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GENETIC ASPECT OF MULTIPLE QUEEN STATUS IN HONEY BEE *Apis mellifera*

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ABSTRACT

Parents of the three races; *Apis mellifera carnica* (car), *Apis mellifera ligustica* (lig) and *Apis mellifera lamarckii* (lam), as well as their offspring's which were yielded from diallel cross (inbreed pure, cross and reciprocal cross) by using instrumental insemination (II) were used for determination of the number of constructed and reared queen cells in emergency and swarming cases during 2005 and 2006 years at the apiary of Faculty of Agriculture, Ain Shams University at Shoubra El-Kheima.

The pure Egyptian bees (lam) or their hybrids either depend on dams (queens) or sires (drones) reared the highest number queen cells (in emergency and swarming cases) as compared with the other two races, followed by the Italian bees (lig) and Carnica bees (car). The later recorded the lowest number of queen cells.

The queen cells which were constructed and reared by the lam bee race either for emergency or for swarming showed high tendency of combining ability either generally or specifically with the other two races. Also, they had higher maternal inheritance when they used as a dam. Moreover their hybrid vigour showed a decreased in the number of queen cells comparing to the parent or pure line (inbred) when hybrid either as a dam or as a sire, but it was higher than the other hybridization. Although, heritability showed moderately estimates (h^2_d , h^2_s & h^2_P = 0.39, 0.19 & 0.29 for emergency and 0.47, 0.27 & 0.37 for swarming), it was higher than that in lig bee race(h^2_d , h^2_s & h^2_P = 0.23, 0.17 & 0.21 for emergency and 0.30, 0.18 & 0.25 for swarming), which came in the second order, and finally the car bee race(h^2_d , h^2_s & h^2_P = 0.15, 0.10 & 0.14 for emergency and 0.24, 0.16 & 0.20 for swarming). On the other hand, the estimation of the same genetic parameters for the rate of brood cells and colony strength were nearly vice versa where the lig bee race come first, followed by the car bee race and the lam bee race.

Keyword: queen honey bee - *Apis mellifera* - diallel cross - heterosis - instrumental insemination

INTRODUCTION

The honey bee queen, *Apis mellifera* is considered the mother and ruler of the colony, which naturally reared in special cell that extends downward from the face or edge of the comb. Queens are reared in nature under three case conditions; emergency, swarming and supersedure. Different authors were studied the following subjects: Queen cell construction (Punnett and Winston 1983 and Cargel and Rinderer 2004), reciprocal interactions between honeybee and combs (Hepburn 1998), worker regulation during queen rearing (Hatch *et al* 1999), influence of worker behaviour on the development and emergence of honeybee queens (Schneider and Hoffman 2002), effect of temperature on the development of the cupped queen cells. (Feng *et al* 2002), tendency to swarming related to physiographic and climatic factors of the areas and bee races (Nuru *et al* 2002 and Chaline *et al* 2005), effect of pesticides on queen rearing (Haarmann *et al* 2002; Collins *et al* 2004 and Pettis *et al* 2004), queen quality according to larval age (Gilley *et al*, 2003 and Tofilski and Czekonska, 2004), rearing season (Koc and Karacaoglu 2004) and the role of chemical and acoustical stimuli in selective queen cell destruction by virgin queens (Harano and Obara 2004).

In spite of multiple matings of queen bees, the social behaviour of bee colony make difficult to determine the genetic parameters of the honeybee population. The combining ability (general combining ability, g.c.a. & specific combining ability, s.c.a.) and heterosis using diallel cross was estimated under different topics by authors for: Field honey weight gains of six inbred races and three mixed races using 9x9 partial diallel cross (Oldroyed *et al* 1985). Defensive behaviour using 6x6 diallel test cross (Moritz *et al* 1987). Selections for honey production using partial diallel cross (Oldroyed *et al* 1987). Hamuli numbers using 9x9 partial diallel cross (Oldroyed and Morgan 1987). Distinguishing between races using diallelic locus (Biasiolo 1991). Grooming behaviour depending on number of damaged Varroa mites either in cages of field tests using three hybridizing lines of *Apis mellifera* cornice in a complete reciprocal diallel (Hoffmanu 1993). Total colony gains of honey, royal jelly, pollen and wax using incomplete 2x4 diallel design (YanHe and ShengLu 2001).

