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Improving Egyptian Honeybee Characteristics by Crossing with Carniolan Drones using Instrumental Insemination (I) Improve Honeybee Queen Characters

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Abstract: *Apis mellifera*, the Western honeybee, is a widespread species that has differentiated into numerous geographical strains or subspecies. These subspecies differ in various characteristics, such as morphology, behavior, and ecology. In the present investigation, different morphometric characteristics of the Carniolan (*Apis mellifera carnica*) and Egyptian (*Apis mellifera lamarckii*) subspecies were compared with their hybrids and backcrosses. The rates of increase in some morphometric characteristics as evidence of hybridization between an Egyptian virgin queen and Carniolan drones being 1.7, 3.2, 0.15, 0.5. 2.4, 0.33 and 1.43 percent for forewing length, width, ovariole number, spermatheca diameter, third tergit length, width, and fourth tergit length. Moreover, the backcross generation showed reductions for all queen characteristics resulting from hybridization between an Egyptian virgin queen and Carniolan drones being 2.35, 4.058.6, 3.1, 0.93,4.2, 1.04, 0.34,and 11.5% for forewing width, ovariole number, spermatheca diameter, third tergit length, third tergit width, fourth tergit length, fourth tergit width and for queen weight.

Keywords: Carniolan subspecies, Egyptian subspecies, Morphometric parameters, Queen honeybee

Honey bees are one of the most important beneficial insects worldwide. Their positive impact can be measured by the value they contribute to the agricultural economy, their ecological role in providing pollination services, and the hive products they produce. The vital characteristics of a beehive with tens of thousands of honeybee workers largely depend on the extension of the queen's health and its reproductive capacity and the number of mated drones (Rangel et al 2013). The actual attributes and mating achievement characterizing the reproductive quality of queens are associated with colony health and productivity. Queen weight is an actual trademark that is basic for assessing the nature of honeybee queens (Tarpy et al 2012). Such variety is impacted by an assortment of variables, including the hereditary foundation, the age at which the hatchling is first raised as a honeybee queen, the season, and the raising circumstances of the colony (Collins and Pettis 2013). However, the local adaptation of *A. mellifera* subspecies and ecotypes within the same subspecies (Büchler et al 2014) shows that there is potential for targeted selection and selective breeding programs. Modification of the genetics of honey bees has been attempted several times, usually geared towards enhancing specific characteristics that promote disease resistance, and a widely used system for queen genetic evaluation. The European study indicated that local genotypes fared better in their local environment (Büchler et al 2014 and Hatjina et al 2014). Russian honey bee breeding programs have been developed by the United States Department of Agriculture laboratory in Baton Rouge, LA (Rinderer et al 2010) and the Minnesota Hygienic line developed by researchers at the University of Minnesota, St. Paul, MN (Ibrahim et al 2007, Spivak et al 2009).

Mating flight requires a lighter body for suitable lift and flight duration; in any case, a heavier weight might decrease her mating success (Hayworth et al 2009). Numerous reports have shown positive correlations between adult queen weight, mating flight number, and overall mating success (Tarpy et al 2011, 2012).Queens who are heavier at emergence tend to initiate oviposition later than queens with lower weight after being artificially inseminated. The weight of the queen has also been shown to correlate with the weight of the ovaries, the size and number of the ovaries, the diameter of the spermatheca, and the number of stored spermatozoa (Tarpy et al 2011, Collins and Pettis 2013). In any case, external measurements of queen size, such as thorax width, head width, and wing length, are likewise correlated with the queen's reproductive organs. For instance, thorax width was discovered to decidedly correspond with stored sperm number; however, no relationship was found between thorax width and ovariole number or ovary weight (Tarpy et al 2011).Therefore, spermatheca size is another valid measure of the physical quality of a queen because a bigger spermatheca size can hold more sperm (Carreck et al 2013). Spermatheca size is likewise affected by rearing conditions and the queen's genetics and conversely corresponds to larval age. This variable has been used by straightforwardly assessing the volume and indirectly assessing the hypothetical greatest number of sperm stored in the spermatheca (Tarpy et al 2011 and Carreck et al 2013). Wing morphometrics can be successfully used to distinguish between *A. m. mellifera, A. m. ligustica* and *A. m. carnica* (Oleksa and Tofilski 2015) and can be successfully applied to Africanized honey bees (Francoy et al 2018). This process could further be automated making the results rapidly available (Santana et al 2014). However, wing morphometric approach could be less reliable in areas of more recent invasion as is the case with southern USA (Francoy et al 2008).

