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Queen morphometric and reproductive characters of *Apis mellifera jemenitica*, a native honey bee to Saudi Arabia

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Abstract

Ten traits of morphological characters and reproductive organs of newly emerged virgin queens of native and imported honey bee races, *Apis mellifera jemenitica* Ruttner (AMJ) and *Apis mellifera carnica* Pollman (AMC), were measured. The results for the comparison between the AMC and AMJ queens showed significant differences in most cases. The virgin AMC queens showed significantly increased body weight (165.9 ± 9.8 mg) over that of AMJ (137.8 ± 7.9 mg) in the two seasons of the study. In addition, the AMC queens had significantly higher values than those of AMJ for all tested morphological traits, including the head capsule (2.61 × 2.44 mm versus 2.45 × 2.23 mm), right mandible (1.29 × 0.38 mm versus 1.18 × 0.37 mm), forewing (9.39 × 3.10 mm versus 9.17 × 3.00 mm), and the length of the 3rd + 4th abdominal tergites (5.95 mm versus 5.57 mm), respectively. The number of ovarioles of the right ovary in the AMC queens was significantly higher (157.0 ± 14.9) than that (146.6 ± 13.9) of AMJ. The diameters of the spermathecae were 1.253 and 1.230 for AMC and AMJ, respectively, with significant differences between the two races in the 2nd season of the study.

Key words: *Apis mellifera jemenitica*, *Apis mellifera carnica*, morphometrics, reproductive organs, Saudi Arabia.

Introduction

Beekeeping has been practiced throughout the Arabian Peninsula as early as 2000 BC (Crane, 1983). Only the native race *Apis mellifera jemenitica* Ruttner is able to survive in the center of Saudi Arabia, with the highest national summer temperature, whereas other subspecies fail to persist successfully (Alqarni, 2006; Alqarni *et al*., 2011). *A. m. jemenitica* is found in the zone of lowest and most irregular rainfall in addition to the area of highest seasonal temperature (Ruttner, 1976; Radloff and Hepburn, 1997; Amssalu *et al.*, 2004; Shaibi *et al.*, 2009). It is commonly found in the Arabian Peninsula, including Saudi Arabia and other Arab countries such as Yemen and Oman (Ruttner, 1976; Fletcher, 1978; Radloff and Hepburn, 1997; Amsalu *et al.,* 2004; Shaibi *et al*., 2009; Ali, 2007; 2011; Alqarni *et al*., 2011; Alghamdi *et al*., 2012).

There are reports that this native Saudi honey bee has some significant morphological, biological, and genetic differences in comparison to its conspecifics and some other populations of this same race in Africa (Alqarni, 1995; 2006; 2010; Alqarni *et al*., 2011; AlGhamdi, 2002; 2005; 2006; Alsharhi, 2013). However, most of the parameters reported to date have been compared using worker populations.

It is well known that colony productivity depends mainly on the queen characters regarding genetic factors, environmental conditions, and beekeeping practices (Hoopingarner and Farrar, 1959; Merozov *et al.*, 1971; Severson and Erickson, 1989). Matilla and Seeley (2007) reported that productivity (swarm production, foraging rates, food storage, and population growth) of genetically diverse colonies was greater than that of genetically uniform colonies. Sammataro and Weiss (2013) stated that beeswax production was significantly increased in colonies fed with sucrose syrup compared to those fed with high fructose corn syrup.

Morphometric and reproductive organ traits of honey bee queens are affected by many factors, including bee race (Komarov and Alpatov, 1936; Moukayess, 1979; Casagrande-Jaloretto *et al.*, 1984), rearing season (Avetisyan *et al.*, 1967; Mirza *et al.*, 1967; Pain *et al.*, 1974; Shawer, 1980), age of grafted larvae (Vagt, 1955; Rawash *et al*., 1983; Woyke, 1987), and food availability (Cale, 1963). Consequently, the quality of the queens produced is associated with these factors (Laidlaw, 1992; Kaftanoglu *et al*., 2000).

