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EFFECT OF REARING SEASON ON QUEEN AND THE REPRODUCTION OF *APIS MELLIFERA CARNICA* AND *A MELLIFERA LIGUSTICA*

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ABSTRACT

Hybrids of *Apis mellifera carnica* and *Apis mellifera ligustica* queen bees were reared to investigate their physiological and reproductive characters as well as spermatheca semen volume to determine the suitable time for queen rearing under Giza conditions in Egypt. Rearing seasons were found to affect the queens' quality determined based on their weight/ volume of the spermatheca, and quantity and quality of stored sperms in the spermatheca. Results indicated that the Carniolian hybrid gynes were heavier than Italian hybrid ones, with greatest mating success being observed in March and April, while it was worst during June. Spermatheca was less voluminuos with less spermatozoa in Italian hybrid queen bees compared to the Carniolian hybrid ones. Thus, under Giza region conditions, it is highly recommended to rear queens during the spring months.

Key words: *Apis mellifera carnica*, *A. mellifera ligustica*, queen, reproductive characters, spermatheca, sperm quantity, spermatozoa

The queen's ability to reproduce is essential to the colony's survival, and thus the queen is the most significant individual within the colony. Analyzing the queens' reproductive potential can reveal the fitness of the colony. A new queen bee is raised wither in emergency scenarios (to replace deceased or missing queens) or in supersedure (to replace ageing, damaged, or diseased queens) and swarm situations. Queens produced from old larvae are of poor quality in terms of morphology and sperm count, and as a result, they mate with considerably fewer males (Amiri et al., 2017). The start of oviposition, queen acceptance ratio, quantity of spermatozoa in the spermatheca, spermatheca diameter, and laying rate are all significantly influenced by the weight of the queen (Wei et al., 2019). More honey is produced when colonies are regularly re-queened with young ones under a year old compared to older ones. Queen rearing is thus a crucial step in beekeeping (Yi et al., 2020). Requeening colonies led by queen bees whose bee's exhibit thigh disease susceptibility or robust defensive behaviour is a common practice among beekeepers (Chuda-Mickiewicz and Samborski, 2015). Hybrid of *Apis mellifera carnica*, the most spread of the bee races in Giza region, may continue to exist in the area. This might be leading to queens mating with low number of drone and colonies that are unsuitable for commercial apiculture (Elenany and Abdallah, 2016). Commercial beekeepers have to be critical of the development of queens, and existence of only one queen in a colony is crucial (Adham, 2000; Ahmet and Hakan, 2013). The colony's economic traits depend largely on the caliber of queen (Harris, 2009), and this in turn, depends on the genetic/ environmental factors, the breeding conditions and queen rearing methods (Zedan, 2002; Taha, 2005; John et al., 2011).

The mating of *A. mellifera* queen is influenced by-environmental factors (Cengiz et al., 2019), presence of worker brood (Stankus, 2008), age of associate nurse workers (Delaney et al., 2011), drone raising cycle (Rangel et al., 2013) and the number of queens being raised (Koywiwattrakul and Sittipraneed, 2009). Beekeepers either breed their own queens or purchase from others (Wei et al., 2019), and hence many do not know the features of the queens. Adapting the region's hot and dry is an additional problem (Amiri et al., 2017). Local honey bee genotypes can be improved for queen rearing and impending breeding initiatives. The quality of the queen, which is defined by the number of ovarioles in ovaries and the size of spermatheca, is a significant factor in the strength and production of the colony. Producing one's own queen under the right circumstances take long time and also queens thus produced in a year may have varied qualities (Sakla and El-shafeiy, 2022). This study investigates the physiological and reproductive traits of the hybrid

queens of *Apis mellifera carnica* and *A. m. ligustica*. The aim is to find out the traits that could be employed in breeding and selection programmes, as well as to choose the ideal period for producing high-quality queens.