The number of queen cells reared in emergency and swarming cases depending mainly on the race and strain moreover the size of the colony where it's genetically character originally at first. Therefore, the present study aimed to find out the role of the honey bee races for constructing and rearing the honey bee queens.

MATERIAL, METHODS AND TECHNIQUES

The present study was carried out during 2005/2006 years at the apiary of the Faculty of Agriculture, Ain Shams University, Shoubra El- Kheima. Three pure honey bee races; Carniolan *Apis mellifera carnica*, Italian *A. m. ligustica* and Egyptian *A. m. lamarckii* were used as parents. Drones (sires) and queens (dams) from each race were used for producing the first generation using diallel cross.

An artificial mating was done using instrumental insemination (II) with 8 ml of semen (recommended dose) for each queen to produce the F1 generation as follows: Fifteen queens from each race were divided into three groups (5 queens/ group). The first group of queens was inseminated by semen from their brothers, i.e. brother- sister mating (inbreed line) .The other two groups were inseminated from drones of the other two races. So the offspring of the first generation for each race include inbreed line, cross and reciprocal cross with other races.

Queens which II (during June 2005) were introduced into nucleus colonies containing three combs covered with adult bees (one full of honey and pollen and the other 2 combs were empty). After starting egg laying the nuclei were left undisturbed (except offering food) for 12 weeks to replace the worker offspring of the inseminated queens with those of the original nuclei colony.

Tendency of workers to rear emergency queen cells

Queens of all experimental colonies were picked up carefully with its serial order (during September 2005) and kept in queens' bank for three days, during which workers of each colony started to rear queen cells (emergency).

The number of constructed queen cells were counted and recorded, and then the queens were reintroduced to its colonies with its serial order.

Tendency of workers to rear swarming queen cells

After reintroducing the queens, the colonies were kept carefully by offering its food requirements and good wintering till the next spring (during March 2006). At that time the colonies were monitored until began to arrange itself to natural swarms. The following parameters were recorded; colony strength (number of combs covered from both sides with adult bees), number of brood cells and the number of appeared queen cells.

The genetic parameters were calculated using SAS (2001) by adopting the following model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk} \quad (1)$$

where:

Y_{ijk} = yield for generations(i); generations(j) and replicates(k).
μ = generation mean
g_i = dam generation effect
g_j = sire generation effect
s_{ij} = interaction
e_{ijk} = error

The general combining ability; specific combining ability; maternal effect; heterosis and heritability for dam, sire & mid parent were estimated according to Falconer (1989) as follows:

$$\text{g.c.a.} = \mu\ (\Sigma\ g_i + \Sigma\ g_j) \quad (2)$$

$$\text{s.c.a.} = cross_{ij} + reciprocal\ \ cross_{ji} \big/ 2\ -\ g.c.a._{i} + g.c.a._{j} \big/ 2 \quad (3)$$

$$\text{mt.ef.} = \text{cross} - \text{reciprocal cross} \quad (4)$$

$$H\ \% = \frac{F_1 - \overline{P}}{\overline{P}}\ x100 \quad (5)$$

$$h^2_d = \frac{4\delta^2_d}{\delta_t} \quad (6)$$

$$h^2_s = \frac{4\delta^2_s}{\delta_t} \quad (7)$$

$$h^2_{s\&d} = \frac{2(\delta^2_d + \delta^2_s)}{\delta_t} \quad (8)$$

where:

g.c.a. = general combining ability;
s.c.a. = specific combining ability;
mt.ef. = maternal effect;
H % = heterosis
h^2_d = heritability of dam component.
h^2_s = heritability of sire component
$h^2_{s\&d}$ = heritability of mid parent

