for *A.m.carnica, A.m.ligustica* and *A.m.*

bukfast (as breeding colonies) respectively, without significant differences between BC or RC.

Table (12): The effect of breeding and rearing colonies on queen cell length of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica* colonies.

RC	BC			Mean
	A.m.	*A.m. ligustica*	*A.m.bukfast*	
A. m. carnica	1.90a	1.93a	1.98a	1.94a
A. m. ligustica	1.88[a]	1.93[a]	1.89[a]	1.90[a]
Mean	1.89[a]	1.93[a]	1.94[a]	1.92

LSD for R C = 0.05 ***LSD for B C = 0.075*** ***LSD for interaction = 0.10***

3-1- Effect of BC, RC and the positions of grafted larvae on queen cell length

Data in Table (13) indicate that for the larvae of BC reared in *carnica* colonies, the average length of queen cell for Center position was insignificantly longer (1.97cm) than those of Edge, and N. edge positions (1.96 and 1.94 cm, respectively). For *ligustica,* the average length of queen cell was insignificantly shorter for Center (1.88 cm) than for the other two positions; Edge (1.90 cm) and N. edge (1.89 cm).

Table (13): The effect of breeding, rearing colonies and positions of grafted larvae on queen cell length of larvae from *carnica*, *ligustica,* and *bukfast*, reared in *carnica* or *ligustica colonies*

RC		Breeding colonies			
	Position	*A.m.Carnica*	*A.m.ligustica*	*A.m.bukfast*	Mean
A.m.Carnica	E	1.95	1.96	1.96	1.96
	N.E	1.93	1.92	1.98	1.94
	C	1.95	1.97	2.00	1.97
A.m.ligustica	E	1.91	1.90	1.90	1.90
	N.E	1.90	1.89	1.89	1.89
	C	1.89	1.90	1.86	1.88

LSD for interaction = 0.086 ***LSD for Mean = 0.05***

3-2-Effect of BC, RC and bars on queen cell length.

Data in Table (14) indicate that the average length of queen cell for *A. m.carnica* (as rearing colonies) was insignificantly longer (1.96 cm) for the 1st bar than that of the 2nd bar (1.95 cm). For *ligustica,* the average length of queen cell was insignificantly shorter (1.89 cm) for the 1st bar than the 2nd bar (1.90 cm).

Table (14): The effect of breeding, rearing colonies and bars on queen cell length of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica colonies* (cm):

RC		Breeding colonies			
	Bar	*A.m. carnica*	*A.m. ligustica*	*A.m.bukfast*	Mean
A.m. carnica	1st	1.94a	1.95[a]	1.97a	1.96[a]
	2nd	1.94a	1.94a	1.98a	1.95[a]
A.m. ligustica	1st	1.87b	1.90b	1.88b	1.89[b]
	2nd	1.92b	1.89b	1.877b	1.90[b]

LSD for interaction = 0.07 ***LSD Mean = 0.04***

3-3- Effect of BC, RC and batches, on queen cell length.

Data in Table (15) indicate that the average length of queen cells for *A. m. carnica* (as rearing colonies) were similar (1.96 cm) for the two batches. For *ligustica* the average length of queen cell was significantly longer (1.96 cm) for batch 1 than for batch 2 (1.83 cm). For BC (*carnica, ligustica* and *bukfast*) reared in *carnica* colonies, the average lengths of queen cells did not differ significantly.

For the same previous breeding colonies reared in *ligustica,* the average lengths of queen cells were significantly longer (1.96,195 and 1.96 cm) for batch 1 than batch 2 (1.83, 185 and 1.81 cm).

Table (15): The effect of breeding, rearing colonies and batches on queen cell length of larvae from *carnica*, *ligustica,* and *bukfast*, reared in *carnica* or *ligustica colonies*

R C		Breeding colonies			
	Batches	*A.m.Carnica*	*A.m.ligustica*	*A.m.bukfast*	Mean
A.m.Carnica	Batch1	1.96a	1.93a	1.98a	1.96a
	Batch2	1.94a	1.97a	1.97a	1.96a
A.m.ligustica	Batch1	1.96a	1.95a	1.96a	1.96a
	Batch2	1.83b	1.85b	1.81b	1.83b

LSD for interaction = 0.069 ***LSD for Mean = 0.04***

3- 4 -Effect of BC, RC and months on queen cell length.

