Saudi Journal of Biological Sciences 26 (2019) 1581–1586

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Reproductive biology and morphology of *Apis mellifera jemenitica* (Apidae) queens and drones

Ramzi Al-Sarhan [a], Nuru Adgaba [a], Yilma Tadesse [a], Yehya Alattal [a], Amal Al-Abbadi [b], Arif Single [a], Ahmad Al-Ghamdi [a,*]

[a] Abdullah Bagshan Chair for Bee Research, Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
[b] Plant Production and Protection Department, Faculty of Agricultural Technology, Al-Balqa Applied University, Jordan

ARTICLE INFO

Article history:
Received 6 September 2018
Revised 10 October 2018
Accepted 15 October 2018
Available online 16 October 2018

Keywords:
Apis mellifera jemenitica
Characterization
Reproductive quality
Ovarioules
Spermathecae
Semen viability

ABSTRACT

The current study aimed to investigate the important reproductive biology and morphology of *A.m. jemenitica* queens and drones through measuring the weight of virgin and mated queens, size and weight of spermathecae, weight of ovaries, number of ovarioles, quantity and viability of semen in queen and drones. Accordingly, the average weights of 0.139 ± 0.01 g and 0.143 ± 0.013 g recorded for virgin and mated queens respectively. The sizes of spermathecae were 1.248 ± 0.103 mm and 1.25 ± 0.022 mm for virgin and mated queens respectively. The mean weight of ovaries was 0.013 ± 0.003 g and the numbers of ovarioles varied from 124 to 163 with the mean of 142.9 ± 9.47 and with no significant difference between virgin and mated queens. The average number of stored sperm per spermathecae of mated queen was estimated to be 4.202 ± 0.613 million with the viability of 80.39%. The average number of sperm per drone recorded was 8,763,950 ± 1,633,203.15 with viability of 79.54 ± 6.70%. In general, the current study revealed that the values recorded for reproductive biology and morphological characters of *A. m. jemenitica* queens and drones were relatively lower than values recorded for other *Apis mellifera* races. This mainly could be associated with the body size of the race which is known to be the smallest race among *A. mellifera* races. Moreover, the harsh environmental conditions of the regions, high temperature, low humidity and limited resources may have contributed for the smaller biological and morphological values. The information will serve as a base in future selection and breeding of program of the race.

1. Introduction

Honey bee queen is important because, many of desirable traits of a colony such as productivity, gentleness and disease resistance are governed by the nature of the queen (Laidlaw, 1979; Morse, 1979; Ruttner, 1983; Ratnieks and Nowogrodzki, 1988). As a result, honey bee queen rearing is one of the important parts of beekeeping to re-queen colonies, to enhance brood and honey production, and to improve their genetic characteristics (Morse, 1979; 1994; Carne, 1990; Laidlaw and Page, 1997), which indicate the importance of rearing of queens.

Not only the numbers of reared queens are important but also the qualities of reared queens are very necessary. Hence, it is becoming essential to evaluate the reproductive biology and morphological characteristics of queens. The productivity of a honeybee queen depends on some of its characters such as: weight at emergency, number of ovarioles and size of spermathecae (Woyke, 1976; Wen-Cheng and Chong-Yuan, 1985; Kaftanoglu and Peng, 1980; Nelson, 1989). Moreover, weight of virgin queen and diameter of spermatheca were considered as important quality parameter of a queen and also noted the presence of significant correlations between the two variables (Kahya et al., 2008).

Since the mating frequency of honey bee queens is highly variable (up to 21 drones during its nuptial flight) (Estoup et al., 1994), it can store variable sperm volumes to be used randomly in egg fertilization (Haberl and Tautz, 1998; Cobey, 2007). In this regard,

* Corresponding author at: Bee Research Chair, Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, PO Box 2460, Riyadh 11451, Saudi Arabia.
E-mail address: alkhazim@ksu.edu.sa (A. Al-Ghamdi).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2018.10.012
1319-562X/

mating quality in terms of stored sperm counts suggested as an important gauge for evaluating reproductive potential or quality of a queen (Delaney et al., 2011). Moreover, Guler and Alpay (2005) considered the volume of spermatheca and number of spermatozoa per spermatheca as important reproductive quality parameters and they found significant positive correlations between the two characters.