RESULTS AND DISCUSSION

Rearing queen cells in emergency and swarming cases depending mainly on the race and strain as well as the size of the colony (colony strength) tested where it's genetically character originally at first. Therefore, parents of the three races; *A. m. carnica* (car), *A. m. ligustica* (lig) and *A. m. lamarckii* (lam) and their offspring's which were yielded from diallel cross [inbreed (pure), cross and reciprocal cross] were used to determine the number of constructed and reared queen cells in these two cases.

In emergency case, where the colonies had nearly same strength (each with 3 combs covered with adult bees), the numbers of appeared queen cells 3 days after dequeening the experimental colonies are recorded in (Table 1). The results shows a significant differences between the number of queen cells among lines (F = 12.07 & LSD = 8.137). The pure Egyptian bees (lam) or their hybrids either depend on dams (queens) or sires (drones) reared the highest number of queen cells followed by the Italian bees (lig) and the Carnica bees (car).

Table (1): Number of Queen cells of the three experimental races in emergency case for parents and their offspring

Genetic groups	Mean ± S.D.	C.V.
Parents		
car	31	
lig	48	
lam	60	
Pure lines		
car x car	28.4 ^{f} ± 5.5	19.37
lig x lig	45.2 ^{cd} ± 5.1	11.28
lam x lam	66.8 ^{a} ± 6.7	10.03
Cross		
<u>lig</u> x car	40.0 ^{de} ± 5.6	14.00
<u>lam</u> x car	53.2 ^{bc} ± 4.9	9.21
<u>lam</u> x lig	56.4 ^{b} ± 6.1	10.81
Reciprocal Cross		
<u>car</u> x lig	35.0 ^{ef} ± 4.7	13.43
<u>car</u> x lam	49.8 ^{bc} ± 6.2	12.45
<u>lig</u> x lam	52.2 ^{bc} ± 7.1	13.60
F value	12.07	
LSD	8.137	

N.B. Under line = dame (♀) **car = *carnica* ; lig = *ligustica* ; lam = *lamarckii***
Prob. < 0.0001

In swarming period (during March, 2006) where the colonies have different status, the colony strength (number of combs covered with adult bees), the number of worker brood cells and the number of reared queen cells for each experimental colony are tabulated in Table (2). The data show that significant differences within all parameters among lines were obtained (F values & LSD's are 43.28 & 23.712, 40.92 & 412.777 and 7.49 & 1.068 for queen cells, number of brood cells and colony strength, respectively). The highest number of reared queen cells were found in lam bees (pure, depends on dams then sires), followed by lig bees (depends on dams, sires then pure), and finally the pure line of car bees which produced the lowest number of queen cells in spite of the adverse values for the rate of brood cells and colony strength for this arrangement.

Table (2): Colony status of the experimental honey bee races for parents and their offspring during swarmes

Genetic groups	Queen cells		Brood		Colony strength	
	Mean ±S.D	C.V	Mean ±S.D	C.V	Mean ±S.D	C.V
Parents						
car	49		4178		5	
lig	74		5387		6	
lam	189		2570		3	
Pure lines						
car x car	45.4 ^{j} ± 7.3	16.08	4062 ^{c}±182	4.48	5.2 ^{bcd} ±0.4	7.69
lig x lig	66.2 ^{fj}± 6.9	10.42	5060 ^{a}±357	7.05	6.6 ^{a}±1.1	16.67
lam x lam	215.8 ^{a}±22.3	5.53	2411 ^{j}±164	6.80	3.0 ^{f}±0.7	23.33
Cross						
lig x car	92.8 ^{e} ± 9.3	10.02	4606 ^{ab}±341	7.40	6.0^{ab}±0.7	11.67
lam x car	161.8^{bc}±19.4	11.99	2732 ^{fj}±276	10.10	3.2 ^{f}±0.4	12.50
lam x lig	177.2^{b}±11.6	6.55	2918 ^{f}±312	10.69	3.6 ^{ef}±0.9	25.00
Reciprocal Cross						
car x lig	86.0 ^{ef} ±13.2	15.35	4205 ^{bc}±117	2.78	5.6^{abc}±1.1	19.64
car x lam	117.2 ^{d}±18.5	15.78	3165 ^{de}±155	4.90	4.6 ^{de}±0.5	10.87
lig x lam	140.8 ^{c}±15.2	10.79	3317 ^{d}±177	5.34	4.8 ^{cd}±0.8	16.67
F value	43.28		40.92		7.49	
LSD	23.712		412.777		1.068	