MATERIALS AND METHODS

The study was conducted in the apiary of Faculty of Agriculture, Cairo University during 2020 and 2021. Twenty honey bee colonies (10 each of Carniolian and Italian hybrids) were used and the queens were open mated ensuring that the colonies were equal in strength and exposed to the routine work. The technique used for rearing of the queens in seasons (late winter, spring and summer) is based on the Doolittle larval grafting method. The grafted larvae werer <24hr, as taken from the stock colony. Mated queens were removed from the rearings, and made queenless builder colonies. The introduced larval queen cells to the queenless builder colonies were grafted from the same genetic origin of rearing colonies in bee wax cups fixed on wooden bars hanging in the rearing frame. Each frame contained 45 grafted queen cells (Moretto et al., 2004).

The following parameters were recorded for each reared frame for each hybrid: weight (mg) of newly emerged virgin queens (<24 hr) /month/ hybrid (Akyol et al., 2008); number of ovarioles/ queen/ month/ hybrid- both ovaries were delicately extracted after queens were dissected in insect saline. The ovarioles were spread out on a microscope slide and counted; For measuring volume of spermatheca, the queens were placed in the freezer for 4-6 min (- 20°C is sufficient) or until immobilized; euthanize by removing her head and pinning body in a dissecting tray to extract the spermatheca; cut both sides of abdomen using forceps, grasp the stinger and gradually draw it out until the ovaries are visible; carefully remove the spermatheca and lay it on a plate by using a binocular; then the radius was measured ($V = 4/3\ \pi r^3$). For count of sperm in mated queen's spermatheca (million), lay spermatheca on a plate with 0.1 ml brine diluent and rupture it with forceps to release sperm; fill a glass petriplate with 0.9 ml water, and u sing a clean glass pipette mix well around 40 times until everything is combined; and finally tha haemocytometer count chamber was used to count the sperm, under 250x magnification- start counts on the gridded section after the sperm have settled (~20 sec), 4 squares at haemocytometer grid each square has 16 squares; and thus sperms were counted from 64 squares and the resulting number is multiplied by 10000 and divided by 0.4 (Woyke, 1979). Measurement of total protein in virgin bee queen's (< 24 hr) haemolymph was done with Foss Tecator 2100. One-way ANOVA/ independent samples t-tests were used to determine significant differences ($p < 0.05$) using the SPSS 25.00. These differences included samples taken from the inside or entrance of the beehives as well as samples related to the seasons.

RESULTS AND DISCUSSION

The results revealed significant differences in the mean weight of newly emerged virgin queens in two hybrids during the active season (Figs. 1, 2); *A. m. carnica* queens were significantly ($p \leq 0.05$) heavier (219.2 mg) than *A. m. ligustica* queens (179.2 mg), with maximum being during the summer season, and the least during the late winter (152.1 and 135.1 mg for *A. m.carnica* and *A. m. ligustica*, respectively). Figures 3 and 4 illustrates the difference between the number

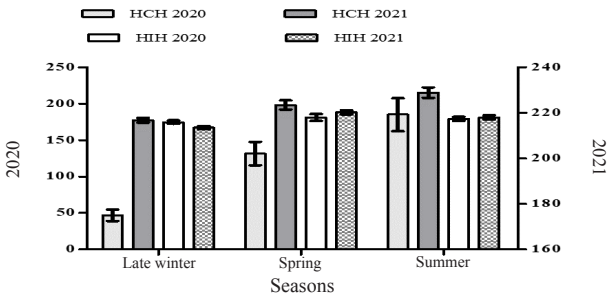

Fig. 1. Weight of heavy queen hybrids of *A. m. carnica* and *A. m. ligustica* (HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)

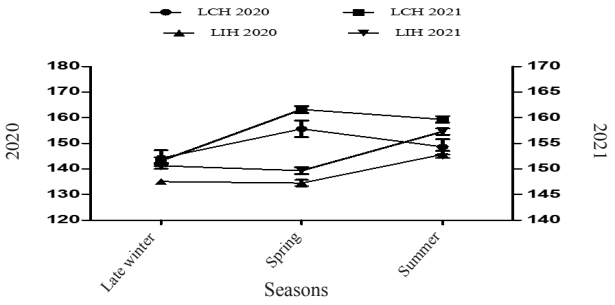

Fig. 2. Weight of light queen hybrids (LCH: Light Carniolian hybrid; LIH: Light Italian hybrid)

