

Morphometric and Reproductive Organs Characters of *Apis mellifera jemenitica* Drones in Comparison to *Apis mellifera carnica*

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ABSTRACT— This is the first measurements carried out on the drones of the native honeybees of Saudi Arabia *Apis mellifera jemenitica*. The mean body weight, length and width of the forewing, number of hamuli on the hind wing, and size of reproductive organs (testes, seminal vesicle and mucus gland) of newly emerged drones, as well as sperm numbers of drones aged 14-days were significantly ($P < 0.01$) different between honeybee, *A. m. jemenitica* and *A. m. carnica* in Saudi Arabia. Drones of *A. m. jemenitica* had smaller body size, wings, reproductive organs compared to *A. m. carnica* drones. In addition, native drones produced fewer spermatozoa than those of Carniolan ones (9.33×10^6 vs. 12.67×10^6). There were significant positive correlations between body weight and forewing dimensions, size of reproductive organs and sperm number.

Key words: drones, *Apis mellifera jemenitica*, body weight, sperm number, reproductive organs.

1 INTRODUCTION

Sufficient rearing of healthy drones is the prime step for successful queen mating. Honey bee queen can mate with an average of 12 drones (range 1–32) and able to hold 4.3-7.0 million spermatozoa in her spermatheca. Throughout 55 days life span, drones mature within 16 days and become less suitable for mating after 28 days [1].

Drone production in the colony is seasonal and depends upon environmental conditions [2], [3], [4] and availability of young and foraging workers [5]. Drones were available from November until June in central region of Saudi Arabia, with the native bees reared significantly higher amounts of drones than other subspecies [6]. The main function of drones within the colony is to fertilize virgin queens. One of the most neglected elements of queen rearing in honeybees is provision of suitable mates for the virgin queens [7]. Production of viable drones is a limiting factor in successful queen rearing. Studies on drones can lead to maintain highly improved breeding programs. This can be achieved by improving the efficiency and quality of mating.

Quality of drone bees is influenced by several factors such as species or subspecies of honey bees [8], [9], age of drones [10], rearing season [11], [12], [13], food supply [14], [15], size of comb cells [16], [17], [18], [19], infestation with parasites, e.g. *Varroa mites* [20], [21], [22], [23], [13], and colony strength [24]. Johnson et al. [25] reported that exposure to sublethal doses of miticides did not affect sperm viability of adult drones.

In the semi-arid conditions that cover the Arabian

Peninsula, particularly Saudi Arabia, *A. m. jemenitica* Ruttner, a native subspecies plays a vital role in the bee-keeping industry. It is highly adapted to the harsh extremes of the Saudi environment [26]. During the summer season, temperature often exceeds 45°C in different regions of the country. Only these native bees survive whereas other subspecies struggle until finally die off [27]. With the annual importation of more than 359 tons of bees (about 240,000 bee packages, mostly from Egypt) of other subspecies (mostly Carniolan hybrids) [28], the genotype structure of the native bees may have been affected to some degree. Out of twelve, eight morphometric characters showed significant differences within native *A. m. jemenitica* colonies, which may indicate differences in the genotype structure [6]. Currently, several studies are underway to evaluate the genotype structure of the native bees using molecular genetics, and quality of queens' reproductive organs of the native bees.

The present investigation is the first attempt to examine the quality of drones of the native race in comparison with that of imported hybrid Carniolan honey bees reared under the environmental conditions of Saudi Arabia.

2 MATERIALS AND METHODS

2.1 Honey bee colonies

The study was carried out at the apiary of Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa, Saudi Arabia during March and April, 2012 season. Three colonies of each subspecies were kept in Langstroth hives. Native colonies of *A. m. jemenitica* were brought from a non-migrating apiary in Jazan, southwest of Saudi Arabia, while Carniolan hybrid bees, *A. m. carnica*, were brought from a reliable source in Egypt. Colonies of each group were headed by young open-mated sister queens of the same subspecies. All colonies were equalized for brood, bee strength and stored food.

