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The Role of the Egyptian Honey Bee Workers in Selecting Adult Queen Kinships, and the Impact on the Colony's Biological Activities

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Abstract This study was conducted at Dirut region, Assiut Governorate, Egypt. Through the Egyptian bee swarming season in mud tube hives, three groups of virgin queens were selected for this study. Group one (attacked by balling worker bees), group two (moved free without any attack of worker bees) and group three (virgin queens were left their hives with the swarm to new home). The effect of Egyptian honey bee workers behaviour toward relatedness virgin queens on the queen acceptance and their biological activities were determined. The obtained results show that the acceptance percentage recorded 43.75% and 100% of the virgin queens of group one and group two after one week of introducing, respectively. The mated queens of group three resulted a highly mean of brood area (inch^2), number of combs covered with bees, mean stored honey and pollen areas (inch^2) followed by group two. While, group one recorded the lowest values.

Keywords: Egyptian bee, virgin queen, bee workers, mud hives, balling bees.

Introduction

In Egypt, rustic beekeeping still uses the Egyptian honey bee, *Apis mellifera lamarkii*. In a few areas of Egypt, perhaps most notably around the town of Assiut about 400 Km upriver from Cairo on the Nile, this native honey bee race is still managed in mud-tube hives much as it was in the times of the pharaohs. The Egyptian bee was first introduced into U.S. in 1866 and was considered extremely beautiful, due to its orange coloration and white pubescence (Schiff and Sheppard, 1993). In Assiut Governorate 500 or more colonies are commonly maintained together in a single apiary (Kamel, 1991 and Sheppard *et al*., 2001).

The ability of workers in social insect societies to discriminate among conspecific groups or individuals on the basis of nestmate or kinship

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recognition has become a control issue in socio-biology (Crozier and Dix, 1979; Holldobler and Michener, 1980 and Getz *et al*., 1982). Honey bees (*Apis mellifera* L.) provide some special challenges for kin selection theory and the study of kin recognition because of their high degree of polyandry, resulting in numerous sibships within colonies (Page and Metcalf, 1982), and because of their extensive use of chemicals to mediate behaviour (Gary, 1974). Honey bee queens mate with up to 18 males (Adams *et al*., 1977). Each colony therefore, is a collection of many subfamilies, each subfamily having a high coefficient of relationship among its members, who have a common father, respect to members of the other subfamilies which having different unrelated fathers. Where adult honey bee workers have the ability to discriminate among sibship or nestmate groups of adult worker and adult queens, possibly on the basis of genetic factors (Wright, 1922, Breed, 1981; Getz *et al*., 1982, Page and Metcalf, 1982 and Getz &Smith 1983).

Mandibular glands of the queen honey bee are source of substance called pheromone that influences the level of aggressive response by workers towards her (Walton and Smith, 1969 & Yadava and Smith, 1971). In other study, Alkattea *et al*. (2008), noticed that kin related recognition cues do not depend on volatile bouquets but rather on stimuli elicited through non-volatile compounds of the cuticle of the queen. Worker bees perceive these compounds in the bioassay by direct contact and liking of the queen. Schneider *et al*. (2001) observed that once queens emerges, they are often bitten, chased and vibrated by workers, which may inhibitor interrupt their aggressive interactions and help to determine the ultimate victor in polygyny reduction.

The objective of the present work is to study the effect of Egyptian honey bee workers behaviour in mud-tube hives toward relatedness adult queens on the queen acceptance and their biological activities.

Materials and methods

The proposal of this study was carried out in a private apiary at Dirut region, Assiut Governorate, Egypt, during the period from 1/3/2011 to 12/7/2011. In this region some beekeepers are still rearing the Egyptian honey bees *Apis mellifera lamarkii* in mud-tube hives. During the swarming season in the mud-tube hives (From mid February to the end of March) many of virgin queens of Egyptian bees were naturally produced, three groups of them (Fig. 1) were discriminated to procedure the experiments as follows:

Group one: virgin queens attacked by balling worker bees, which around them in the same colony.

Group two: virgin queens were moved free without any attack of worker bees.