Although workers of *A. m. jemenitica* morphometrics have been described (Ruttner, 1976; Alqarni, 1995; Alqarni *et al*., 2011; Alghamdi *et al.*, 2012), no reports have been found with regard to queen morphometrics and histology for this race, which is a crucial point to be clarified. The morphometric and reproductive characters of queens of *Apis mellifera carnica* Pollman and *A. m. jemenitica* are presumed to be different. Some authors consider morphometric traits as quality indicators of the reproductive organs of queens, e.g. the weight of the virgin queen was positively correlated with the volume of the spermathecae (Eid *et al*., 1980; Al-Abbadi, 2005; Akyol *et al*., 2008). Other researchers have reported that the variations in such organs, e.g. the ovariole number, could not be associated with morphometric traits, *i.e.* queen size, body weight, and thorax width (Casagrande-Jaloretto *et al.*, 1984; Hatch *et al.*, 1999).

The native bee race has displayed good adaptation to the harsh conditions in Saudi Arabia especially in the central region of the country. However, beekeepers reports and some studies showed that its honey production rates were lower compared to *A.m. carnica*, the most imported race to Saudi Arabia (Alqarni, 1995; Balhareth, 2012).

The present study comprises an attempt to provide details and report on some queen physical traits which have not been previously addressed. Indeed, such studies are necessary to investigate the important aspects of

queen characters for the indication of potential quality. It would be helpful for the selection and breeding programs for the native bee race. Therefore, this work is the initial step in the study of certain *A. m. jemenitica* queen morphological and reproductive organ traits.

Materials and methods

Ten traits were measured in newly emerged queens of *A. m. jemenitica*, the native honey bee race of Saudi Arabia, and those parameters were compared with those of newly emerged *A. m. carnica* queens, the most imported race to Saudi Arabia, under the same conditions. This study was conducted in the apiary of Bee Research Unit, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, during the spring (April) seasons of 2010 and 2011.

Honey bee colonies

Ten honey bee colonies of the two races (five colonies/ race) of equal density were used for the experiment. The native honey bee colonies of *A. m. jemenitica* were collected from the Assir region, southwestern Saudi Arabia; those of the Carniolan honey bee, *A. m. carnica*, were imported from a reliable source in Egypt that maintains only Carniolan bees. All the colonies were maintained in wooden Langstroth hives with mother queens of the same age. The performance of the colonies was enhanced by feeding them sugar syrup (1:1, w/v) and pollen grains at rates of 0.5 L and 200 g/ colony, respectively.

Queen rearing

The experimental queens of the two tested honey bee races were produced in the above-mentioned apiary, with standard queen rearing techniques (Doolittle, 1909) using 5 mother queens per race. After being completely sealed, the queen cells were caged using plastic cages and kept in their colonies until queen emergence.

Measurements of morphometric traits

Newly emerged virgin queens (12 hrs old; 20 queens/ race) were transported to the laboratory to be weighed (Szabo, 1973). The dimensions (length and width) of the head capsule and right mandible (Ruttner, 1980), fore wing (Woyke, 1987), and the length of the 3^{rd} + 4^{th} abdominal (abd.) tergites (Abdalla, 2001) were measured using a binocular microscope with the aid of micrometric lenses.

Measurements of reproductive organs

Virgin queens were anesthetized with chloroform and fixed onto a dissecting tray. Using fine scissors, the two lateral sides of the queen were carefully cut, and the tergal parts were gently removed. The entire specimen was washed with distilled water for clear observation of the ovaries. The right ovary was carefully isolated and placed in a drop of xylene on a glass slide (Abdalla, 2001). For clarification, a drop of Puri's medium (100 mL distilled water + 50 ml glycerin + 30 ml glacial acetic acid + 70 g chloral hydrate) was placed onto the ovary for two minutes to separate the ovarioles, which were carefully washed with water to remove any residue (Ibrahim, 1977). The loosened ovarioles were counted with the aid of binocular magnification. The diameter and volume of the spermathecae were calculated (Szabo, 1982) using a graded eyepiece, and the specimens were examined under a stereomicroscope (SZX 10, Olympus, Japan) at magnification of 100-400X (zoom ratio of 10: 1).

Statistical analysis

The experimental colonies (queens) of both honey bee races were randomized in a complete block design. The data were calculated as the mean values and tested for significance at a 5% probability by Duncan's test and also correlation coefficients were calculated (Duncan, 1955). The software program SAS (SAS, 2008) was applied for the data computing and analysis.