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2.2 Drone rearing

A new comb from each replicate colony was selected and a strip at 5 cm from its top was horizontally cut off, then inserted back into the colony. Each colony was provided with 1L sucrose syrup (1:1, w/v). The comb was left for worker bees to complete the space with drone cells. After completion, the comb and the queen were inserted into a complete comb cage (45.25 × 25.35 × 9.45 cm) with sides consisting of queen excluders. After egg laying, the combs were left in their colonies until drone emergence. A piece of queen excluder was fixed to the hive entrance. These colonies were checked to remove all drones. The objective of this procedure was to facilitate determining the actual age of drones to be used for sperm counts.

2.3 Body weight

Thirty newly emerged drones (within six hours of emergence) of each subspecies (ten from each replicate), were used to determine the fresh body weight (mg) using an electrical balance after being anesthetized with chloroform. Weighed drones were then kept for two days in 70% ethanol to harden the reproductive organs and facilitate subsequent measurements.

2.4 Size of drone cells

The size of newly constructed cells of both Yemeni and Carniolan drones (100 cells per each) were measured using a pipette and distilled water. The volume of water (mm³) needed to fill the drone cell was obtained.

2.5 Wing measurements

The right fore wing and hind wing of the kept drones (ten from each replicate), were removed and put on a glass slide to measure the maximum length and width (mm) using a dissecting binocular microscope supplied with a micrometer lens. In the same drones, the number of hamuli on the right hind wings were counted.

2.6 Reproductive organs

The same drones (ten from each replicate), were dissected to measure the maximum length and width (mm) of the right testis, seminal vesicle and mucus glands using a dissecting binocular microscope fitted with an ocular micrometer. The sizes (mm³) of these organs were calculated. As testis, seminal vesicle, and mucus gland of drones assuming cylindrical shape [29], the estimated volumes = $\pi \times (w/2)^2 \times l$, where: $\pi = 3.14$, w = maximum width and l = maximum length.

2.7 Sperm number

Twenty drones of 14-days old, from each group, were chilled to prevent their ejaculation when dissected. The drones were dissected in a physiological saline solution to remove the seminal vesicle. The seminal vesicle was placed in a small Petri dish containing 1 ml distilled water and macerated with a fine pair of needles, this mixture was agitated with an eyedropper. Distilled water was added to 5 ml as a total volume. The semen was mixed to the solution in both steps. Sperm numbers were counted under a phase contrast microscope using a Thoma

counting chamber in a total volume of 0.064 μ L. Sixteen replicate fields were counted and their average spermatozoa was used as the best estimate count for that drone [17].

2.8 Statistical analysis

Data obtained were statistically analyzed by the analysis of variance using the general linear model procedure [30]. Treatment means were compared by Duncan's Multiple Range Test [31]. A Pearson's correlations were calculated to test for an association between body weights, wing measurements, sizes of reproductive organs and sperm numbers.

3 RESULTS

3.1 Morphometric characters

Data in Table 1 indicated that, the averages of body weight and cell size (190.90 and 0.40 v.s 227.22 mg and 0.43 cm³) of the newly emerged Carniolan and Yemeni drones were significantly ($P < 0.01$) different. The mean length and width of the right forewing, and the mean numbers of hamuli on the right hind wing of newly emerged drones were influenced by honey bee subspecies (Table 2). The length and width of the right forewing and the mean numbers of hamuli on the right hind wing of Carniolan drones were significantly ($P < 0.01$) higher than those of the Yemeni subspecies. The length and width of the right hind wing of Carniolan and Yemeni drones were not significantly different ($P > 0.05$).

TABLE 1

Body weight (mg) and cell size (cm³) of the Yemeni and Carniolan honey bee drones.

Parameters	Subspecies		Significant
	Yemeni	Carniolan	
Body weight	190.90±0.33b	227.22±0.63a	**
Size of drone cell	0.40±0.01b	0.43±0.01a	**

Values are mean ± S.E. Means of each row followed by the same letter are insignificantly different. ** indicate $P < 0.01$.

