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

Ramzi Al-Sarhana, Nuru Adgaba, Yilma Tadessea, Yehya Alattala, Amal Al-Abbadi, Arif Single, Ahmad Al-Ghamdi

**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 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,
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 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.

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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 Donogh (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 queen’s 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 range 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 ± 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 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 ± 1.60) × 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 spermathecae was closer to Alqarni et al. (2013) who reported a spermathecan diameter of 1.23 ± 0.055 mm for the same races and also 1.25 ± 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 queen 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 spermathecae (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 Gencer 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% whereas 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 |
|-------------------------|--------------------------|----|---------|---------|
| Queen weight at emergence | (Mean ± SD) | (Mean ± SD) | 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 |
Kovačić et al., 2016 reported the presence of positive correlation between the queens’ weight and the size of spermatica. 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 ± 1.60) × 10^6 obtained in the current study was lower than the average number of sperm (12.01 ± 0.186) × 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 recorded for other *A. m. ligustica* races. The smaller body size of the race *A. m. Ligustica* in Canada (Rousseau et al., 2015).

The 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. jenemcita* 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.

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References

Abdellaat, M.A., El Gaair, F.H., Mohana, N.F., 1970. Untersuchungen zur Königinnenzucht und-paarung. Apicarta 5, 9–10.

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

Al-Ghamdi, A., Alsharihi, 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., Keiz, 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.1.52.4.09.

Carne, E., 1990. Bees and Beekeeping: Science, Practice and World Resources. Cornell University Press, Ithaca, NY, p. xvi+141.

Careck, N.M., Andree, M., Brent, C.S., Cox-Foster, D., Dade, H.A., Ellis, J.D., Hatjina, F., Engelsdorp, 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 instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie 38, 390–410. https://doi.org/10.1515/aid.2007.029.

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

Dedej, S., Hartfelder, K., Aumener, P., Rosenkrantz, P., Engels, W., 1998. Caste determination is a sequential process: effect of larval age at grafting on ovary size, hind leg size and cephalic volatiles in the honey bee (*Apis mellifera carnica*). J. Apicult. 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/apeid/2010027.

Dodosiou, 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.

Gencer, 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 survival of honey bee or syrup. J. Apic. Res. 43, 3–8.

Güler, 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. Apicult. Res. 23, 31–36.

Table 3

| 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) | 0.3045 | 10 | 0.5710 |
| No. of ovarioles | Weight (gm) | 0.4696 | 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 |
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.

Hatina, F., Malgorzata, B., Leonidas, C., Robert, C., Cecilia, C., Marica, M.D., Janja, F., Aleš, G., Evgenyva, N.L., Nikola, K., Jan, K., Per, K., Marco, L., Vesna, L., Mica, M., Beata, P., Plamen, P.P., Sladan, 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, L. 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., Özkyriym, A., Pirk, C., Rose, R., Straus, U., Tanner, G., Tarpy, D., Steen, J., Vauio, A., Vejsnats, 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 New York, USA, p. 224.

Kahya, Y., Gençer, H.V., Woyke, J., 2008. Weight at emergence of honey bee (Apis mellifera carnica) 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 (Hymenoptera: Apidae) drone sperm quality in relation to age, genetic line, and time of mating periods. J. Apic. Res. and Bee World. 47, 118–125.

Koeniger, N., Koeniger, G., Pechhacker, H., 2005. The nearer the better? Drones (Hymenoptera: Apidae) drone sperm quality in relation to age, genetic line, and time of mating periods. J. Apic. Res. and Bee World. 47, 118–125.

Košir, J., Williams, G., Zheng, H., 2013. Miscellaneous standard methods for Apis mellifera (Hymenoptera: Apidae) drone sperm quality in relation to age, genetic line, and time of breeding. Can. Entomol. 147, 702–711.

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

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 honeybee 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.

Welsh, C., 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. 104, 205–211.

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. 104, 205–211.