Results

The data in table 1 show that the *A. m. carnica* virgin queens had significantly ($P < 0.05$) higher body weights (165.9 ± 9.8 mg, $n = 20$) than those of *A. m. jemenitica* (137.8 ± 7.9 mg) in the two seasons of the study. Similarly, the *A. m. carnica* queens showed significantly ($P < 0.05$) higher values than *A. m. jemenitica* for all the tested morphological traits, including the dimensions (general mean) of the head capsule (2.61 × 2.44 mm versus 2.45 × 2.23 mm), right mandible (1.29 × 0.38 mm versus 1.18 × 0.37 mm), forewing (9.39 × 3.10 mm versus 9.17 × 3.00 mm), and length of the 3^{rd} + 4^{th} abd. tergites (5.95 mm versus 5.57), respectively (table 2).

With regard to the reproductive organs, table 3 shows that the number of ovarioles in the right ovary of the *A. m. carnica* queens (156.98 ± 14.9; n = 20) was significantly ($P < 0.05$) higher than that of *A. m. jemenitica* (146.6 ± 13.9). In contrast, the mean values for the diameters of spermathecae did not differ significantly ($P > 0.05$) for the two races, with values of 1.253 mm and 1.230 mm for *A. m. carnica* and *A. m. jemenitica*, respectively (table 4), and a significant difference ($P < 0.05$) between the two races was only observed in the 2^{nd} season. Additionally, an insignificant difference ($P > 0.05$) was found in the spermathecal volume in the 1^{st} season between the two races, but this difference was significant ($P < 0.05$) in the 2^{nd} season, with values of 1.038 mm^3 and 0.979 mm^3 for *A. m. carnica* and *A. m. jemenitica*, respectively (table 5).

Highly positive correlations were found between the virgin queen body weight and head capsule dimensions ($r = 0.6771$), length of mandible ($r = 0.6618$), length of forewing ($r = 0.6056$), and length of the 3 + 4 abd. tergites. In contrast, only a weak correlation was found between the body weight and ovariole number ($r = 0.2225$), diameter of spermathecae ($r = 0.2413$), and volume of spermathecae ($r = 0.2439$).

Positive correlations were found between the ovariole number and head capsule dimensions ($r = 0.3749$ for length and $r = 0.5383$ for width), length of mandible ($r = 0.3102$), and length of the 3 + 4 abd. tergites. In addition, positive correlations were found between the

Table 1. Mean body weight (± SD) of virgin queens of *A. m. carnica* and *A. m. jemenitica* reared by the grafting method during spring of 2010 and 2011 in Riyadh, Saudi Arabia.

Honeybee race	Mean body weight (mg) of queens (n = 20 / race)			
	2010	2011	Range	General mean
A. m. carnica	169.050 a ± 2.798	162.700 a ± 0.974	153.0 - 195.0	165.875 A ± 9.791
A. m. jemenitica	133.750 c ± 1.534	141.875 b ± 0.907	121.0 - 149.0	137.813 B ± 7.919

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

Table 2. Mean values (± SD) of morphometric characters (mm) of virgin queens of *A. m. carnica* and *A. m. jemenitica* (n = 20 / race) reared by the grafting method during spring of 2010 and 2011 in Riyadh, Saudi Arabia.

		A. m. carnica		*A. m. jemenitica*		General mean	
		2010	2011	2010	2011	*A. m. carnica*	*A. m jemenitica*
Head capsule	Length	2.608 ± 0.0200	2.615 ± 0.0139	2.468 ± 0.0247	2.423 ± 0.0143	2.6113 a ± 0.0120	2.4450 b ± 0.0145
	Width	2.400 ± 0.0218	2.482 ± 0.0148	2.213 ± 0.0158	2.254 ± 0.0167	2.4408 a ± 0.0145	2.2333 b ± 0.0118
Right mandible	Length	1.273 ± 0.5600	1.300 ± 0.0600	1.199 ± 0.7700	1.164 ± 0.1210	1.2865 a ± 0.0046	1.1813 b ± 0.0076
	Width	0.373 ± 0.0076	0.388 ± 0.0032	0.369 ± 0.0053	0.371 ± 0.0043	0.3803 a ± 0.0042	0.3695 b ± 0.0034
Right fore wing	Length	9.400 ± 0.0435	9.385 ± 0.0343	9.185 ± 0.0372	9.155 ± 0.0312	9.3925 a ± 0.0274	9.1700 b ± 0.0241
	Width	3.048 ± 0.0219	3.125 ± 0.0190	2.980 ± 0.0401	3.025 ± 0.0143	3.0863 a ± 0.0156	3.0025 b ± 0.0213
Length of 3^{rd} + 4^{th} abd. tergites		5.883 ± 0.0540	6.025 ± 0.0315	5.450 ± 0.0309	5.688 ± 0.0198	5.9538 a ± 0.0329	5.5688 b ± 0.2630