TABLE 2

Length and width (mm) of fore and hind wings and number of hamuli on the hind wing of Yemeni and Carniolan drones.

Parameters		Subspecies		Significant
		Yemeni	Carniolan	
Fore wing	Length	12.33±0.09b	14.01±0.18a	**
	Width	3.62±0.04b	4.17±0.08a	**
Hind wing	Length	7.23±0.06	7.50±0.10	NS
	Width	3.15±0.05	3.23±0.05	NS
	No. hamuli	19.50±0.29b	22.00±0.58a	**

Values are mean ± S.E. Means of each row followed by the same letter are insignificantly different. ** and NS indicate $P < 0.01$ and insignificant, respectively.

Significant positive correlations (Table 3) were shown between weight of drone in one hand and each of length ($r = 0.98$; $P < 0.001$) and width ($r = 0.96$; $P < 0.01$) of the right forewing, length of right hind wing ($r = 0.80$; $P < 0.05$), and number of hamuli on the right hind wing ($r = 0.90$; $P < 0.01$) on the other hand.

TABLE 3

Pearson's correlation coefficients of traits of the tested honey Bees drones.

Parameters	Body weight	No. hamuli	Length of forewing	Width of forewing	Length of hind wing	Width of hind wing	Size of testis	Size of seminal vesicle	Size of mucus gland
Body weight									
No. hamuli	0.90**								
Length of forewing	0.98**	0.97**							
Width of forewing	0.96**	0.96**	0.99**						
Length of hind wing	0.80*	0.98**	0.90**	0.88*					
Width of hind wing	0.58	0.85*	0.73	0.73	0.93**				
Size of testis	0.99**	0.94**	0.99**	0.99**	0.85*	0.67			
Size of seminal vesicle	0.99**	0.91**	0.98**	0.98**	0.80*	0.60	0.99**		
Size of mucus gland	0.96**	0.92**	0.97**	0.98**	0.84*	0.64	0.97**	0.97**	
No. sperms	0.99**	0.89**	0.97**	0.96**	0.77	0.57	0.99**	0.99**	0.97**

* and ** indicate that correlation is significant at 0.05 and 0.01 levels (2-tailed), respectively.

3.2 Reproductive organs characters

As shown in Table 4, the mean size of testis, seminal vesicle and mucus gland were influenced by honey bee subspecies. They were significantly ($P < 0.001$) higher in Carniolan drones than those of Yemeni subspecies.

Significant positive correlations were obtained between mean volume of testis, seminal vesicle and mucus gland in one hand and mean weight of drone ($r = 0.99, 0.99$ & 0.96 ; $P < 0.01$), length of fore wing ($r = 0.99, 0.98$ & 0.97 ; $P < 0.01$), width of forewing ($r = 0.99, 0.98$ & 0.98 ; $P < 0.01$), length of hind wing ($r = 0.85, 0.80$ & 0.84 ; $P < 0.05$), and number of hamuli ($r = 0.94, 0.91$ & 0.92 ; $P < 0.01$) on the other hand, respectively (Table 3).

TABLE 4

Size of reproductive organs and sperm numbers of Yemeni and Carniolan drones.

Parameters	Subspecies		Significant
	Yemeni	Carniolan	
Testis (mm ³)	20.76±0.33 ^b	30.43±0.63 ^a	**
Seminal vesicle (mm ³)	0.86±0.02 ^b	1.34±0.01 ^a	**
Mucus gland (mm ³)	3.33±0.12 ^b	4.48±0.10 ^a	**
Sperm numbers ($\times 10^6$)	9.33±2886.75 ^b	12.67±2886.75 ^a	**

Values are mean \pm S.E. Means of each row followed by the same letter are insignificantly different. ** indicate $P < 0.01$.