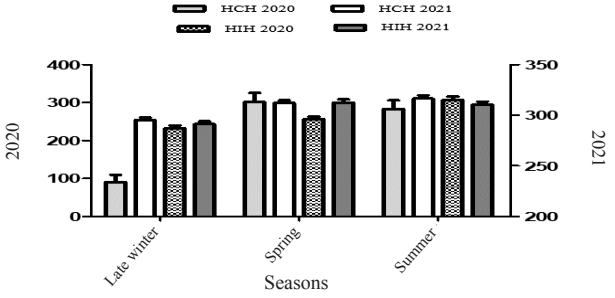

Fig. 3. Ovarioles in queens (HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)

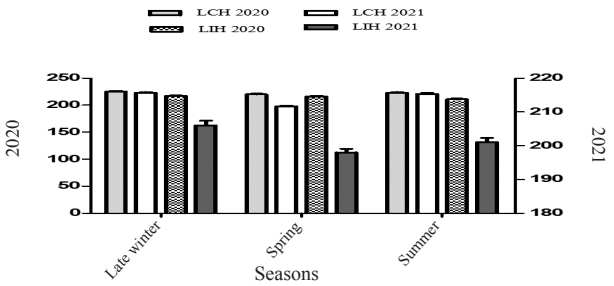

Fig. 4. Ovarioles (LCH: Light Carniolian hybrid; LIH: Light Italian hybrid)

of ovarioles- 313 ovarioles in HCH and 256 in HIH during the spring season; however during late winter, these decreased in both; mean number of ovarioles in hybrids varied significantly during 2020, but without significant differences in 2021. The least spermatheca volume was observed in late winter- 0.05 mm for both hybrids in heavy queens and 0.04 mm in light queens; maximum spermathecal volume was observed during summer season for carniolian hybrid, This was due to the abundance of nectar offering plants. There was no clear significant differences between hybrids for the sperm count in spermatheca; maximum count was during spring (6.2 million) for Italian hybrid, whereas the least was observed during summer (5.8 million) in heavy queens; while in the light queens, the Italian hybrid revealed maximum count in spring (4.6 million) (Table 1). Total protein in haemolymph was maximum in summer for heavy queen of carniolian hybrid (9.4 KDa) while it was only (7.5 KDa) in the summer for Italian hybrid in light queen (Table 2). The *A. m. carnica* queens were heavier than those of *A. m. ligustica*, and spring-reared queens were noticeably heavier. Such seasonal weight variations across various queens may be due to the workers' effective foraging efforts and better environmental circumstances.

The weight of queens at emergence was reportedly influenced by the rearing season and the origin of the queen (Szabo et al.,1987; Güler et al.,1999). The recipient colonies tended to accept the Carniolan virgin queens more readily than the Italian virgin queens; this might be attributed to the Caniolian bees being more docile (Guzmán-Novova et al., 1998), the mother queen's genetic influence (Moretto et al., 2004), and the presence of elderly workers in the mating nuclei, which can be advantageous because they can prompt the queen to make earlier mating. Additionally, recipient colonies prefer queens with high body weights than those with low body weights (Szabo, 1977; Taranov, 1973). This may account for the increased acceptance rate during the spring, when pollen and nectar are more readily available. These findings are in line with those of Kaftanolu and Kumova (1992), who investigated the impact of queen bee rearing season on quality and discovered that acceptance rates varied from 81.7 to

Table 1. Spermatheca, sperm count in mated queens of *A. m. carnica* and *A. m. ligustica*