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

Table 3. Mean ovariole number (± SD) of virgin queens of *A. m. carnica* and *A. m. jemenitica* reared by the grafting method during spring of 2010 and 2011 in Riyadh, Saudi Arabia.

Honey bee race	Ovariole number (right ovary) (n = 20 / race)			
	2010	2011	Range	General mean
A. m. carnica	149.750 a ± 3.313	164.200 a ± 4.153	109.00 - 197.00	156.98 A ± 14.889
A. m. jemenitica	147.400 b ± 2.507	145.750 b ± 1.542	118.00 - 178.00	146.58 B ± 13.854

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

Table 4. Mean values (± SD) of the spermathecal diameter of virgin queens of *A. m. carnica* and *A. m. jemenitica* reared by the grafting method during spring 2010 and 2011 in Riyadh, Saudi Arabia.

Honey bee race	Spermathecal diameter (mm) (n = 20 / race)			
	2010	2011	Range	General mean
A. m. carnica	1.201 c ± 0.009	1.305 a ± 0.009	1.15 - 1.35	1.253 A ± 0.066
A. m. jemenitica	1.187 c ± 0.008	1.273 b ± 0.008	1.12 - 1.30	1.230 A ± 0.055

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

Table 5. Mean values of the spermathecal volume of virgin queens of *A. m. carnica* and *A. m. jemenitica* reared by the grafting method during spring of 2010 and 2011 in Riyadh, Saudi Arabia.

Honey bee race	Spermathecal volume (mm^3) (n = 20 / race)			
	2010	2011	Range	General mean
A. m. carnica	0.909 c ± 0.095	1.166 a ± 0.104	0.80 - 1.29	1.038 A ± 0.163
A. m. jemenitica	0.876 c ± 0.081	1.080 b ± 0.083	0.70 - 1.15	0.979 B ± 0.131

The mean values with different letters in the same column are significantly different ($P \leq 0.05$).

spermathecal diameter and width of head capsule ($r = 0.3421$), width of forewing ($r = 0.3271$), and length of the 3 + 4 abd. tergites ($r = 0.4904$). However, the correlation between the ovariole number and length and width of the forewing was insignificant ($r = 0.1832$ and 0.0190, respectively).

Discussion

The results of this study showed clear differences between the queens of *A. m. jemenitica* and *A. m. carnica*. Alqarni *et al*. (2011) and Alghamdi *et al.* (2012) reported various details of worker morphometrics, de-

scribing significant differences among the workers of *A. m. jemenitica* and *A. m. carnica*. Additionally, the ongoing research at the molecular level, combining the characteristics of queens, workers, and drones reveal clear differences between *A. m. jemenitica* and other races. Alsharhi (2013) examined the genetic diversity of the native Saudi bees and stated that gene flow between this race and the imported Carniolan bees was very low.

Table 2 shows the results of the morphological traits, whereby the values for the queens of *A. m. carnica* significantly exceeded those of *A. m. jemenitica*. These differences were also found in the morphometric traits of workers of Carniolan bees compared to those of *A. m. jemenitica* or their 1st generation hybrid (Alqarni, 1995) and in morphometric and reproductive characters of drones from the same two races (Alqarni and Taha, unpublished data). The present data for *A. m. carnica* (table 2) are in accordance with the results of Abdalla (2001), who recorded 9.01 × 3.01 mm for the forewing dimensions and 6.10 mm for the length of the 3rd + 4th abd. tergites. In contrast, our measurements are relatively smaller than those of Al-Abbadi (2005), who reported 10.15 × 3.18 mm for the forewing dimensions and 4.40 mm for the length of the 3rd + 4th abd. tergites in the queens of the Carniolan hybrid. Although these differences were relatively small, they could be due to hybridization or differences in queens' source.