The queen takes 1-5 mating flights during which mates with an average of 12-14 dronesstoring all the sperm she will use during her lifetime(Tarpy and Pettis 2013). After mating, queens undergo behavioral, physiological and transcriptional changes signaling their new role in the hive. The both seminal fluid components (such as proteins) and insemination volume trigger and maintain particular aspects of queen post-mating changes (Ninoet al 2013) which can in turn affect the entire colony (Peso et al 2013). Numerous mating has also been shown to provide benefits, including an expanded number of stored sperm, improved appeal of the queen (Richard et al 2007), upgraded division of work inside the colony , stabilized brood nest temperature (Oldroyd and Fewell 2007), further developed correspondence with laborers (Mattila et al 2008, Carr-Markell et al 2013), diminished occurrence of disease, and further developed colony fitness (Mattila and Seeley 2007), all of which positively influence colony development and survival . The mating number is generally used as a proxy for genetic diversity. The assessed sperm number stored in a queen spermatheca was used to evaluate the queen's reproductive quality (Tarpy et al 2012). Factors influencing results of instrumental insemination: (1) The optimal age for insemination of queens is 5 to 14 days post emergence. Queens inseminated older than 2 weeks tend to store less sperm in their spermathecae. Queens inseminated less than 4 days old have high mortality. (2) The standard semen dosage given to each queen is 8 to 12 μl. An insufficient semen dose can result in premature queen supersedure or premature queen failure. The (3) Post-insemination care of queens influences sperm storage, (a) Active movement of queen, appropriate brood nest temperatures, and attendance by worker bees promote sperm migration into the queen's spermatheca. (b) Queens confined in cages after insemination tend to store less sperm and retain semen in their oviducts (Cobey et al 2013). This work focuses on the important morphological characteristics of the honey bee queens of Egyptian and Carniolan honey bee races and their hybrids.

MATERIAL AND METHODS

The present investigation was carried out during the seasons of 2020 at the Department of Beekeeping, Plant Protection Research Institute, Dokki, Giza, Egypt. There are two regions that were chosen as isolated areas in Egypt according to their special geographical races. The first region was Al-Manzala center, which is in the northern part of the Nile Delta, where the pure Carniolan race is found. The second region was Manfalot Province, Assiut Governorate, where the pure Egyptian race is found. Three colonies from each of the Carniolan strain and Egyptian races were included in this study.

Hybridization between Carniolan and Egyptian races: Hybridization was performed via instrumental insemination at the Department of Beekeeping, Plant Protection Research Institute. The first hybrid of the mentioned pure subspecies was established as follows: Egyptian queen × Carniolan drone. The hybrids from backcrossing between the Carniolan strain and the Egyptian strain were established as follows: (Egyptian queen × Carniolan drone) × Carniolan drone.

Instrumental Insemination Techniques According to Cobey et al 2013

Saline diluent method: Two saline diluent methods are recommended. The simple formula (0.9% NaCl, 0.1% glucose and antibiotic) is for insemination with fresh collected semen used for insemination the same day (Cobey et al 2013). The second formula is recommended when mixing and storing semen, including storage at temperatures above freezing and in liquid nitrogen (Hopkins et al 2012).

Insemination Techniques

Eversion of the endophallus: Semen is collected directly from mature drones, 14 days post-emergence or older. For identification purposes, drones can be collected immediately after emergence (i.e. capturing “fuzzy” drones that are newly enclosed) and stored in cages placed in a bank colony (another honey bee colony that will tend the drones (Büchler et al 2013).

Semen collection: Semen is collected directly from the endophallus of many drones into a syringe and stored in glass capillary tubes. The amount and consistency of semen obtained from each drone varies and depends on skill and experience. Generally, each drone will yield approximately 1 µl of semen. The standard volume of semen to inseminate one queen is ~8 to 12 µl. Maintain sanitary conditions, as drones often defecate during eversion.

Insemination of the queen: Inseminate queens between 5 and 12 days post-emergence. Carbon dioxide is used to anesthetize the queen during the procedure and also stimulates oviposition. Queens can be emerged in a queenless bank or, preferably, in their own colonies .If mating nuclei are used, cage the queen cells or be sure that the hive entrances are covered with queen excluder material to prevent unwanted natural mating flights. Check the spermatheca to determine the degree of insemination success. Sperm migration requires about 40 hours post insemination. After insemination, a subset of queens can be held in a nursery colony until tested.