Several authors (Fischer and Maul, 1991; Dedej et al., 1998; Hatch et al., 1999; Gilley et al., 2003; Dodologlu et al., 2004; Kahya et al., 2008) also indicated that morphological characters such as wet or dry weight, thorax width, head width, and wing lengths are standard morphological measures and are significantly correlated with queen reproductive success or fecundity (Woyke, 1971; Nelson and Gary, 1983). Generally, high live weight, high number of ovarioles, large size of spermatheca and high number of spermatozoa in spermathecae have been considered as important quality parameters of a queen (Hatjina et al., 2014). Recently, body weight, number of ovarioles, size of spermatheca, and number of spermatozoa have been considered as standardized queen quality parameters (Carreck et al., 2013; Human et al., 2013).

Honey bee queen performance is reported to depend not only on quantity but also quality of sperm and this also known to be linked to colony performance (Pettis et al, 2016). Pettis et al, (2016) reported the presence of high variations in average viability of stored sperm 92% and 55% for good and failing colonies, respectively. It is well known that the performance of a honey bee colony is not only depends on the quality of the queen but also on the quality of drones that would mate with the queen (Hatjina et al., 2014). So, it is becoming essential to analyze the quality of drone and its semen. Many authors showed the presence of links between physical characteristics of queens and the performance of colonies such as brood rearing, honey production, colony weight and survival rate of the queens (Harbo and Szabo, 1984; Cobey, 1998; Collins, 2000; Pritsch and Bienefeld, 2002; Rhodes et al., 2004; Skowronek et al., 2004).

Study on reproductive and morphometric characteristics of *A. m. jemenitica* queen is very limited except that of Alqarni et al. (2013), who studied the *A. m. jemenitica* virgin queen characters in comparison to *A. m. carnica.* However, this particular study was aimed at characterizing *A. m. jemenitica's* queens and drones related to their reproductive and morphological parameters. Hence in this study, we considered both the virgin and mated queens' anatomical and physiological parameters such as: fresh weight of virgin and mated queens, size & weight of spermathecae, number of ovarioles; numbers and viability of stored semen in spermathecae. Moreover, we assessed the size and weight of drones and numbers and viability of sperms in matured drones.

2. Materials and methods

For this study, the native *A. m. jemenitica* colonies in their natural homeland (Arabian Peninsula) with no introgression with other races were taken.

2.1. Reproductive biology and morphological characteristics of virgin queens

For the determination of morphological characteristics of virgin queen; first, several queens were reared under queen less colony conditions following the standard queen rearing techniques (Büchler et al., 2013). From the reared queens, sixty virgin queens were randomly taken and some important morphological characteristics such as: fresh weight, size of right forewing, dimension of thorax, length of abdomen, width of the third abdominal segment tergite, number of ovarioles and size of spermatheca were measured following Carreck et al. (2013); Cobey et al. (2013) and Human et al. (2013) protocols.

In body weight determination, the selected queens were anesthetized with CO_2 and when the queens become immobilized their fresh weight was measured with sensitive electronic balance (Kern ABS, Kern & Sohn GmbH, Germany) with a precision of 0.0001 mg sensitivity. In determining the length and width of the right forewing; and the length, width and height of the thorax; width of the third abdominal segment tergite and length of the abdomen at a relaxed position, a digital caliper (Vogel vernier caliper, Vogel, Germany) with accuracy of 0.01 mm was used following the protocols of Rangel et al. (2013).

Moreover, to determine the size of spermatheca and numbers of ovarioles, 60 sampled virgin queens were dissected following Rhodes and Somerville (2003) and Cobey et al. (2013) protocols. Then, the spermathecae and ovarioles were carefully removed and kept in saline buffer solution. After that, the diameter of spermatheca was measured using dissecting microscope with ocular micrometer. And the numbers of ovarioles were determined by carefully counting each ovarioles under dissecting microscope.

2.2. Reproductive biology and morphological characteristics of mated queens

2.2.1. Introduction of virgin queens in to mating nuclei colonies for natural mating

To determine the characteristics of mated queens, sixty caged virgin queens were introduced in to mating nuclei colonies for mating purpose as described by Cobey et al. (2013). The mating nuclei colonies were placed near to the drone source colonies. For this purpose, eight drone rearing colonies were prepared and strengthened well and finally induced to rear drones about one month prior to the scheduled queen mating time according to Tiesler and Englert (1989). Mating yard was located in undulated area where enough pollen source plants exist. In addition, sufficient supplementary sugar was fed continuously in a candy form. To prevent any genetic introgression from other honeybee lineages or colonies, absence of nearby apiaries was insured for at least 2 km distance (Hopkins, 2011).

2.2.2. Investigation of the reproductive biology of mated queens

For mated queens' reproductive biology parameters determination, out of 60 introduced virgin queens; a total of 30 successfully mated queens were used. Before measuring the parameters, the mating success of the queens was verified based on the presence of different stages of normal brood in the nuclei colonies led by the respective queens.