Group three: virgin queens were left their hives with the swarm to new home. The swarm was captured and introduced into Langstroth wood hive and left until the queen mating.

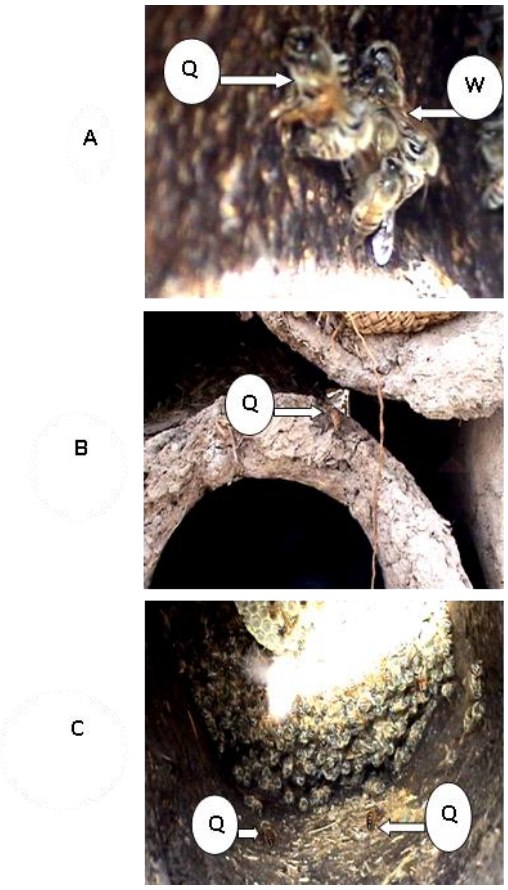


Fig. 1. Tested Egyptian virgin queens were obtained from mud tube hives. A, queens (Q) isolated from balling bee workers (W) inside the hive. Free queens without balling isolated from out (B) or inside (C) of mud tube hives.

Experimental procedures

Experiment 1: Acceptance percentages of the virgin queens introduced into new honey bee divisions

Sixteen Egyptian virgin queens of group one and three virgin queens of group two were captured from 18-19 March 2011. Each captured virgin queen was introduced into new queenless honey bee division of Langstroth wood hive under the semi-ball cage and was released from the cage after 48 hours. All

new divisions in this experiment were of Carniolian hybrid and almost equal strength of about three brood combs and two combs of honey and pollen. One week later, the number and percentage of accepted, died and mated queens were recorded.

Experiment 2: Effect of bee workers behaviour toward relatedness queens on the biological activities

Nine colonies were used for this experiment; six new divisions with Egyptian mated bee queens from experiment-1 were selected. Three colonies of group one and group two were used. Also three colonies of group three were prepared for this study.

The following biological activities were measured in each tested colony during the experimental period (from 7/4/2011 to 12/7/2011), i.e., area of worker brood (inch²), area of stored honey and pollen (inch²) were measured at 12 days intervals according to Fresnay (1962). Also, the number of combs covered with bees and queen cups production was counted at 12 days intervals according to Nour (1992).

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) through SPSS computer program. Means were compared using Duncan's Multiple Range tests.

Results

Acceptance percentages of the virgin queens introduced into new honey bee divisions

Data in Table (1) indicate the effect of tested Egyptian virgin bee queens of group one (attacked by balling worker bees) and group two (moved free without any attack of worker bees) on the acceptance percentages during introducing into new honey bee divisions. The acceptance percentage for the queens of group one recorded 43.75%, while the virgin queens of group two were 100% after one week of introducing. Also, the queen mating percentages were 57.14 and 100% for group one and group two, respectively.

Table 1. Acceptance percentages of the virgin queens introduced into new honey bee divisions

Treatment	Total number of the introducing virgin queens	Number of accepted queens after one week.		Number of mated queens	
		No.	%	No.	%
Virgin queens of group one	16	7	43.75	4	57.14
Virgin queens of group two	3	3	100	3	100

Group one, virgin queens attacked by balling worker bees.
Group two, virgin queens were moved free without any attack of worker bees.