Maintenance of queens and drones and factors affecting queen performance: The quality of the insemination, in terms of technique and sanitation, are critical. The treatment of queens before and after the insemination will influence the amount of semen stored and queen performance. Natural conditions should be maintained as much as possible.

Maintenance of drones for instrumental insemination: Producing a large number of mature drones from select sources can be more challenging then queen rearing, especially if seasonal conditions are not optimal. Drones have a high rate of attrition and drift heavily among colonies. Free flying drones have better survival and have a prior opportunity to void faeces.

Maintenance of queens before and after instrumental insemination: The pre-and post-insemination treatment of queens will influence their performance. Maintain queens with a high proportion of nurse bees in well-fed nursery and/or nucleus colonies (Büchler et al 2013). Direct release of queens into colonies after insemination enhances sperm migration.

Statistical analysis: Data of all treatments were analyzed in a randomized complete block design analysis of variance. All means were compared using Duncan's multiple range tests at level of 0.05.

RESULTS AND DISCUSSION

Measurement of various parameters: Various anatomical measurements of honeybee queens are important for identifying bee subspecies for breeding. In the present investigation, the morphometric parameters of different vital organs were compared between the Carniolan and Egyptian subspecies and their hybrids and the backcross. The morphometric parameters of honeybee queens that were analyzed were length and width of the forewing, number of the ovarioles, diameter of the spermatheca, length and width of the third tergit, length and width of the fourth tergit, and the weight of the queen.

Honeybee queen characteristics: There were no significant differences in the mean length of forewing of honeybee queens for Egyptian and Carniolan subspecies and their F_1 hybrids and backcross being 8.98, 9.35, 9.13, and 8.92 mm, respectively (Table 1). Regarding the mean width of forewing, there were no significant differences among the Egyptian and Carniolan races and their F_1 hybrids and backcross at 2.98, 3.15, 3.08, and 2.96 mm, respectively. The number of ovarioles of honeybee queens showed a significant difference among the Egyptian and Carniolan subspecies, their F_1 hybrids, and the backcross being 130.77, 168.47, 131.07, and 120.65, respectively. Moreover, for the diameter of the spermatheca of honeybee queens, there were no significant differences among the Egyptian and Carniolan subspecies, their F_1hybrids, and the backcross at 1.005, 1.04, 1.01, and 0.98 mm, respectively. For the length of the third tergit of honeybee queens, there were no significant differences among the Egyptian and Carniolan subspecies, their F_1 hybrids, and the backcross at 8.40, 8.91, 8.60, and 8.52 mm, respectively. Furthermore, for the width of the third tergit, there were no significant differences among the Egyptian and Carniolan strains, their F_1 hybrids, and the backcross at 2.89, 3.05, 2.99, and 2.87 mm, respectively. However, the statistical analysis showed a significant difference in the length of the fourth tergit among the Egyptian and Carniolan subspecies, their F_1 hybrids, and the backcross at 9.62, 10.13, 9.76, and 9.65 mm, respectively. In addition, for the width of the fourth tergit, there was a significant difference among the means for Egyptian and Carniolan races, their F_1 hybrids, and the backcross at 2.89, 3.36, 2.92, and 2.91 mm, respectively. Regarding the weight of honeybee queens, there were significant differences among the Egyptian and Carniolan races, their F_1 hybrids, and the backcross at 0.143, 0.173, 0.165, and 0.148 mg, respectively.

Increasing percentage of honeybee queen characteristics: There was increase in characteristics as evidence of hybridization between Egyptian virgin queens and Carniolan drones including 1.7, 3.2, 0.15, 0.5, 2.4, 0.33, 1.43, 1.03 and 13.03 percent for forewing length, forewing width, ovariole number, spermatheca diameter, third tergit length, third tergit width, fourth tergit length, fourth tergit width and queen weight (Table 1). The backcross generation showed reductions for all queen characteristics because of hybridization between Egyptian virgin queens and Carniolan drones: 2.35, 4.05, 8.6, 3.1, 0.93, 4.2, 1.04, 0.34 and 11.5 percent for forewing length, forewing width, ovariole number, spermatheca diameter, third tergit length, third tergit width, fourth tergit length, width and queen weight. Searching to improve honey bee queen reproductive quality is one of the main goals of apicultural research. The queens reared naturally or artificially are critical component to colony fitness and productivity. Thus, maximizing their reproductive potential is of fundamental importance to both colonies and beekeepers (Amiri et al 2017). The body weight of the queen is impacted by the age at which the young larva is grafted, genetic factors, and generally external rearing conditions. Carr-Markell et al (2013) showed that queens reared from 1-day-old hatchings had an average of 154 ovarioles and queens reared from 2-day-old hatchings had 146 ovarioles, and those from 3-day-old hatchings had 136 ovarioles, while high-quality queens ought to have 150 ovarioles. The technique overcomes the problems resulting from lack of rigorous mating control under natural conditions. The queen is typically inseminated with semen from 8 to 12 drones (Cobey et al 2013).