Data in Table (16) indicate that, the average length of queen cells from breeding colonies (*A.m. carnica, A.m.ligustica* and *A.m. bukfast*) reared in *A. m.carnica* differed significantly during the different months (2.19, 1.88, 1.81 and 1.95 cm) for April, May, July and August, respectively.

For *ligustica* colonies, when used to rear the larvae of the same breeding colonies, the average length of queen cells differed significantly during April and May (2.07 and 1.77), while it was the same during July and August (1.87 cm).

The average length of queen cells reached its maximum (2.25 cm) during April, for *bukfast* reared in *carnica* colonies. On the other hand, it

reached its minimum (1.76 cm) during May for *ligustica* reared in *ligustica* colonies.

Table (16): The effect of breeding, rearing colonies and months on queen cell length (cm) of larvae from *carnica*, *ligustica*, and *bukfast*, reared in *carnica* or *ligustica colonies*.

R C		Breeding colonies (BC)			
	Month	*A.m. carnica*	*A.m. ligustica*	*A.m.bukfast*	Mean
A.m. carnica	April	2.16a	2.16a	2.25a	2.19a
	May	1.88	1.88	1.84	1.88d
	July	1.78	1.81	1.82	1.81e
	August	1.96	1.95	1.95	1.95c
A.m. ligustica	April	2.07	2.11	2.04	2.07b
	May	1.78	1.76	1.77	1.77e
	July	1.87	1.85	1.88	1.87d
	August	1.88	1.88	1.83	1.87d

LSD for interaction = 0.086 *LSD for Mean = 0.049*

DISCUSSION

In this study the weight was considered as a qualitative criterion of the honeybee queens (**Taranov, 1973; Schaper, 1985; Page and Erickson, 1986; Mazeed, 1992; and Zeedan, 2002**),while the acceptance rate of grafted larvae was considered as a quantitative criterion of the honeybee queens. The different weights of the resulting queens may be attributed to the different numbers of the introduced queen cells (**EcKert and Shaw, 1960; Zhu, 1981; Rawash *et al.*, 1983; Abd Al – Fattah *et al.*, 2007 and Abd Al-Fattah, *et al.*2011**). We used a fixed number of queen cells, to neutralize this parameter.

In this study, significant differences were found between genotypes in terms of acceptance rate of grafted larvae. The acceptance rate of grafted larvae was found to be significantly higher for *A. m. ligustica* (either as breeding or rearing colonies) than *carnica* or *bukfast*. The obtained results are in general agreement with that of **Şahinler and Kaftanoğlu (2005) ; Sharaf El-din (2010) and Ahmad *et al.* (2013)**.The acceptance rate of grafted larvae, reached its maximum when the larvae were grafted in reared colonies of the same genetic origin (larvae from *carnica,* and reared in *carnica,* or larvae from *ligustica* and reared in *ligustica* colonies). The present findings were supported by the works of **Mohammedi and Le Conte (2000)**; **Tarpy *et al.*, (2004)**; and **Hammad, (2012)** who found that the genotype of grafted larvae and nurse bees influenced larval acceptance and concluded that worker bees have the ability to discriminate between related and unrelated larvae. On the contrary, **Breed *et al.*, (1984); Visscher (1986a); Tarpy and Fletcher (1998); Albarracín *et al.*, (2006) and Masry (2010)** revealed that nurse bees do not functionally discriminate between related and unrelated larvae during queen rearing.

The obtained results indicated that certain conditions may also affect the chosen larvae, this is seen when workers choose larvae located in a specific position on the rearing frame. The acceptance rate of grafted larvae was affected by the level and position at which the queen cells were held or constructed within the rearing frame (the Center position significantly exceeded the Edge or Near edge positions, and the Middle bar significantly exceeded the Upper one). These findings were supported by the works of **Abd Al-Fattah, *et al* (2007) and Abd Al-Fattah, *et al.* (2011).** On the contrary, **Sharaf El-Din *et al.* (2000)** reported that the lower level of bars induced the highest acceptance rate. **Albarracín *et al.* (2006)** stated that bar positions had no significant effect on the acceptance rate of larvae.