N.B. Under line = dame (♀) **car = *carnica* ; lig = *ligustica* ; lam = *lamarckii***
Prob. < 0.0001

The correlations coefficients between parameters for the hybridization of the experimental races during swarmes are given in Table (3). The data clearly show that the correlations between reared queen cells and colony strength were significant either in case of car bees (pure, depends on dams or sires) or pure line of lam bees and insignificant in case of lam bees (pure or depends on dams and sires).

Table (3): Correlations coefficient between parameters for the hybridization of the experimental races during swarmes

Genetic groups	Parameters		
	Qc / Cs	Qc / B	Cs / B
Pure lines			
car x car	0.6818**	0.5923*	0.6707**
lig x lig	0.5087*	0.٥672*	0.6278*
lam x lam	0.3594	0.4١94	0.٥276*
Cross			
lig x car	0.٥٥06*	0.5226*	0.8925**
lam x car	0.3651	- 0.٢614	0.4773
lam x lig	0.2607	- 0.٢125	0.4213
Reciprocal Cross			
car x lig	0.5217*	0.5586*	0.9473**
car x lam	0.3928	- 0.3380	0.6285**
lig x lam	0.3034	- 0.2607	0.7537**

N.B. Under line = dame (♀) Qc = Queen cells ; Cs = Colony strength ; B = Brood

The correlations between queen cells and brood were relatively the same as previously described, but the cross and reciprocal cross of lam bees appeared negative sighing.

The general combining ability (g.c.a.) is defined as the average performance of an infinitely numbers of progeny of an individual or line when mated to a random sample of gametes from specified tester population. The data in Table (4) show that the three experimental races were combining generally with each others. The high rate of g.c.a. for the number of queen cells either in emergency or swarms for lam bees followed by lig bees and car bees. The vice versa was obtained in cases of number of brood cells and colony strength where lig bees showed high rate of g.c.a. followed by car bees and lam bees.

Table (4): General combining ability for different parameters of the experimental races.

parameters	Genetic groups		
	car	**lig**	**Lam**
Emergency			
Queen cells	41.28	45.76	55.68
Swarms			
Queen cells	100.64	112.60	162.56
Brood	3753.92	4021.16	2908.52
Colony strength	4.92	5.32	3.84

The specific combining ability (s.c.a.) is defined as the deviation of the average numbers of progeny of two individuals or lines from the values that would be expected on the basis of known general combining abilities (deviations from additively of g.c.a.'s). In case of the number of queen cells either in emergency or swarms, lam bees appeared tendency of s.c.a. with the other two races, however the hybridization between lig and car bees were decreased each others. In cases of number of brood cells and colony strength the hybridization between lam bees and the other two races decreased its rates. On the other hand, lig and car bees appeared tendency of s.c.a. with each others by increasing its rates, Table (5).

Table (5): Specific combining ability for different parameters between the hybridization of the experimental races.