Large volume sperm mixing may set the stage to implement novel breeding strategies. It allows for homogenous mixing of 3000 µL sperm (approx. 7000 drones), sufficient to inseminate up to 250 honeybee queens (Van Praagh et al 2014). The technique may set the stage to establish custom design colonies not just for honey production but also for other colony phenotypes of apicultural interest. Migration of sperm from the oviducts into the spermatheca is a complex process involving contraction of muscles mediated by the specialized composition of fluids in the semen and the oviduct as well as active sperm movement. Queens are very active after natural mating which also promotes sperm migration; therefore, use a direct queen introduction release method (Büchler et al 2013). The after effects of this investigation should assist queen breeders to work on improving rearing technologies and queen mating conditions, which are fundamental for affirming queens for commercial sale. The present study will add to a better understanding of how morphological measurements can give a norm by which to rear higher-quality queens with more prominent mating success and improved productive fitness.

The selection and improvement program could be developed in the native strain population. Oldroyd and Fewell (2007) suggested that this selective breeding should be able to produce a suitable genotype. Insemination is one measure of queen mating success, but arising data shows that mating variety is also significant for queen and colony productivity. The genetic variety inside a colony is an immediate expression of the number of drones and worker offspring (Tarpy et al 2004). Numerous experimental investigations have shown that genetically diverse colonies improve the function of laborers and decrease the degree of pathogens and parasites in the brood. The aims of queen breeding are both to preserve native wild-type strains and maintain desirable honeybee traits, such as honey production, population brood viability, brood quality and quantity, swarming tendency, disease resistance, pollen production, morphometric measurements and genetic traits (Eliza et al 2010). Al-Abbadi (2005) reported that the length and width of the forewings of new Carniolan hybrid queens were 10.15 and 3.18 mm, respectively. Furthermore, Kamel et al (2013) found that the forewing length and width, hindwing length and width, and head capsule length and width were 9.7, 3.05,

Table 1. Morphometric characteristics (mm) of *Apis mellifera lamarckii* and *Apis mellifera carnica* queens, F_1 hybrids, and backcross

Character	Subspecies		F_1 hybrid	Backcross hybrid	Significance	Increasing percentage (F1)	Reduction percentage (backcross)
	Egyptian	Carnica					
Forewing length	8.98±0.03	9.35±0.14	9.13±0.06	8.92±0.20	NS	1.7%	2.35 %
Forewing width	2.98±0.03	3.15±0.07	3.08±0.03	2.96±0.05	NS	3.2%	4.05%
Number of ovariole	130.77±2.5	168.47±5.42	131.07±2.36	120.65±5.17	*	0.15%	8.6%
Diameter of spermatheca	1.005±0.005	1.041±0.10	1.010±0.01	0.98±0.07	NS	0.5%	3.1%
3rd tergite length	8.40±0.19	8.91±0.23	8.60±0.14	8.52±0.14	NS	2.4%	0.93%
3rd tergite width	2.98±0.015	3.05±0.15	2.99 ±0.06	2.87±0.14	NS	0.33%	4.2%
4th tergite length	9.62±0.19	10.13±0.21	9.76±0.14	9.65±014	*	1.43%	1.04%
4th tergite width	2.89±0.27	3.36±0.27	2.92±0.58	2.91±0.58	*	1.03%	0.34%
Weight of queen	0.143±0.007	0.173±0.006	0.165±0.005	0.148±0.08	*	13.03%	11.5%

Values are the mean ± S.D.; *P<0.05 between subspecies, hybrid(F1) and backcross; NS P>0.05 between subspecies, hybrid(F1) and backcross