Bee hybrids / Seasons	2020				2021			
	HCH	LCH	HIH	LIH	HCH	LCH	HIH	LIH
Spermatheca								
Late winter	0.05± 0.02	0.05± 0.02	0.05± 0.02	0.04± 0.02	0.04± 0.02	0.04± 0.01	0.09± 0.02	0.05± 0.02
Spring	0.17± 0.04	0.09± 0.02	0.21± 0.05	0.08± 0.03	0.18± 0.04	0.12± 0.01	0.11± 0.02	0.10± 0.01
Summer	0.20± 0.05	0.11± 0.03	0.16± 0.04	0.06± 0.02	0.23± 0.06	0.13± 0.04	0.19± 0.02	0.07± 0.03
Sperm count								
Late winter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spring	6.10± 0.35	4.50± 0.15	6.30± 0.37	4.60± 0.16	5.10± 0.27	4.70± 0.14	5.10± 0.22	4.60± 0.15
Summer	6.20± 0.32	4.50± 0.13	5.80± 0.28	4.50± 0.18	5.80± 0.26	5.10± 0.11	5.20± 0.24	4.40± 0.10

(HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)(LCH: Light Carniolian hybrid; LIH: Light Italian hybrid).

Table 2. Total protein (KDa) in virgin bee queen's haemolymph *A. m. carnica* and *A. m. ligustica*

Bee hybrids / Seasons	2020				2021			
	HCH	LCH	HIH	LIH	HCH	LCH	HIH	LIH
Late winter	5.63± 0.41	5.63± 0.43	5.56± 0.41	6.00± 0.50	5.54± 0.42	4.38± 0.38	5.23± 0.41	5.11± 0.42
Spring	6.88± 0.52	5.94± 0.51	7.81± 0.62	6.13± 0.52	5.63± 0.50	4.69± 0.43	5.63± 0.44	5.63± 0.45
Summer	9.38± 0.87	6.56± 0.52	8.75± 0.74	7.50± 0.61	6.25± 0.56	4.81± 0.49	5.63± 0.45	5.31± 0.39

(HCH: Heavy Carniolian hybrid; HIH: Heavy Italian hybrid)(LCH: Light Carniolian hybrid; LIH: Light Italian hybrid).

91.4% from April to July but were lower in August (60.0%) and September (58.3%). In contrast to the origin of the queens, the raising season certainly had an impact on mating success. For both honey bee hybrids, the highest meaningful percentages of mating success were observed between March and June. The lowest rates of successful mating occurred in July and August, despite 80% of queens being accepted. The air temperature and the availability of nectar and pollen throughout the spring months appeared to be the main environmental factors impacting the success of mating (Szabo et al., 1987). Most queen losses occur during mating flight (Ruttner, 1983 and Czekoska, 2000) due to bad weather, particularly wind (da Silva et al., 1995), predators, or the queens becoming lost (Hellmich et al., 1986). This explains why queen mortality is so high in the summer and in the late winter. Compared to *A. m. ligustica* queens, *A. m. carnica* queens had a substantially greater spermatheca volume (mm^3). Our findings concur with those made public by (Kaftanolu and Kumova, 1992), who discovered that among the ecotypes in Turkey, Caucasian queens had the largest spermatheca. In comparison to other months of our study period, queens reared in May have noticeably larger spermatheca. After July, the worker bees destroyed the majority of the drones due to a lack of pollen and nectar. Due to this circumstance, the amount of sperm in the spermatheca may have fluctuated throughout the course of the year, resulting in variations in spermatheca size.

The amount of sperm in the spermatheca increased in *A. m. carnica* queens due to the bigger spermatheca size. The average sperm count of *A. m. carnica* queens was substantially higher than that of *A. m. ligustica* queens. For *A. m. carnica* and *A. m. ligustica*, the total number of sperm per queen peaked in May and fell to a minimum in September. Throughout June to September, there may have been a decrease in the amount of nectar and pollen flowing in from the field along with a rise in daily temperature. According to research in different fields (Woyke and Jasinki, 1973; Kaftanolu and Peng, 1982; Koeniger and Ruttner, 1989; Kaftanolu and Kumova, 1992), the average quantity of spermatozoa was discovered to be less than the quantities reaching the spermatheca.

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AUTHOR CONTRIBUTION STATEMENT

AG, AE and YE conceived the presented idea. YE and AG developed the theory and performed the computations. YE verified the analytical methods. YE and AE analyzed data. All authors discussed the results and contributed to the final manuscript. YE and AG carried out the experiments. YE and AG wrote the draft manuscript. All authors contributed to the final version, read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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