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IMPACT OF HONEY BEE COLONIES OF DIFFERENT RACES ON REARING *APIS MELLIFERA LAMARCKII* QUEEN LARVAE

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ABSTRACT

Egyptian honey bees *Apis mellifera lamarckii* is the native honey bee race in Egypt. Strong colony of *A. m. lamarckii* headed with young open-mated queen that procedure sufficient eggs was used in this study as breeding colony. The rearing colonies of Egyptian honey bees(*A. m. lamarckii*), Carniolian honey bees(*Apis mellifera carnica*) and Italian honey bees (*Apis mellifera ligustica*) were used in this study for rearing Egyptian honey bees grafted larvae. Procedure of Doolittle method for queen rearing was used for grafting 24 h old worker larvae. Acceptance percentage of grafted queen larvae, queen cell size, virgin queen weight, mean number of ovariols and spermatheca volume were determined. Results revealed that reared colonies of *A. m. lgustica* significantly recorded lowest percentage of accepted grafted larvae, smallest size of queen cell and highest queen weight than the other tested honey bee races. The highest quality of reared queens resulted in *A. m. carnica* and *A. m. lamarckii* colonies, that had longest queen cell size, heaviest virgin queen,highest mean number of ovarioles and longest volume of spermatheca.

Key words: *Apis mellifera lamarckii*, grafted larvae, spermatheca and ovarioles.

INTRODUCTION

Beekeeping has a very old history in Egypt; the old drawings that are found in the Nile river civilization, suggest that bees were kept for the large-scale production of precious wax and honey (Crane, 1983 and Hussein 2000). Egyptian honeybees *Apis mellifera lamarckii*is the native race of Egypt (Ruttner, 1988). It is considered defensive, build up early and exhibiting good hygienic behaviour but low in nectar collect. Also, it is more resistance against a major bee pest, the brood mite *Varro destructor* (Kamel, *et al*. 2003). A relatively large enclave of *A. m. lamarckii* in traditional mud-tube hives (estimated 400,000 colonies) can still be found in Assiut governorate and minor populations remain in isolated oases within Egypt (Garnery *et al*., 2001).

*A. m. lamarckii*s hows a significantly higher mass specific metabolism than the Carniolian bee *A. m. carnica*. It is more active, more aggressive and well adapted to its tropical environment. It neither forms winter clusters nor stores food for overwintering and breed nearly throughout the year. It is regarded as a typical representative of tropical African bees (Ruttner, 1988 and Schmolz *et al*., 2001).

Around the beginning of the twentieth century, beekeepers replace the aggressive native Egyptian race with the European honeybee *A. m. carnica* because of its higher foraging efficiency and its easier way of handling. Meanwhile, it expels *A. m. lamarckii* from its ancestral foraging sites to areas with scarce forage (Simonthomas and Simonthomas, 1980; Mazeed 1988 and Schmolz *et al*., 2001).

Due to transport of honeybee queens of *A. m. carnica*, and *A. m. ligustica*there are considerable risks for the conservation of wild honeybee populations in their native range (Moritz *et al*., 2005). Since the mating range of queens and drones can exceed 80 km² (Ruttner and Ruttner, 1972), genes from wild populations and imported strains are constantly mixing. On the other hand, populations of wild bees could be wiped out by gene-flow or diseases from imported stocks. However, the native honeybee populations of

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Egyptian bees are often well adapted to the local conditions including resistance to specific pests and diseases.

Because of mating and mixing between imported and native races, queen breeders in Egypt do not consider the genetic origin of donor as well as rearing colonies for queen production. Therefore, commercial beekeepers have lower acceptance percentage of grafted larvae and poor quality of queens (Masry, 2010).

The racial origin of grafted larvae affect acceptance for queen rearing (Albarracín *et al*., 2006**)**. Pickard and Kither (1983) and Mohammedi and Le Conte (2000) observed significant differences in rearing colonies (*Apis mellifera mellifera* and *A. m. ligustica*) with respect to the rate of acceptance of larvae for queen rearing depending on whether the larvae belong to the own or a different honeybee race. Furthermore, interaction between genotype of grafted larvae and rearing colonies is affected also the quality of the produced queen (Breed *et al*., 1984; Casagrande-Jaloretto *et al*., 1984; Skowronek *et al*., 2004; Guler and Alpay, 2005; Al-Ghzawi and Zaitoun, 2008, and Masry, 2010).Thus, in the present study we investigate the effect of honeybee racial origin of rearing colonies on acceptance percentage and quality of produced queens' of *A. m. lamarckii*.

MATERIALS AND METHODS

The study was carried out in the apiary yard of the experimental station of the Faculty of Agriculture, Cairo University at Giza region, Egypt, from the 1st of April to the end of September 2011.