2.2.3. Queen weight

For weight determination 30 mated queens were anesthetized with CO_2 and weighed (mg) using an analytical balance with precision of (0.0001 mg) (Kern ABS, Kern & Sohn GmbH, Germany). The same queens were used for investigation of other parameters.

2.2.4. Number of ovarioles

For the determination of ovarioles numbers; 30 mated queens were sacrificed and dissected immediately after killing using ethanol. The dissected queens were also used to study the weight of ovary and number of ovarioles, size of spermathecae, and number and viability of stored semen. Dissection was done according to Buchler *et al.* (2013) protocols. To detach the paired oviducts and ovaries, first, the abdominal sternum was removed up to the second segment. Then, the spermathecae and other glands become visible and the spermathecae were easily removed and transferred in to a saline solution.

2.2.5. Size and weight of spermathecae

First, the spermathecae were weighed immediately after dissection (wet weight) using precision balance (0.0001 mg) (Kern ABS, Kern & Sohn GmbH, Germany) according to Carreck et al. (2013). Then the dimensions of the spermathecae were measured using an eye-piece ocular micrometer (Nikon SMZ1500, Nikon, Japan). "The two cross diameters of spermathecae were measured and averaged to get the diameter of the spermatheca.

2.2.6. Weight of ovaries

The wet weight of the right ovary of the mated queens were weighed immediately after dissection using precision balance (0.0001 mg) (Kern ABS, Kern & Sohn GmbH, Germany) following Carreck et al. (2013) protocol.

2.2.7. Number of ovarioles

The numbers of ovarioles was determined by immediately transferring the ovaries into saline solution. Then, the right ovary was separated and mounted by a drop of Xylene for 4 min on a microscopic slide to make the ovarioles clear and visible (Woyke, 1987; Abdalla, 2001). To make the counting easier and clear, Puri's media was used according to Ibrahim (1977). The Puri's media was composed of 10 ml distilled water, 5 ml glycerin, 3 ml glacial acetic acid, 7 gm chloral hydrate, and 8 gm Arabic gum. We add a drop of this media on the ovary and we left it only for about 4 min to avoid the damage of the tissues by the media, and washed with warm water 3 times. After that, each ovariole become visible and were counted easily by dividing it into small portions under binuclear microscope following Abdalla (2001) protocol.

2.2.8. Stored semen quantity and viability determination

The stored sperm quantity and viability of the mated queens were determined following Cobey et al., 2013 and Human et al., 2013 protocols.

2.3. Drone size and weight:

To determine the average weight and size of *A. m. jemenitica* drones, 30 drones from each of eight drone mother colonies (total: 240) were investigated. The drone weight was simply determined using electronic balance with precision of (0.0001 mg) (Kern ABS, Kern & Sohn GmbH, Germany) after the drone being immobilized using a smooth flush of carbon dioxide. The same sample was used to assess the size of the drone by volumetric procedure (Cobey et al., 2013). First, the drones were immobilized and then placed in 2 ml graduated glass tube and the tube was filled with water. The size of the drone was obtained by subtracting the volume of water displaced by the drone from the total volume of the water.

2.4. Collection and determination of number and viability of drones' semen

Drone semen was collected from 40 days old drones and their age was determined according to Human et al., (2013) procedure. For this, from each drone producing colony 20 drones a total: 160 were tested. After eversion of endophallus, the semen was collected using glass syringe as described by Cobey et al. (2013). Semen was then loaded on hemocytometer on which a cover slip was placed. Capillary action was used to fill each chamber (the space between the cover slip and slide) with solution. Then it was examined under 250x magnification. The counting was started on the gridded section after the sperm have settled (~20 s). Count of sperms was calculated as described in details by Cobey et al. (2013). Finally, the viability of sperms was evaluated using dual fluorescent staining (SYBR-14 with propidium iodide (PI)) as described in Collins and Donoghu (1999).

3. Results

3.1. Reproductive biology and morphological characteristics of virgin queens

The average fresh weight of *A. m. jemenitica* virgin queens (at emergence) was 0.136 ± 0.01 g ($N = 70$) and 0.141 ± 0.01 g ($N = 70$) for queens reared under queen-right and queen-less conditions respectively and the variation between the techniques was significant at $P < 0.05$. The numbers of ovarioles recorded for *A. m. jemenitica* virgin queens were varied from 124 to 163 with the mean of 142.9 ± 9.47 ($N = 60$). Moreover, the average size of spermathecae of virgin queens was 1.248 ± 0.103 mm.