Parameters	Genetic groups		
	lig x car	**lam x car**	**lam x lig**
Emergency			
Queen cells	- 6.02	3.02	3.58
Swarms			
Queen cells	- 17.22	7.90	21.42
Brood	517.96	- 382.92	- 347.34
Colony strength	0.68	- 0.48	- 0.38

N.B. Under line = dame (♀)

The maternal effect indicates to the preference for using the race either as a dam or a sire (cross and reciprocal cross) according to the increase or decrease of the tested character. The value of maternal effect (Table,6) for the number of constructed and reared queen cells either in case of emergency or in swarming period were positive when depending on yellow dams (lig & lam). It's noticeable that lam bees when it used as a dam with the other two races gave higher maternal inheritance in case of swarming queen cells, in spite of the opposite direction noticed for the number of brood cells and colony strength which were decreased.

Table (6): Maternal effect for different parameters between the hybridization of the experimental races.

Parameters	Genetic groups		
	lig x car	**lam x car**	**lam x lig**
Emergency			
Queen cells	5.00	3.40	4.20
Swarms			
Queen cells	6.80	44.60	36.40
Brood	401.80	- 433.40	- 400.00
Colony strength	0.40	- 1.40	- 1.20

N.B. Under line = dame (♀)

The heterosis (hybrid vigour) showed that, lam bees when hybrid either as a dam or as a sire with the other two races caused a decrease in the number of queen cells and vice versa in cases of brood and colony strength comparing to its parent or pure line. The lig bees when hybrid with car bees increased each others, except after using as a sire in cases of emergency queen cells, brood and colony strength and as a dam in case of brood, Table (7).

Table (7): Heterotic effect for different parameters between the hybridization of the experimental races.

Parameters	Genetic groups					
	lig x car	**car x lig**	**lam x car**	**car x lam**	**lam x lig**	**lig x lam**
Emergency						
Queen cells						
Dam (d)	5.26	12.90	- 11.33	60.64	- 6.00	37.37
Sir (s)	29.03	- 7.89	71.61	- 17.00	48.40	- 13.00
Mid parents(P)	15.94	1.45	16.90	9.45	15.10	6.53
Swarms						
Queen cells						
Dam (d)	25.40	75.51	-14.39	139.18	-6.24	90.27
Sir (s)	89.39	16.22	230.20	-37.99	139.46	-25.50
Mid parents(P)	50.89	39.84	35.97	-1.51	34.75	7.07
Brood						
Dam (d)	-14.50	0.65	6.30	-24.25	13.542	-38.42
Sir (s)	10.24	-21.94	-34.61	23.15	-45.83	29.07
Mid parents(P)	-3.69	-12.07	-19.03	-6.19	-26.66	-16.63
Colony strength						
Dam (d)	0.00	12.00	6.67	-8.00	20.00	-20.00
Sir (s)	16.67	-6.67	-36.00	53.33	-40.00	60.00
Mid parents(P)	9.09	1.82	-20.00	15.00	-20.00	6.67

N.B. Under line = dame (♀)

The heritability estimates (h^2) is determined the value of inheritance of the character from parents to offspring's. Table (8) indicate that the heritability which depends on dam h^2_d or mid parents h^2_P for queen cells in emergency and swarming cases were higher in lam bees than the other two races, in spite of the moderate heritable obtained. On the other hand, it could be noticed that h^2_d & h^2_P for brood and colony strength were moderate in both lig and car bees. However, this values were low in lam one.

Table (8): Heritability estimates (h^2) for different parameters of the three experimental races.

Parents	Genetic groups		
	Car	Lig	Lam
Emergency			
Queen cells			
Dam (d)	0.15	0.23	0.39
Sir (s)	0.10	0.17	0.19
Mid parents(P)	0.14	0.21	0.29
Swarms			
Queen cells			
Dam (d)	0.24	0.30	0.47
Sir (s)	0.16	0.18	0.27
Mid parents(P)	0.20	0.25	0.37
Brood			
Dam (d)	0.33	0.40	0.23
Sir (s)	0.25	0.22	0.17
Mid parents(P)	0.27	0.34	0.19
Colony strength			
Dam (d)	0.48	0.51	0.39
Sir (s)	0.31	0.29	0.22
Mid parents(P)	0.40	0.43	0.31

In general, the queen cells which were constructed and reared by lam bee race either for emergency or for swarming showed high tendency of combining ability either generally or specifically with the other two races. Also, it had higher maternal inheritance when it used as a dam and moderately heritable estimates h^2_d & h^2_P than the others. Its hybrid vigour showed decrease in the number of queen cells comparing to its parent or pure line when hybrid either as a dam or as a sire but it was higher than the other hybridization. The lig bee race came in the second order and the car bee race gave the least.