1.1. Honey bee races:

Three honeybee genotypes were used. Two colonies of *A. m. lamarckii* were used to produce sufficient eggs for grafting as breeding colonies, while three strong honey bee colonies of (*A. m. lamarckii*, local *A. m. carnica* and local *A. m. ligustica*) were used as queenless colonies. All experimental colonies were headed with young open-mated queen.

1.2. Preparation of queen rearing colonies:

Seventy two hours before being grafted larvae, one colony of each reared race was requeened. The queen were confined and removed with uncapped brood comb to another place in the apiary. Reared colonies respected daily to remove queen cells that naturally built and fed with sugar syrup (Ruttner, 1988). Doolittle method was the procedure used for grafting 24 h old larvae (Laidlaw and Page, 1997). The grafted larvae were collected from three places on the grafted comb as follow:

1- Middle (Me)

2- Side (Si)

3- Lower edge (Be) to adjust the position effect (Masry *et al*., 2010). The grafted cells were placed in the queenless colonies (reared colonies) for ten days (Woyke, 1971). Two days before the onset of emergence the sealed queen cells were caged using semi-ball cages till emergence.

1.3. Percentage of acceptance grafted larvae and queen characteristics:

Acceptance percentage of grafted larvae was calculated after 24 h after grafting. Newly emerged virgin queens were collected and the following characteristics were measured:

Size of queen cell (cm), queen weight (mg), volume of spermatheca (mμ**)** and number of ovarioles (Eckert, 1934; Hatch *et al*., 1999; Skowronek *et al*., 2004 and Masry *et al*. 2010).

1.4. Statistical Analysis:

The effect of queen cell size and queen weight, volume of spermatheca and number of ovarioles and also acceptance percentage were analysed using Statistix 9 (Statistix analysis Software) (Thomas and Maurice, 2008); which were performed with General Linear Model's procedure with different positions as the fixed effect; interactions between queen races and size of cell and queen weight were also studied. This procedure computes the analysis of variance for acceptance. The F test assumes that the within-group variances are the same for all groups. The null hypothesis of these tests is that different queen races are equal. A large F test and

corresponding small p-value (say, smaller than 0.05) is evidence that there are differences, by using L.S.D. test to compare means of treatments.

RESULTS

Percentage of acceptance (%) of *A. m. lamarckii* grafted larvae:

The differences among genotype in acceptance percentage were significant ($p < 0.05$). *A. m. carnica* and *A. m. lamarckii* significantly had the highest accepted percentage of *A. m. lamarckii* grafted larvae than *A. m. ligustica*, where the acceptance % were (93.75± 1.3, 87.2± 1.9 and 62.5± 4.5) in *A. m. carnica*, *A. m. lamarckii* and *A. m. ligustica* respectively. However, larvae collected from the lower edge (Be) and side (Si) had significant the highest acceptance percentage than middle (Me) of the comb (Fig.1).

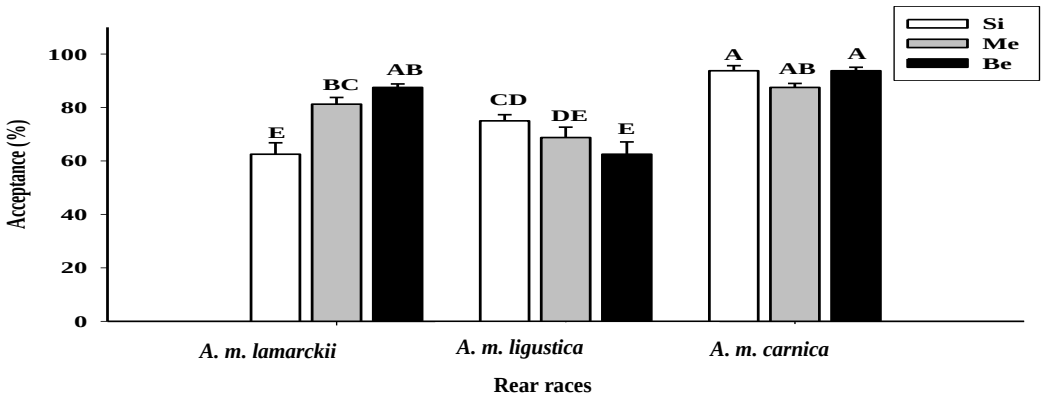

Fig.(1): Acceptance percentage (%) of *A. m. lamarckii* grafted larvae collected from three position of brood comb side (Si), middle (Me) and lower edge reared in *lamarckii*, *ligustica* and *carnica* colonies.
A,B,C,D and E: Figures differ at 5% level.

Table 1. Mean queen cell size (cm) of *A. m. lamarckii* reared in different honey bees genotypes(mean ± S.E).