6.97, 2.03, 4.01, and 3.81, respectively. Taha and Alqarni (2013) observed that the mean body weight and forewing length and width of newly emerged Carniolan queens were 165.87 mg, 9.37 mm, and 3.08 mm, respectively. Shaheen (2018) indicated that the *Apis mellifera carnica* honeybee queens have a mean fresh body weight ranging from 137 to 177 mg. *A. mellifera carnica* honeybee virgin queens have a mean ovariole number ranging from 238.3 to 318.7, and the mean forewing length ranged from 9.40 to 9.71 mm, while the forewing width ranged from 3.10 to 3.27 mm. Taha and Alqarni (2013) found that the ovariole number in the right ovary of *A. mellifera carnica* queens was 156.89. Hasanat (2018) observed that the mean body weight of all queens collected from different districts was 160.0 mg, mean thorax width was 4.28 mm, mean queen spermatheca radius was 0.48.0 mm and mean spermatheca area was 0.75 mm. However, there was an attempt to prove that there is a very strong correlation of the queen size with the reproductive potentiality of the queens where larger queens have larger spermathecae, store greater number of sperm and full spermathecae are heavier than the smaller one (Collins and Pettis, 2013). The average weight of queen is 160.75 mg which may vary (Tarpy et al 2011) .Theaverage weight of queens was 206.6 mg in United States. Gonzalez et al (2017) reported their naturally mated queens' fresh body weight as 199.01 mg and the thorax width as 4.55 mm, in California. Delaney et al (2010), in another experiment in United States, reported an average naturally mated queen wet weight as 184.8 mg and an average thorax width as 4.35 mm, But, the queens collected from different districts of Bangladesh produced an average thorax width of 4.28 mm. Hasanat (2018) likely other researchers, the study also revealed very strong significant positive correlations between body weights, body lengths with spermatheca radius, spermatheca area of the queens.Also found that significantly correlated with strong queens and heavier spermatheca with good storage capacity of sperms. So, bigger queens' progenies should be bigger than the usual.

CONCLUSIONS

The characteristics of the Egyptian honeybee queen were improved because of hybridization between an Egyptian virgin queen and Carniolan drones (F1), while the backcross generation showed a reduced percentage for all queen characteristics.

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REFERENCES

AL-Abbadi AA 2005. *Honeybee queen rearing methods and their relation to morphological and physiological characteristics and queen productivity*. Ph.D. thesis, Faculty of Agriculture, Alexandria University, Egypt.

Amiri E, Strand MK, Rueppell O and Tarpy DR 2017. Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. *Insects* **8**(2): 22-26.

Büchler R, Andonov S, Bienefeld K, Costa C, Hatjina F, Kezic N, Kryger P, Spivak M, Uzunov A and Wilde J 2013. Standard methods for rearing and selection of *Apis mellifera* queens. *Journal of Apicultural Research* **52**(1): 1-30.

Büchler R, Costa C, Hatjina F, Andonov S, Meixner MD, Le Conte Y, Uzunov A, Berg S, Bienkowska M and Bouga M 2014. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. *Journal of Apicultural Research* **53**: 205-214.

Carreck NL , And M, Brent CS, Cox-Foster D, Dade HA , Ellis J D, Hatiina F and Van-Englesdor P 2013. Standard methods for *Apis mellifera* anatomy and dissection. *Journal of Apicultural Research* **52**: 1-40.

Carr-markell MK, Mcdonald KM and Mattila HR 2013. Intracolonial genetic diversity increases chemical signaling by waggle-dancing honey bees, *Apis mellifera*. *Insectes Sociaux* **60**(4): 485-496.

Cobey Susan W, David R Tarpy and Jerzy Woyke 2013. Standard methods for instrumental insemination of Apismellifera queens. *Journal of Apicultural Research* **52**(4): 1-18.

Collins AM and Pettis JS 2013. Correlation of queen size and spermatheca contents and effects of minced exposure during development. *Apidologie* **44**: 351-356.

Eliza C, Adrian S, Agripina S, Maria M, Aurelia C and Dumitru C 2010. Beekeeping research and development institute. Bucharest.

Delaney DA, Keller JJ, Caren JR and Tarpy DR 2011. The physical, insemination and reproductive quality of honey bee queens (*Apis Mellifera* L.). *Apidologie* **42:** 1-13.

Francoy TM, Wittmann D, Drauschke M , Muller S, Steinhage V, Bezerra-Laure MA, De Jong D and Gonczalves LS 2008. Identification of Africanized honey bees through wing morphometrics: Two fast and efficient procedures. *Apidologie* **39**: 488-494.

Gonzalez AN, Ing N and Rangel J 2017. *Upgradation of antioxidant genes in the spermatheca of honey bee (Apis mellifera) queens after mating*. Apidologie, cINRA, DIB and Springer-Verlag France SAS.

Hasanat MA 2018. *Reproductive Potential Difference of Artificially Inseminated and Naturally Mated Honey Bee Queens* (*Apis mellifera* L.). Thesis for: Master of Biology, Department of Entomology Faculty of Agriculture Sher-e-Bangla Agricultural University.