The number of brood cells is considered one of the indicators for colony strength. For this character lig bee race appeared high rates of general and specific combining ability, maternal effect and hybrid vigour followed by car and lam bee races, respectively. Although the heritability either depends on dam or mid parent for both characters were low in lam bee race than that in lig and car bees which were moderate, it was lie within that estimated by Vesely and Siler, 1963 (0.30), Soller and Bar-Cohen, 1967 (0.33) and EL-Banby, 1969 (0.51 in citrus season and 0.34 in clover season 0.34).

Consequently these results emphasized that the multiple queen status in lam bee race is the genetically inheritance and not affected by brood or colony strength. This may be due to behavioural characteristic unique adaptations to its environment

Finally, the previous results give an economic value in the queen rearing centers where the starter colonies must be first generation of lam bees to get high successful results.

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المظهر الوراثى لحالة تعدد الملكات فى نحل العسل

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قسم وقاية النبات – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر

استخدم ثلاث سلالات لنحل العسل هى الكرينولى و الايطالى و المصرى وهجنها الناتجة من الخلط المتبادل باستخدام التلقيح الآلى لدراسة حالة تعدد الملكات فى فترات الطوارى والتطريد وذلك فى منحل كلية الزراعة جامعة عين شمس خلال عامى ٢٠٠٥ / ٢٠٠٦.

اظهرت النتائج ان النحل المصرى سواء النقى او هجنة المعتمدة على الام (الملكة) او المعتمدة على الاب (الذكر) يربى اعداد كبيرة من البيوت الملكية فى حالة الطوارى او فى فترة التطريد يلية النحل الايطالى واخيرا النحل الكرينولى وعلى العكس من ذلك بالنسبة للحضنة وبالتالى قوة الطوائف.

تشير النتائج الى ان النحل المصرى اظهر استعداد عالي على التوافق سواء العام او الخاص مع السلالات الاخرى وايضا لة تآثير أمى عالى ,بالاضافة الى ان قوة الهجين اظهرت معدلات اقل من أباءة الاانها اعلى من اى سلالة اخرى. وبالرغم من ان تقديرات المكافئ الوراثى للنحل المصرى تندرج فى المستوى المتوسط حيث سجلت للأم و الأب و متوسط الاباء ٠,٣٩ و ٠,١٩ و ٠,٢٩ فى حالة الطوارى و ٠,٤٧ و ٠,٢٧ و ٠,٣٧ فى حالة التطريد على التوالى الا انها اعلى من النحل الايطالى حيث سجلت للأم و الأب و متوسط الاباء ٠,٢٣ و ٠,١٧ و ٠,٢١ فى حالة الطوارى و ٠,٣٠ و ٠,١٨ و ٠,٢٥ فى حالة التطريد على التوالى واخيرا تأتى معدلات النحل الكرينولى حيث سجلت للأم و الأب و متوسط الاباء ٠,١٥ و ٠,١٠ و ٠,١٤ فى حالة الطوارى و ٠,٢٤ و ٠,١٦ و ٠,٢٠ فى حالة التطريد على التوالى. ومن جهة آخرى فان المحددات الوراثية السابقة فى حالة الحضنة وقوة الطوائف كانت معدلاتها معكوسة حيث اظهر النحل الايطالى افضل المعدلات يلية النحل الكرينولى واخيرا النحل المصرى.