Genotypes	Size of queen cells (cm)	Position of grafted larvae on brood comb		
		Si	Me	Be
A. m. lamarckii	1.5±0.15ab	1.1±0.15b	1.4±0.18ab	1.5±0.15ab
A. m. ligustica	1.1±0.11 b	1.3±0.13ab	1.2±0.16ab	1.1±0.11 b
A. m. carnica	1.6±0.17 a	1.5±0.15 a	1.5±0.16ab	1.6±0.17 a

Means designated with the same letter are not significantly different at 0.05 level of probability (F= 5.8, P ≤ 0.004).Si= the side of brood comb, Me= middle of brood comb and Be= lower edge of brood comb

Table 2. Mean queen weight (mg) of *A. m. lamarckii* reared in different honey bees genotypes (mean ± S.E).

Genotypes	Queen weight (mg)	Position of grafted larvae on brood comb		
		Si	Me	Be
A. m. lamarckii	144.4±6.3ab	105±4.5c	139.4±4.9abc	144.4±6.3abc
A. m. ligustica	115±4.3bc	118.1±4.3bc	116.3±4.25bc	115±4.4bc
A. m. carnica	148.1±6.6 a	156.9±6.6ab	166.3±7.7 a	148.1±6.6abc

Means designated with the same letter are not significantly different at 0.05 level of probability(F=10.1, P ≤ 0.0002).Si= the side of brood comb, Me= middle of brood comb and Be= lower edge of brood comb

Table 3. Mean number ovarioles of *A. m. lamarckii* queen reared in different honey bees genotypes (mean ± S.E).

Genotypes	Number of ovarioles	Position of grafted larvae on brood comb		
		Si	Me	Be
A. m. lamarckii	225.3±17ab	225.3±17ab	204.3±15 b	225.3±17ab
A. m. ligustica	219±16 b	240±19ab	244.7±19ab	219±16 b
A. m. carnica	264±21 a	235.7±18ab	242.7±19ab	264±21 a

Means designated with the same letter are not significantly different at 0.05 level of probability(F= 3.5, P ≤ 0.07). Si= the side of brood comb, Me= middle of brood comb and Be= lower edge of brood comb

Table 4. Mean volume of spermatheca (mμ)of *A. m. lamarckii* queen reared in different honey bee genotypes(mean ± S.E).

Genotypes	Volume of spermatheca (mμ)	Position of grafted larvae on brood comb		
		Si	Me	Be
A. m. lamarckii	1220±102ab	1183.3±98bc	1200±100bc	1220±102ab
A. m. ligustica	1186.7±108 a	1100±90c	1133.3±93 c	1186.7±108bc
A. m. carnica	1250±105 b	1266.7±106 a	1216.7±101bc	1250±105ab

Means designated with the same letter are not significantly different at 0.05level of probability(F= 5.2, P ≤ 0.03). Si= the side of brood comb, Me= middle of brood comb and Be= lower edge of brood comb

Weight of produced queen (mg):

Apis mellifera carnica significantly produced the heaviest weightof queens (148.1±6.6 mg) than *A. m. lamarckii* (144.4±6.3 mg) and *A. m. ligustica* (115±4.3 mg). However, the queens produced from larvae collected from the middle of brood comb and reared in *A. m. carnica* colonies had the heaviest weight of queen (166.3±7.7mg) than the other place of collected larvae (Table 2).

Mean number of ovariolesfor produced queen: *A. m. carnica* significantly produced higher number of ovarioles(264±21) than *A. m. lamarckii* (225.3±17) and *A. m. ligustica* (219±16). However, the queens produced from larvae collected from the blow edge of brood comb and reared in *A. m. carnica* had the highest number of ovarioles (264±21) than those collected from other places (Table 3).

Spermatheca volume(mμ)of produced queen: *Apis mellifera carnica* and *A. m. lamarckii* significantly had the largest volume of spermatheca for produced queen (1250±105 and 1220±102mμ) respectively. While, *A. m. ligustica* produced the smallest volume of spermatheca (1186.7±108mμ). Also, the queens produced from larvae which collected from the side of brood comb and reared in *A. m. carnica* colonies had largest spermatheca volume (1266.7±106mμ) than other places of collected larvae (Table 4).

DISCUSSION

In this study the significant differences were found among genotypes in terms of acceptance percentage of grafted larvae and queen quality. The highest acceptance rate of grafted larvae occurred with *A. m. carnica*as reared race for nursing *A. m. lamarckii* queen larvae. The obtained results are in agrees with the Król (1974), Mohammedi and Le conte (2000) and Hammad (2012) they 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 not related larvae. Furthermore, Masry *et al*.,

2010 mentioned that *A. m. lamarckii* was found to record the highest acceptance percentage of grafted larvae as reared colonies followed by *A. m. carnica* and *A. m. ligustica*. They indicate that there were highly significant difference between honeybee genotypes of grafted larvae and reared colonies and this difference influence larval acceptance. On the other hand, Guler and Alpay (2005) and Albarracin *et al*., (2006) indicate that honeybee genotypes did not affect the acceptance percentage of grafted larvae.