The length of abdomens and the widths of the 3rd abdominal tergite segments of virgin queens were 8.99 ± 0.43 mm and 3.27 ± 0.24 mm respectively. The average dimension of verging queens head capsule was 3.51 ± 0.23 mm which is slightly bigger than values (2.34 ± 0.01 mm) reported for the same race by Alqarni et al. (2013). The length and width of the right forewing obtained in the current study were 9.361 ± 0.41 mm and 3.217 ± 0.20 mm respectively. Some of the important morphometric values of *A.m. jemenitica* virgin queens are shown in Table 1.

3.2. Reproductive biology and morphological characteristics of mated queen

The average weight of mated queens reared during this study was 0.143 ± 0.013 g with range of 0.119–0.169 g. The average size of mated queens spermathecae was 1.25 ± 0.022 mm with range of 1.22–1.28 mm. The mean numbers of stored sperm per spermatheca of mated queen was 4.202 ± 0.613 million with range of 2.63–4.89 million. The average viability of stored sperm of mated queen recorded in this study was 80.39%.

Table 1
Summary of some of the morphometric values of *A. m. jemenitica* virgin queens.

Parameters	N	Mean	STD	Min	Max
Weight (gm)	60	0.141	0.013	0.108	0.167
Width of the head (mm)	60	3.509	0.225	3.11	4.01
Length of right forewing (mm)	60	9.361	0.413	8.38	10.5
Width of right forewing (mm)	60	3.217	0.202	2.67	4.08
Length of abdomen (mm)	60	8.989	0.427	8.51	10.59
Height of thorax (mm)	60	4.226	0.186	3.77	4.97
Length of thorax (mm)	60	4.341	0.218	3.63	4.89
Width of thorax (mm)	60	4.101	0.151	3.72	4.47
Width of the 3rd abdominal tergite segment (mm)	60	3.270	0.237	2.79	3.9

The average weight of the right ovary of mated queens was 0.013 ± 0.003 g with rang of 0.007–0.017 g. The number of mated queen ovarioles was 142.8 ± 5.67 with range of 134–154. Moreover, the average weight of spermatheca was $0.0007 \pm 8E^{-05}$ with range of 0.0006–0.0008.

3.3. Morphometric and reproductive characteristics of drone

The average weight of drones was 0.16 ± 0.02 g while the average volume of drone was 0.26 ± 0.06 ml^3 and the correlation between the two variables was very significant $P < 0.0001$. The average number of sperm per drone recorded in this study was $(8.80 \pm 1.60) \times 10^6$. The average viability of the semen in drone was 79.54 ± 6.70% and there was no significant correlation between number of semen and their viability status.

4. Discussion

4.1. Morphological and reproductive characteristics of virgin queens

The average fresh weight value of virgin queens obtained in this study was closer to value recorded (137.813 ± 7.919 mg) for the same race by Alqarni et al. (2013). However, the average value obtained in the current study was much smaller than the average weight of 165.875 ± 9.791 mg recorded for *A. m. carnica* by the same authors. The mean ovarioles numbers recorded in the current study were relatively smaller than the records of Alqarni et al. (2013) who reported 146.58 ± 13.85 and 156.98 ± 14.89 for the *A. m. jemenitica* and *A. m. carnica* races respectively. The average size of spermathechae was closer to Alqarni et al. (2013) who reported a spermathecae diameter of 1.230 ± 0.055 mm for the same races and also 1.253 ± 0.066 mm for *A. m. carnica* virgin queens. The sizes of right forewings were slightly higher than records of Alqarni et al. (2013) who reported 9.170 ± 0.02 and 3.00 ± 0.02 mm for length and width of right forewing respectively for the same race.

The slight variations observed between the two studies for the same race could be due to the variations in ecotypes of the bees taken from the two study sites. In this regard, the presence of different ecotypes within *A.m. jemenitica* of Arabian Peninsula was well recorded (Al-Ghamdi et al., 2012). In addition, the influence of higher weather temperatures and insufficient nectar and pollen resources on the weight of queens at emergence is well reported (Abdellatif et al., 1970).

Moreover, Alqarni et al. (2013) reported the presence of seasonal variability in live weight of virgin queens of *A. m. jemenitica* and similarly Hatjina et al. (2014) reported seasonal variations in live weight of Bulgarian honey bee (*Apis mellifera macedonica*) queens. Generally, in some of morphometric characters such as: the average live weight of queen at emergence was higher for queens reared under queen less conditions, while for thorax length, width and height of virgin queens reared from queen right colonies were significantly bigger than queens less colonies (Table 2). This indicates, the absence of clear variations in general morphometric characters of queens reared between queen right and queen less colony conditions and it may require further investigations.