Hatjina F, Costa C , Büchler R, Uzunov A, Drazic M, Filipi J, Charistos L, Ruottinen L, Andonov S and Meixner MD 2014. Population dynamics of European honey bee genotypes under different environmental conditions. *Journal of Apicultural Research* **53**: 233-247.

Hayworth MK, Johnson NG, Wilhelm ME, Gove RP, Metheny J and Rueppell O 2009. Added weights lead to reduced flight behavior and mating success in polyandrous honey bee queens (*Apis mellifera*). *Ethology* **115**: 698-706.

Hopkins BK, Herr C and Sheppard WS 2012. Sequential generations of honey bee (*Apis mellifera*) queens produced using cryopreserved semen. *Reproduction, Fertility and Development* **24**(8): 1079-1083.

Kamel SM, Osman MA, Mahmoud MF, Mohamed KM and Abd Allah SM 2013. Morphometric study of newly emerged unmated queens of honey bee *Apis mellifera lamarckii* in Ismailia Governorate, Egypt. *Arthropods* **2**: 80-88.

Ibrahim A, Reuter GS and Spivak M 2007. Field trial of honey bee colonies bred for mechanisms of resistance against *Varroa destructor*. *Apidologie* **38** :67-76.

Mattila HR and Seeley TD 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317:** 362-364.

Mattila HR, Burke KM and Seeley TD 2008. Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers. *Proceedings of the Royal Society B: Biological Sciences* **275**: 809-816.

Nino EL, Tarpy DR and Grozinger CM 2013. Differential effects of insemination volume and substance on reproductive changes in honey bee queens (*Apis mellifera* L.). *Insect Molecular Biology* **22**: 233-244.

Oldroyd BP and Fewell J H 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology & Evolution* **22**: 408-413.

Oleksa A and Tofilski A 2015. Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. *Apidologie* **46**: 49-60.

Peso M, Nino EL, Grozinger C and Barron A 2013. Effect of honey bee queen mating condition on worker ovary activation. *Insectes Sociaux* **60**: 123-133.

Rangel J, Keller JJ and Tarpy DR 2013. The effects of honey bee (*Apis mellifera* lamarckii) queen reproductive potential on colony growth. *Insectes Sociaux* **60**: 65-73.

Richard FJ, Tarpy PR and Grozinger CM 2007. Effects of insemination quantity on honey bee queen physiology. *PLOS ONE* **2**: 79-80.

Rinderer TE, Harris JW, Hunt GJ and De Guzman LI 2010. Breeding for resistance to Varroa destructor in North America. *Apidologie* **41**: 409-424.

Shaheen AA 2018. *Studies on improvement of local Carniolan honeybee colonies in Manazla region*. Ph.D. Department of Plant Protection Cairo: Faculty of Agriculture, Al-Azhar University.

Santana FS, Costa AHR, Truzzi FS, Silva FL, Santos SL, Francoy TM and Saraiva AM 2014. A reference process for automating bee species identification based on wing images and digital image processing. *Ecological Informatics* **24**: 248-260.

Spivak M, Reuter GS, Lee K and Ranum B 2009. The future of the MN hygienic stock of bees is in good hands. *American Bee Journal* **149**: 965-967.

Taha EA and Alqarni AS 2013. Morphometric and reproductive organs characters of *Apis mellifera jemenitica* Drones in comparison to *Apis mellifera carnica*. *International Journal of Scientific & Engineering Research* **4**(10): 411-414.

Tarpy DR and Pettis JS 2013. Genetic diversity affects colony survivorship in commercial honey bee colonies. *Naturwissenschaften* **100**(8): 723-728.

Tarpy DR, Keller JJ, Caren JR and Delaney DA 2011. Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens. *Insectes Sociaux* **58**: 569-574.

Tarpy DR, Keller JJ, Caren JR and Delaney DA 2012. Assessing the mating 'health' of commercial honey bee queens. *Journal of Economic Entomology* **105**: 20-25.

Tarpy DR, Nielsen R and Nielsen DI 2004. A scientific note on the revised estimates of effective paternity frequency in *Apis*. *Insectes Sociaux* **51**: 203-204.

Van Praagh JP, Brauße J and Titera D 2014. Mixing and storing large volumes of honeybee (*Apis mellifera* L.) sperm integrated in a breeding program. *Proceedings of the Netherlands Entomological Society Meeting* **25**: 39-45.

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