A. m. carnica build the largest queen cell than *A. m. lamarckii* and *A. m. ligustica* (Table 1). The present results indicate that Carniolan honeybee race secretes large amount of wax. Król, 1985 found that Carniolan race produced largest and heaviest queen cell than Caucasian and Italian races. The same trend reported by Masry, 2010 and Masry, *et al*., 2010, they found that *A. m. carnica* produced the longest queen cell than Italian race and Egyptian race. Queen cell length affected by larval age, supplemental feeding, colony strength, environment conditions and rearing methods (Robinson, 1989; Gençer *et al*., 2000; Dodologlu *et al*., 2004 and Cengiz *et al*., 2009).

A high variation was observed among honeybee genotypes in terms of the queen quality. Carniolan bees had the heaviest queen weight than Egyptian and Italian race (table 2). Tarpy *et al*., (2000) declared that queens, who have significantly heavier weight at emergence, were of higher reproductive quality. Furthermore, Skowronek *et al.* (2004) reported that body weight of queens is significantly affected by inherited characters as well as by rearing conditions such as nursing colony. Masry, 2010 and Masry *et al*., 2010 demonstrated that honeybee race of reared and breeding colonies affected the weight of obtained queens and conclude that high weight queens may be carefully selected from the acceptance larvae to be a high quality queens for the commercial beekeeping.

The reproductive quality of honeybee queens depends on her genotype and the environment conditions. Number of ovarioles and volume of spermatheca consider as the important potential affected the honeybee queen reproductive (Woyke, 1971; Tarpy *et al*., 2000 and Masry, 2010). The native honeybee race of Egypt *A. m. lamarckii* - indeed; it is adapted to the environment since from thousands of years (B.C.). Also, this race may has a percentage of genetic crossing from *A. m. carnica*. Therefore, may produce more food than the others race and resulting high quality queens (Masry *et al*. 2010). Honey bee queens which have significantly heavier weight at emergence, larger spermatheca and highly number of ovarioles suggesting that they were of higher reproductive quality (Tarpy *et al*., 2000). Furthermore, Guler and Alpay (2005) mentioned that there are no differences among genotypes in respect of the number of spermatozoa in the spermatheca but the volume of spermathica is correlated with the number of spermatozoa (Woyke, 1971; Emsen, 2004 and Dodologlu *et al*., 2004). Furthermore, the perfect queens that has heavy weight, high number of ovarioles and big volume of spermatheca - indeed- has high quality glands and her abdomen has much larger surface. Such queens have the maximum chance to be accepted during introduction (Masry, 2010).

Finally, most queen breeders in Egypt do not consider the genetic origin of donor as well as rearing colonies for queen production. This may provide an explanation for the problems of acceptance larvae for queen rearing and poor quality of queens reported by commercial beekeeper (Masry, 2010). Therefore we recommended reared Egyptian honeybee queens in local Carniolan colonies to maintenance our race of extinction and produced high quality queens.

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الملخص العربى

تربية يرقات ملكات نحل *APIS MELLIFERA LAMARCKII* (السلالة المحلية) فى طوائف نحل العسل من سلالات مختلفة

سلالة النحل المصرى *Apis mellifera lamarckii* هى السلالة المحلية. طوائف قوية من سلالة النحل المصرى *A. m. lamarckii* ذات ملكات صغيرة السن حديثة التلقيح الطبيعى منتجة للبيض بكثافة عالية أُستخدمت فى هذه الدراسة للحصول على اليرقات اللازمة للتطعيم كمصدر لليرقات. طوائف نحل المصرى *A. m. lamarckii* وطوائف نحل كرينولى *A. m. carnica* وآخرى من النحل الإيطالى *A. m. ligustica* أُستخدمت كطوائف لتربية يرقات سلالة النحل المصرى التى تم تطعيمها بالكؤوس الملكية فى عمر 24 ساعة بإستخدام طريقة دولتيل لتربية ملكات النحل على نطاق تجارى.

نسبة قبول اليرقات الملكية، حجم البيت الملكى، وزن الملكة العذراء، عدد فروع المبيض وحجم القابلة المنوية تم تقديرها فى هذه الدراسة. سجلت طوائف التربية من سلالة النحل الإيطالى أقل نسبة قبول لليرقات المطعومة وأصغر حجم بيت ملكى وأخف الملكات وزناً عنه فى حالة سلالات النحل الأخرى.

أعلى الملكات جودة سُجلت عند إستخدام طوائف من سلالة النحل الكرينولى *A. m. carnica* والنحل المصرى *A. m. lamarckii* على الترتيب، حيث أنتجت أطول بيت ملكى وأثقل الملكات وزناً وأكثرهم فى عدد فروع المبيض وحجم القابلة المنوية.