4.2. Biological and morphological characteristics mated queen

The average weight of mated queens recorded in the current study was much smaller than the reports for other races: *A.* m. *ligustica* mated queen (0.221 ± 0.031 g) and Slovenia queens (*A. m. carnica*) 0.208 ± 0.015 g (Hatjina et al., 2014). The average size of spermathechae (1.25 ± 0.022 mm) obtained in the current study was closer to the values (1.210 ± 0.024–1.270 ± 0.020 mm) recorded for Bulgarian bee (*A. m. macedonica*) mated queens (Hatjina et al., 2014). In addition, the average weight of ovary recorded in this study was much smaller than the average weight of ovary (0.071 ± 0.011 g) reported for *A. m. carnica* (Hatjina et al., 2014).

The number of stored sperm (4.202 ± 0.613 million) recorded per spermathecae of mated queens was within the range of number of stored sperm (3.30 ± 1.68–4.96 ± 1.14) reported for European evolved bees (Hatjina et al., 2014). Moreover, the current result of stored semen (3.99 ± 1.504 million)/per spermathecal was within the range of stored semen (0.20–9.03 million) reported by Delaney et al. (2011) for naturally mated queens from different commercial queen producers. According to Jay and Dixon (1984) study report, the number of stored sperms in 11% of tested queens was less than three million while in 45–64% were more than five million sperm which is slightly higher than the current findings. The numbers of sperms in spermathecae of naturally mated queens reported to vary depending on different circumstances such as mating period weather conditions (Koeniger et al., 2005), frequency of mating and quality of drones (Haberl and Tautz, 1999; Schlüns et al., 2003).

The stored sperm viability percentage obtained in this study was within the normal range of viability (60–90%) recorded by Pettis et al. (2016) but the current viability result was relatively lower than the viability (97.8%) reported by Gençer and Kayha (2011). Low sperm viability is an important indicative parameter for the low performance of a queen and a colony (Pettis et al., 2016) and they found that in good colonies the average viability was 92% where as in other normal and failing colonies the average viabilities of sperm were 57 and 55% respectively. Similarly, the average numbers of ovarioles recorded in this study was relatively fewer than the average number of ovarioles (173 ± 2.48) reported for *A. m. ligustica* and (160.94 ± 14.97) and for Slovenia bees (*A. m. carnica*), (Hatjina et al., 2014).

Generally, positive correlations were noted among the different reproductive characteristics of the mated queens, however the correlations were not statistically significant (Table 3). The absence of significant correlations between queen weight and ovarioles numbers were also noted by Hatjina et al. (2014). However, significant positive correlations were observed between numbers of sperms in spermathecae with the size of spermathecae and also the weight of ovarioles with the weight of the queens at $P < 0.05$. Similarly,

Table 2
Comparison of some morphometric parameters by queen rearing technique.

Morphometric parameters	Queen rearing techniques		DF	t-value	P-value
	Queenright (Mean ± SD)	Queen-less			
Queen weight at emergence	(0.136 ± 0.01)	(0.141 ± 0.01)	1	2.302	0.023
Width of head	3.35 ± 0.178	3.50 ± 0.223	1	3.613	0.001
Height of thorax	4.33 ± 0.194	4.24 ± 0.193	1	−1.255	0.035
Length of thorax	4.65 ± 0.155	4.37 ± 0.239	1	−3.317	0.002
Width of thorax	4.30 ± 0.227	4.11 ± 0.161	1	−4.270	0.000

Table 3
Correlation between some parameters of naturally mated honeybee queens.

Variable	By variable	Correlation	Count	P-value
Size of spermatica (mm)	Weight (gm)	0.4385	10	0.2049
Weight of spermatica (g)	Weight (gm)	0.4796	10	0.1607
Weight of spermatica (g)	Size of spermatica (mm)	0.3059	10	0.3901
No. of sperms in spermatica (millions)	Weight (gm)	0.2224	10	0.5368
No. of sperms in spermatica (millions)	Size of spermatica (mm)	0.7308	10	0.0164
No. of sperms in spermatica (millions)	Weight of spermatica (g)	0.4525	10	0.1891
Weight of ovarioles (g)	Weight (gm)	0.7630	10	0.0103
Weight of ovarioles (g)	Size of spermatica (mm)	0.3752	10	0.2854
Weight of ovarioles (g)	Weight of spermatica (g)	0.3534	10	0.3165
Weight of ovarioles (g)	No. of sperms in spermatica (millions)	0.2045	10	0.5710
No. of ovarioles	Weight (gm)	0.4606	10	0.1804
No, of ovarioles	Size of spermatica (mm)	0.0872	10	0.8106
No. of ovarioles	Weight of spermatica (g)	−0.4328	10	0.2116
No. of ovarioles	No# of sperms spermatica (millions)	−0.0730	10	0.8411
No. of ovarioles	Weight of ovarioles (g)	0.3132	10	0.3782

Kovačić et al., 2016 reported the presence of positive correlation between the queens' weight and the size of spermathecae. Similarly, the presence of significant positive correlation was reported between the average queen weight and ovary weight (Hatjina et al., 2014).