Effect of bee worker behaviours toward relatedness queens on the biological activities

Biological activities of tested queens in group one (attacked by balling worker bees), group two (moved free without any attack of worker bees) and group three (virgin queens were left their hives with the swarm to new home) are presented in Tables (2&3). Results show that there were a highly significant difference in the mean area of brood (inch^2) and mean number of combs covered with bees in all dates of the experimental period. Whereas, the colonies of group three resulted a highly mean of brood area and number of combs covered with bees followed by group two. While, group one recorded the lowest values. Insignificant differences were found between tested groups in the mean number of queen cups in most dates of the experiment. While, slightly increasing of queen cups were recorded in colonies of group three compared with the other tested groups (Table 2).

Concerning to the different dates of the experimental period, the data presented in Table (3) clarify that the mean area of stored honey (inch^2) was significantly increased in group three followed by group two then group one.

Also, the obtained results recorded higher values in the mean area (inch^2) of stored pollen in colonies of group three than group one that recorded the lowest values. Although there was a variation in recorded means, but it was significantly different in the almost experimental periods.

The general mean of the obtained results for the biological activities of tested queens at the end of experiment (Fig. 2 A&B) illustrates the following:

The mean worker brood area reached 330.8, 88.04 and 203.5 inch^2 for group three, group two and group one, respectively.

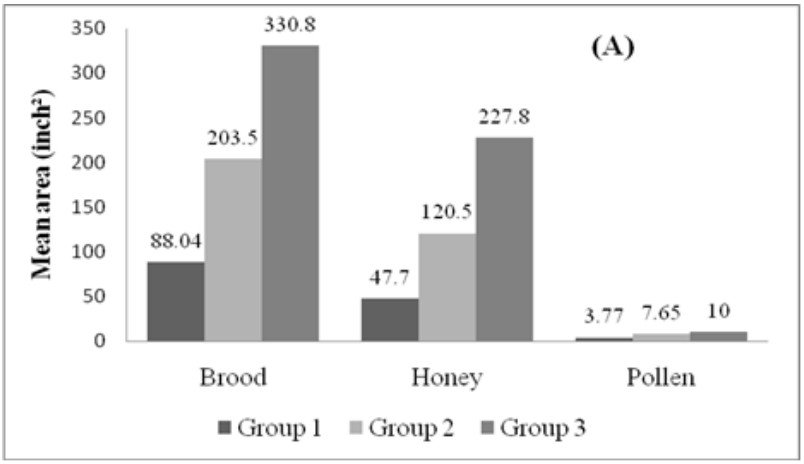
Tested colonies of group three produced the highest mean area of stored honey (227.8 inch^2) and pollen (10.0 inch^2) in compared with group two (120.5& 7.65 inch^2) and group one (47.7& 3.77 inch^2) for stored honey and pollen, respectively (Fig. 2A).

The colonies of group three recorded the highest mean number of combs covered with bees (5.96) and queen cups (1.7), while group two and group one recorded (4.21& 0.36) and (2.96 & 0.1), respectively (Fig. 2B).

Table 3. Effect of bee workers behaviour toward relatedness queens on the mean amount of stored honey and pollen (inch ^{2})

Date	Mean amount of stored honey (inch^2)				Mean amount of stored pollen (inch^2)			
	Group one	Group two	Group three	F value	Group one	Group two	Group three	F value
7/4/11	13.6 b	14.6 b	170.0 a	19.1**	2.3 b	4.3 ab	16.0 a	4.6*
19/4/11	12.0 b	15.6 b	132.6 a	14.8**	1.6 b	3.3 ab	10.0 a	4.3*
1/5/11	11.6 b	19.6 b	68.3 a	14.3**	2.0 a	2.6 a	5.3 a	2.4^{NS}
13/5/11	24.0 b	96.6 c	223.3 a	28.0**	2.3 b	4.6 ab	6.3 a	3.3*
25/5/11	41.6 b	138.3 c	386.6 a	49.1**	4.6 a	7.0 a	7.6 a	0.8^{NS}
6/6/11	61.6 b	153.3 c	483.3 a	141.6**	2.3 b	7.6 a	7.3 a	8.0**
18/6/11	21.0 a	93.3 a	76.6 a	3.4^{NS}	3.3 a	5.3 a	6.6 a	1.2^{NS}
30/6/11	100.0 b	203.3 a	200.0 a	11.3**	4.3 b	12.3 a	12.6 a	5.8*
12/7/11	156.6 a	350.0 b	310.0 ab	4.5*	11.0 a	21.6 a	18.6 a	1.6^{NS}