The acceptance rate of grafted larvae was affected also by the batches as well as the rearing months or seasons of the year, as our results indicated. The acceptance rate of grafted larvae for the 1st batch exceeded the 2nd batch and, the acceptance rate for the 1st batch reached its peak when the larvae were grafted in reared colonies of the same genetic origin, also when larvae of *bukfast* were reared in *ligustica* colonies. The acceptance rate of grafted larvae during July and August (summer) significantly exceeded April or May (spring). The obtained results regarding the months or seasons of the year are in general agreement with that of **Shawer *et al.* (1980), Genc *et al.* (2005)** and **Guler and Alpay (2005).**

A significant difference was observed between honeybee genotypes in terms of the queen weight. *Carnica* had heavier queen weight than *ligustica* (as rearing colonies). **Abou El-Enain (2000), Masry (2010) and Abd-El-Megeed (2011)** found that the Italian race is superior to the Carniolan race. Certain conditions may also affect the weight of the resulting queens, this can be explained by the heavy queens obtained from a defined location on the rearing frame, as our results indicated (Heavier queens were obtained when the queens emerged from queen cells were located at the middle bar level in the rearing frame). These results agree with those of **Visscher, (1986)** and **De Grandi–Hoffman *et al.*, (1993)** who reported that the frequency of heavy queen weights increased when the queen cells were located at the middle location of each bar. **Abd Al Fattah *et al.* (2011)** reported that queens emerged from cells on the middle rearing bars and the middle positions of each bar had a high frequency of heavy weight in comparison with those reared on the upper or lower bars and located at the peripheral of the bars. Our results indicated that, the queens were significantly heavier during April than May, July and August, while the effect of the position of the grafted larvae of each bar as well as the batches had no significant effect on queen weights. The obtained results regarding the months of the year are in general agree with that of **Koç and Karacaoğlu (2004),**Concerning the average length of queen cells,

insignificant differences were found between genotypes where *A.m. carnica* built queen cells larger than *ligustica* (as rearing colonies), while *bukfast* built larger queen cells (as breeding colonies). **Masry (2010)** mentioned that the largest queen cells were obtained from *carnica* race as rearing or breeding colonies. For rearing months, our results indicated that, the average length of queen cells were significantly longer for April than May, July and August, while the level and position of the grafted larvae as well as the batches had no significant effect on queen cell length. The obtained results regarding the months agree with that of **Shawer *et al.* (1980),** and **Genc *et al.* (2005),**

CONCLUSION

As the results indicate, and under the conditions of our queen rearing colonies, the genetic origin of breeding and/or rearing colonies, in addition to the positions of the queen cells on the rearing bar, the location of the rearing bar in queen rearing frame, the batch number, and months of the year affect greatly the acceptance rate of the
grafted larvae, affect moderately the queen weight, and had little effect on queen cell length.

The acceptance rate of the grafted larvae was higher for *ligustica* as breeding or rearing colonies, significantly higher for related than unrelated larvae, for Center than those of Edge or Near edge positions, for the Middle bar than the upper bar, for batch 1 than batch 2, for July or August than April or May. The average weight of queens was heavier for the Middle bar than the Upper bar, significantly heavier during April than May, July and August. The average length of queen cell was significantly longer for April than May, July and August.

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تاثير الاصل الوراثي لطوائف التربية وطوائف اليرقات علي نسبة القبول واوزان الملكات واطوال البيوت الملكية في نحل العسل

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أجري هذ البحث بمحافظة الفيوم وذلك لدراسة تأثيرالأصل الوراثي لطوائف التربية (الكرنيولي والايطالي) وطوائف الأصل لليرقات (الكرنيولى والإيطالى والبكفاست) علي نسبة قبول اليرقات المطعومة واوزان الملكات واطوال البيوت الملكية.

أشارت النتائج إلي أن نسبة قبول اليرقات كانت أعلى بشكل غير معنوي للهجين الإيطالى (سواء كأصل وراثى أو كطوائف تربية) عن الكرنيولي أو البيكفاست وكانت أعلى معنويا لليرقات ذات صلة القرابة عن تلك عديمة صلة القرابة. وللموضع الوسطى عن موضعي الحافة أوقرب إلحافة وللسدابة الثانية عن السدابة الأولى ولدورة التربية الأولى عن الثانية ولشهري يوليو وأغسطس عن أبريل ومايو. كان متوسط أوزان الملكات أعلي بشكل غير معنوي للهجين الكرنيولي عن الايطالي (كطوائف تربية) وللسدابة الثانية عن السدابة الأولى وأعلي معنويا خلال أبريل عن أشهر مايو ويوليو وأغسطس. كانت البيوت الملكية أطول بشكل غير معنوي للهجين الكرنيولي عن الايطالي (كطوائف تربية) وأطول معنويا خلال أبريل عن أشهر مايو ويوليو وأغسطس.