4.3. Morphometric and reproductive characteristics of drone

The average weight of drones recorded in this study was smaller than the weights of 0.1945 ± 0.035 g and 0.202 ± 0.053 g recorded for Africanized and European honey bee drones, respectively (Rinderer et al., 1985). The mean number of sperm per drone $(8.80 \pm 1.60) \times 10^6$ obtained in the current study was lower than the average number of sperm $(12.01 \pm 0.186) \times 10^6$ recorded for *Apis mellifera caucasica* drones in Turkey (Gencer and Firatli, 2005). In contrary smaller numbers of 4.6 ± 0.9 and 5.7 ± 0.9 millions of sperms were recorded for Africanized and European honey bee drones respectively (Rinderer et al., 1985). The drone semen viability percentage of the current study was within the mean percentage of viability of semen (64.2 ± 1.07% (range 36.79–86.66)) recorded for *A. m. ligustica* in Canada (Rousseau et al., 2015).

5. Conclusion

In general, from the current study it can be concluded that most of the reproductive and morphological values recorded for *A. m. jemenitica* queens and drones are relatively lower than values recorded for other *Apis mellifera* races. These mainly could be associated with the body size of the race which is known as the smallest race among *A. mellifera* races. The smaller body size of the race is considered as an adaptive trait to the prevalence harsh environmental conditions of the region, high temperature, low humidity and limited resources. The information generated in the current study will serve as a bases in future selection and breeding program of the race.

Acknowledgments

This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Project Award number 2-17-04-001-0023.

References

Abdalla, M.A., 2001. Studies on certain factors influencing the honeybee queen rearing Ph.D. Thesis. Faculty of Agriculture, Assiut University, Egypt.

Abdellatif, M.A., El-Gaiar, F.H., Mohana, N.F., 1970. Untersuchungen zur Koniginnenzucht und-paarung. Apiacta 5, 9–10.

Alqarni, A.S., Balhareth, H.M., Owayss, A.A., 2013. Queen morphometric and reproductive characters of *Apis mellifera jemenitica*, a native honey bee to Saudi Arabia. Bull. Insectol. 66, 239–244.

Al-Ghamdi, A., Alsharhi, M., Alattal, Y., Nuru, A., 2012. Morphometric diversity of indigenous Honeybees, *Apis mellifera* (Linnaeus, 1758), in Saudi Arabia (Insecta: Apidae). Zool. Middle East. 57, 97–103.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, F.N., Kryger, P., Spivak, M., Uzunov, A., Wilde, J., 2013. Standard methods for rearing and selection of *Apis mellifera* queens. J. Apicult. Res. 52. https://doi.org/10.3896/IBRA.1.52.1.07 pp. 29.

Carne, E., 1990. Bees and Beekeeping: Science, Practice and World Resources. Cornell University Press, Ithaca, NY, p. xvii+614.

Carreck, N.M., Andree, M., Brent, C.S., Cox-Foster, D., Dade, H.A., Ellis, J.D., Hatjina, F., Englesdorp, D.V., 2013. Standard methods for *Apis mellifera* anatomy and dissection. J. Apicult. Res. 52, 1–40. https://doi.org/10.3896/IBRA.1.52.4.03.

Cobey, S.W., 1998. Comparison of colony performance of instrumentally inseminated and naturally mated honey bee queens. Proceedings of American Bee Research Conference, Colorado.

Cobey, S.W., 2007. Comparison studies of instrumental inseminated and naturally mated honeybee queens and factors affecting their performance. Apidologie 38, 390–410. https://doi.org/10.1051/apido:2007029.

Cobey, S.W., Rarpy, D.R., Woyke, J., 2013. Standard methods for instrumental insemination of *Apis mellifera* queens. J. Apicult. Res. 52. https://doi.org/10.3896/IBRA.1.52.4.09.

Collins, A., 2000. Survival of honey bee (Hymenoptera: Apidae) spermatozoa stored at above-freezing temperatures. J. Econ.Entomol. 93, 568–571.