Group one, virgin queens attacked by balling worker bees.
Group two, virgin queens were moved free without any attack of worker bees.
Group three; virgin queens were left their hives with the swarm to new home.
*: Significant; ** : Highly significant; NS : Non significant
a: Between columns, figures followed by same letter do not differ at 5% level (F- test).
ab: Figures differ at 5% level.; C: Figures differ at 5% level.



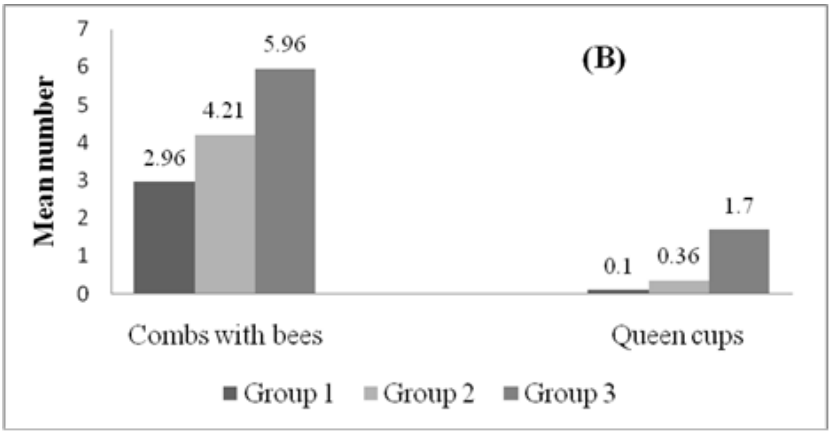


Fig. 2. The effect of Egyptian honey bee worker behaviours towards relatedness adult queens on their biological activities.
Group one, virgin queens attacked by balling worker bees.
Group two, virgin queens were moved free without any attack of worker bees.
Group three; virgin queens were left their hives with the swarm to new home.

Discussions

During our study we found that, the Egyptian honey bee workers *Apis mellifera lamarkii* reared in mud-tube hives could distinguish among queens of different degrees of kinships. Whereas, during swarming seasons, we observed some adult virgin queens which had emerged from their queen cells were attacked by the worker bees (balling behaviour) in the same colony and other queen moved without any attack. This study demonstrates that the balling behaviour of Egyptian bee workers against relatedness virgin queens influence on the acceptance of those queens in new divisions. Also, the balling behaviour of bee workers decreased the biological activities of the queens which attacked by the bee workers. Through the experimental period following biological activities were determined, area of worker brood, stored pollen and honey (inch ^{2}), mean number of combs covered with bees and number of queen cups. Other tested colonies in Langstroth hives which headed by queen movement free without attack by bee workers in their mud-tube hives or queens caught with swarm (move to a new location) results the highest value of biological activities. In case of kin selection honey bee gynes are sometimes immobilized by clusters of workers, known as " balling " (Walton and Smith, 1969; Ambrose, 1975; Lensky *et al*., 1991and Pettis *et al*., 1998), which might make gynes easier to sting by rival during gyne duels (Gilley, 2001 & Tarpy and Fletcher, 2003). In the other study, Schneider *et al*., 2001, observed that once queens emerge, they are often bitten, chased and vibrated by workers, which may inhibitor interrupt their aggressive interactions and help to determine the

ultimate victor in polygyny reduction. The aggressive behaviour of honey bee workers towards gynes seems to occur mainly when a colony is prepared to issue one or more after swarms. Gilley *et al*., (2003), suggested that these behaviours are used to formulate collective decisions during queen production.