It is shown in table 1 that the virgin queens of *A. m. carnica* had significantly higher body weight than the *A. m. jemenitica* queens. Although the body weight of newly emerged queens might be an indicator of queen fecundity (Eid *et al.*, 1980), the difference in the present study is due to the larger size of *A. m. carnica* queens. In Egypt, the weight of Carniolan hybrid queens was reported to be 153.4 mg and 145.9 mg for two successive years (Abdalla, 2001) and was 174.16 mg for queens produced by the grafting method (Al-Abbadi, 2005). Accordingly, the fresh weight of queens is not a good indicator of ovariole number (Corbella and Goncalves, 1982; Hatch *et al.*, 1999), even though this relationship was found to be significant by other authors (El-Sayed, 1977; Ibrahim, 1977; Woyke, 1987). Fischer and Maul (1991) found a significant correlation between the spermathecal volume and ovariole number. Additionally, a weak correlation was found between the body weight and spermathecal diameter (Esmen, 2004).

With regard to the reproductive organs, table 3 shows that the ovariole number in the right ovary of *A. m. carnica* queens was significantly higher than that of the *A. m. jemenitica* queens; an insignificant difference was found between the two studied seasons. Heavier queens have more ovarioles and larger spermathecae, and thus, lay more eggs (Rakhmatov, 1967; Woyke, 1971; Szabo, 1982; Taranov, 1973; Eid *et al.*, 1980; Harbo, 1986). In the present study, the diameter of the spermathecae in the two races differed significantly in the 2nd season. An insignificant difference was found between the two races in the spermathecal volume in the 1st season, whereas this difference was significant in the 2nd season. This result could be due to the different sources of queens in the two seasons. Correlation between spermathecal volume and morphometric traits was considered by some authors as an indicator of queen quality (Eid *et al.*, 1980; Al-Abbadi, 2005).Contrary, correlations between morphometric traits and size of reproductive organs were weak, positive, or insignificant in this study. The only highly positive correlation was found between queen body weight and morphometric traits. Akyol *et al.* (2008) found high correlation between body size and spermathecal diameter. The same authors added that no correlation was found between the ovariole number and other morphological characters, including the thoracic width, wing length, or wet weight, and that the ovariole number ranged from 100 to 180 per ovary in *A. mellifera* queens. It is clear that correlations between queen morphometric and reproductive organs measurements fluctuate among several studies (Eid *et al.*, 1980; Casagrande-Jaloretto *et al.*, 1984; Hatch *et al.*, 1999; Al-Abbadi, 2005; Akyol *et al.*, 2008) and solid conclusion could not be reached. This is possibly due to the effects of several factors such as bee race, larval stage grafted, nutrition, colony strength. Indeed, the ovariole number was affected by honey bee race (Corbella and Goncalves, 1982).

The bee stage used for grafting (eggs or larvae) significantly affects the spermathecal volume, which was reduced from 1.18 mm^3 when using eggs to 0.82 mm^3 in the case of 3-day-old larvae (Woyke, 1987). The histological structure of the ovaries of *A. m. jemenitica* queens was studied in the 3rd and 5th larval instars, in 1-, 2-, and 3-day-old pupae, and in newly emerged queens, and several differences were found from one developmental stage to another and in the different ages of each stage (Almehmadi *et al.*, 2011). The same authors found that the queen's ovaries continue to grow and differentiate after the 5th (final) larval stage and also continue to grow during the three pupal stages.

Throughout the present study, it was found that the morphometric and reproductive characters were significantly different between the queens of *A. m. jemenitica* and *A. m. carnica*. These differences could be associated to the studies and reports regarding the performance of the two races. The native bees (smaller in size) can tolerate harsh conditions but produce less honey. Whereas the imported bees (larger in size) cannot tolerate harsh condition but they produce more honey (Alqarni, 1995; Balhareth, 2012). Our findings strengthen the previous findings for the morphometric description of *A. m. jemenitica* workers and drones in Saudi Arabia.

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