Collins, A.M., Donoghue, A.M., 1999. Viability assessment of honeybee spermatozoa by the use of dual fluorescent staining. Theriogenology 51, 1513–1523.

Dedej, S., Hartfelder, K., Aumeier, P., Rosenkranz, P., Engels, W., 1998. Caste determination is a sequential process: effect of larval age at grafting on ovariole number, hind leg size and cephalic volatiles in the honey bee (*Apis mellifera carnica*). J. Apic. Res. 37, 183–190.

Delaney, D.A., Keller, J.J., Caren, J.R., Tarpy, D.R., 2011. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). Apidologie 42, 1–13. https://doi.org/10.1051/apido/2010027.

Dodologlu, A., Dulger, C., Gence, F., 2004. Colony condition and bee behaviour in honey bees (*Apis mellifera* L.) Housed in wooden or polystyrene hives and fed bee cake or syrup. J. Apic. Res. 43, 3–8.

Estoup, A., Solignac, M., Cornuet, J.M., 1994. Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. Proc. R. Soc. Lond. B 258, 1–7.

Fischer, F., Maul, V., 1991. Untersuchungen zu aufzuchtbedingten königinnenmerkmalen. (An inquiry into the characteristics of queens depending on queen rearing). Apidologie 22, 444–446.

Gencer, H.V., Firatli, C., 2005. Reproductive and morphological comparisons of drones reared in queen right and laying worker colonies. J. Apic. Res. 44, 163–167.

Gençer, H.V., Kayha, Y., 2011. Are sperm traits of drones (*Apis mellifera* L.) from laying worker colonies noteworthy? J. Apic. Res. 50, 130–137.

Gilley, D.C., Tarpy, D.R., Land, B.B., 2003. The effect of queen quality on the interactions of workers and dueling queen honey bees (*Apis mellifera* L.). Behav. Ecol. Sociobiol. 55, 190–196.

Guler, A., Alpay, p., 2005. Reproductive characteristics of some honeybee (*Apis mellifera* L.) genotypes. J. Anim. Vet. Adv. 4, 864–870.

Haberl, M., Tautz, D., 1998. Sperm usage in honeybees. Behav. Ecol. Sociobiol. 42, 247–255.

Haberl, M., Tautz, D., 1999. Paternity and maternity frequencies in *Apis mellifera sicula*. Insectes Soc. 46, 137–145.

Harbo, J.R., Szabo, T.J., 1984. A comparison of instrumentally inseminated and naturally mated queens. J. Apic. Res. 23, 31–36.

Hatch, S., Tarpy, D.R., Fletcher, D.J.C., 1999. Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insect. Soc. 46, 372–377.

Hatjina, F., Malgorzata, B., Leonidas, C., Robert, C., Cecilia, C., Marica, M.D., Janja, F., Aleš, G., Evgeniya, N.I., Nikola, K., Jan, K., Per, K., Marco, L., Vesna, L., Mica, M., Beata, P., Plamen, P.P., Slađan, R., Maja, I.S.S., Flemming, V., Jerzy, W., 2014. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. J. Apic. Res. 53, 337–363. https://doi.org/10.3896/IBRA.1.53.3.02.

Hopkins, I., 2011. Australasian Bee Manual and Complete Guide to Modern Bee Culture in the Southern Hemisphere. X-Star Publishing Company, USA, p. 270.

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J., Forsgren, E., Fries, I., Hatjina, F., Hu, F., Jaffé, R., Jensen, A., Köhler, A., Magyar, J., Özkýrým, A., Pirk, C., Rose, R., Strauss, U., Tanner, G., Tarpy, D., Steen, J., Vaudo, A., Vejsnæs, F., Wilde, J., Williams, G., Zheng, H., 2013. Miscellaneous standard methods for *Apis mellifera*. J. Apic. Res. 52, 1–56.

Ibrahim, S.M.A., 1977. Studies on Queen Honeybee Rearing. Faculty of Agriculture, Alexandria University, Egypt. M.Sc. Thesis.

Jay, C., Dixon, D., 1984. Infertile and Nosema infected honey bees shipped to western Canada. J. Apic. Res. 23, 40–44.

Kaftanoglu, O., Peng, Y.S., 1980. A washing technique for collection of honeybee semen. J. Apic. Res. 23, 205–211. https://doi.org/10.1080/00218839.2008.11101437.

Kahya, Y., Gençer, H.V., Woyke, J., 2008. Weight at emergence of honey bee (*Apis mellifera* caucasica) queens and its effect on live weights at the pre and post mating periods. J. Apic. Res. and Bee World. 47, 118–125.