Schneider and Degrandi Hoffman (2003) stated that honey bee workers also frequently shake emerged gynes. All of worker-gyne interactions could affect the fates of the gynes and thus may represent group decisions over which gynes survive to become queens and which do not (Gilley, 2001). In *Apis mellifera*, temporary polygyny (the presence of multiple queens) occurs during episodes of queen replacement (supercedure and emergency queen rearing) and colony reproduction (swarming), after which supernumerary queens are usually quickly eliminated in the establishment of monogyny. Both worker and queen behaviours may be involved in the queen reduction process. Workers can distinguish between adult queens that are related and unrelated to them possibly by using a chemical signal originating from glands on the tergite (Moritz and Crewe, 1988). Alkattea *et al*., 2008, noticed that kin related recognition cues do not depend on volatile bouquets but rather on stimuli elicited through non-volatile compounds of the cuticle of the queen. Worker bees perceive these compounds in the bioassay by direct contact and liking of the queen. These studies were matched with our results where the workers play an important role in selecting their adult queen-kinships.

Conclusion

From the foregoing results it can be concluded that, the Egyptian honey bee workers naturally inhabit in mud tube hives can discriminate between related and unrelated virgin queen produced through the swarming season in their colony. Whereas, some virgin queens were attacked by workers (balling behaviour) and isolated to pass out of their hives, in contrast the other virgin queens were moved without any attack. Also, the obtained results indicated that the aggressive behaviour of Egyptian workers towards virgin queens affected on acceptance percentages and biological activities during introducing that queens into new honey bee divisions. In recommendation, the beekeepers must be careful during the beekeeping practises and select the virgin queens in the swarming season for building new colonies of Egyptian bees in mud tube hives.

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Table 2. Effect of bee workers behaviour toward relatedness queens on the mean of brood area, No.of combs covered with bees and No. of queen cups

Date	Mean of brood area (inch^2)				Mean No. of combs covered with bees				Mean No. of queen cups			
	Group one	Group two	Group three	F value	Group one	Group two	Group three	F value	Group one	Group two	Group three	F value
7/4/2011	21.0 b	36.6 b	163.3 a	8.5*	2.3 b	3 ab	4.3 a	3.5^{NS}	0.0 a	0.3 a	3.0 a	3.3^{NS}
19/4/2011	34.3 b	64.0 b	180.0 a	16.5**	2.3 b	3.3 b	5.0 a	9.8**	0.0 a	0.0 a	2.3 a	3.7^{NS}
1/5/2011	53.3 b	91.6 b	181.6 a	18.5**	2.3 b	3.3 b	5.3 a	10.5**	0.0 a	0.0 a	1.6 a	1.0^{NS}
13/5/2011	59.0 b	171.6 ab	300.0 a	5.9*	2.3 b	3.3 b	6.0 a	19.4**	0.0 a	0.0 a	2.3 a	3.7^{NS}
25/5/2011	63.3 b	191.6 ab	476.6 a	5.6*	3.0 b	3.6 b	7.5 a	31.0**	0.0 a	0.3 a	2.3 a	3.0^{NS}
6/6/2011	71.6 b	223.3 b	550.0 a	8.2**	3.3 b	4.3 b	6.0 a	9.8**	0.0 a	0.3 a	1.3 a	2.6^{NS}
18/6/2011	100.0 b	226.6 ab	326.6 a	6.3*	3.3 b	5.0 ab	6.0 a	7.0*	0.3 a	1.6 a	1.3 a	1.0^{NS}
30/6/2011	156.6 b	350.0 a	346.6 a	18.4**	3.6 b	5.6 a	6.6 a	21.0**	0.6 a	0.3 a	0.6 a	0.3^{NS}
12/7/2011	233.3 b	476.6 a	453.3 a	4.5*	4.0 b	6.3 a	7.0 a	9.5**	1.0 a	0.0 a	0.3 a	1.7^{NS}

Group one, virgin queens attacked by balling worker bees.
Group two, virgin queens were moved free without any attack of worker bees.
Group three; virgin queens were left their hives with the swarm to new home.

*: Significant; ** : Highly significant; NS : Non significant.
a: Between columns, figures followed by same letter do not differ at 5% level (F- test).
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