Koeniger, N., Koeniger, G., Pechhacker, H., 2005. The nearer the better? Drones (*Apis mellifera*) prefer nearer drone congregation areas. Insectes Soc. 52, 31–35.

Kovačić, M., Puškadija, Z., Ivorurlan, S.I.S., Kranjac, D., 2016. Number of spermatozoa in spermatheca of honey bee (*Apis mellifera carnica*) queens 0–1 year old reared in the conditions of continental Croatia. In: 9th International Scientific/professional Conference, pp. 257–260.

Laidlaw, H.H., 1979. Contemporary Queen Rearing. Dadant and Sons, Hamilton, Illinois.

Laidlaw, H.H., Page, R.E., 1997. Queen Rearing and Bee Breeding. Wicwas Press, New York, USA, p. 224.

Morse, R.A., 1979. Rearing Queen Honeybees. Wicwas Press, U.S.A., pp. 18–116.

Morse, R.A., 1994. Rearing Queen Honeybees. Bd. Wicwas Press, Cheshire, Ct; USA, pp. 64–72.

Nelson, D.L., 1989. Assessment of queen quality in honeybee queens. Can. Beekeeper 14, 207–208.

Nelson, D.L., Gary, N.E., 1983. Honey productivity of honey bee colonies in relation to body weight, attractiveness and fecundity of the queen. J. Apic. Res. 22, 209–213.

Pettis, J.S., Rice, N., Joselow, K., van-Engelsdorp, D., Chaimanee, V., 2016. Correction: colony failure linked to low sperm viability in honey bee (*Apis mellifera*) queens and an exploration of potential causative factors. PLoS One 11 (5), e0155833. https://doi.org/10.1371/journal.pone.0155833.

Pritsch, G., Bienefeld, K., 2002. Comparison of performance of bee colonies with naturally mated and artificially inseminated queens (*A.m. carnica*). Apidologie 33, 513.

Rangel, J., Keller, J.J., Tarpy, D.R., 2013. The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth. Insect. Soc. 60, 65–73.

Ratnieks, F.W., Nowogrodzki, R., 1988. Small Scale Queen Rearing by Beekeepers in the Northeast. Information Bulletin 209, Cornell Cooperative Extension Publication. College of Agriculture and life science Cornell University, p. 12.

Rhodes, J., Somerville, D., 2003. Introduction and Early Performance of Queen Bees. Rural Industries Research and Development Corporation. Publication No. 03/049.

Rhodes, J.W., Somerville, D.C., Harden, S., 2004. Queen honey bee introduction and early survival effects of queen age at introduction. Apidologie 35, 383–388.

Rinderer, T.E., Hellmich, R.L., Danka, R.g., Colins, A.M., 1985. Male reproductive parasitism: a factor in the africanization of european honey-bee population. Science 228, 1119–1121.

Rousseau, A., Fournier, V., Giovenazzo, P., 2015. *Apis mellifera* (Hymenoptera: Apidae) drone sperm quality in relation to age, genetic line, and time of breeding. Can. Entomol. 147, 702–711.

Ruttner, F., 1983. Reliable rearing methods in queen rearing. In: Ruttner, F. (Ed.), Biological Basis and Technical Instruction. Apimondia Publishing House, Bucharest, pp. 179–231.

Schlüns, H., Schlüns, E.A., van-Praagh, J., Moritz, R.F.A., 2003. Sperm numbers in drone honeybees (*Apis mellifera*) depend on body size. Apidologie 34, 577–584.

Skowronek, W., Bienkowska, M., Kruk, C., 2004. Changes in body weight of honeybeé queens during their maturation. J. Apic. Sci. 48, 61–68.

Tiesler, F.K., Englert, E., 1989. Aufzucht, paarung und verwertung von königinnen. Ehrenwirth Verlag, München, Germany.

Wen-Cheng, H., Chong-Yuan, Z., 1985. The relationship between the weight of queen honeybee at various stages and the number of ovarioles, eggs laid and sealed brood produced. Honeybee Sci. 6, 113–116.

Woyke, J., 1971. Correlations between the age at which honey bee brood was grafted, characteristics of the resultant queens, and results of insemination. J. Apic. Res. 10, 45–55.

Woyke, J., 1976. Rearing conditions and number of sperms double grafting. Am. Bee. J. 128, 439–440.

Woyke, J., 1987. Can the number of ovarioles in the ovaries been estimated by external characters of living queens. In: The 31st International Apicultural Congress, 19–25 August 1987, Warsaw, Poland, pp. 152–155.