The number of sperms in the seminal vesicle of Carniolan drones aged 14-years old was significantly ($P < 0.001$) higher than that of the Yemeni one (Table 4). Significant positive correlations were noticed between mean number of sperm on one hand and mean weight of the drone ($r = 0.99$), length of forewing ($r = 0.97$), width of forewing ($r = 0.96$), number of hamuli ($r = 0.89$), size of testis ($r = 0.99$), seminal vesicle ($r = 0.99$) and mucus gland ($r = 0.97$) on the other hand (Table 3).

4 DISCUSSION

4.1 Morphometric characters

The Carniolan drones possessed the highest ($P < 0.01$) body weight (119.03% of the Yemeni race). The reduced weight of the Yemeni drones may be due to the smaller drone cell size of the Yemeni race compared to the cells of the Carniolan bees

(0.40 cm³ vs. 0.43 cm³) (Table 1). Competition between Carniolan and Yemeni drones is expected when mating with queens. Since flight ability and semen production vary among drones [32], sperm number is responsible for reduced reproductive success of small drones [33]. Drone weight effect seems to be specific to bee species [34]. The reduced weight of Africanized drones possibly resulted from poor feeding of drone larvae by nurse bees [35]. Weights of both European and Africanized worker larvae nursed by Africanized nurse bees were less than those nursed by European bees [36]. In our study, Yemeni drones weighed less than those of Carniolan ones (190.90 mg vs. 227.22 mg, respectively). Our results are similar to those reported by Rinderer et al. [35] who found that, Africanized drones weighed less than those of European ones (194.6 mg vs. 220.2 mg, respectively). The average weight (203 mg) of *A. m. ligustica* drones was significantly heavier than that (181 mg) of *A. m. syriaca* [4].

The length and width of the forewing of Yemeni drones were about 13.63% and 15.19% smaller than those of Carniolan ones, respectively. These findings are substantially above those observed by Berg et al. [16] who reported that, wing length of small drones recorded 7% reduction compared to the large ones, while present results are relatively similar to those found by Schlüns et al. [17] who noted that the wing length of small drones emerged from worker cells was about 13% smaller compared to wings of normally sized drones emerged from drone cells.

The length and width of hind wing, and number of hamuli of Yemeni drones were about 3.87%, 2.54% and 12.82% smaller compared to those of Carniolan ones, respectively. The number of hamuli on the hind wings were significantly correlated to the body weight and length of hind wings ($r = 0.90$ & 0.98 ; $P < 0.01$). The number of hamuli and their extension have concernable heritability values and are useful in the classification of honey bee populations [37]. Alqarni [6] reported that workers of *A. m. carnica* significantly surpassed those of *A. m. jemenitica* and their 1st hybrid in 11 morphometric characters including length and width of the fore wing and the number of hamuli on the hind wing

The differences between forewing dimensions is strongly related to body size. Significant positive correlations between wings dimensions and mean weight of drone were found in our study. In this respect, our results are in agreement with those obtained by Taha [24].

4.2 Reproductive organs characters

The mean size of reproductive organs; testis, seminal vesicle and mucus gland of Yemeni drones, were about 46.58, 55.81 and 34.53% smaller compared to those of Carniolan ones, respectively. These differences may be due to the variation in drone body sizes [38]. These results are in harmony with the findings of Gencer & Firatli [19]; Taha et al. [38] as they showed that, large drones have larger reproductive organs than small ones. On the other hand, Rinderer et al. [35] found that although the weights of Africanized drones were smaller than those of European ones, the weights of the seminal vesicles and mucus glands of Africanized drones were similar to those of European bees.

The sperm numbers in the seminal vesicle of Yemeni drones were about 35.80% lesser than those of Carniolan ones. The differences in sperm numbers between drones may be due to the variation in sizes of body, testis and seminal vesicle. The present findings are coincided with those reported by Jarolimek & Otis [33], Schlüns et al. [17], Taha et al. [38] who found significant positive correlation between body size and sperm numbers in honey bee drones. Yemeni drones had fewer spermatozoa than those of Carniolan ones (9.33×10^6 vs. 12.67×10^6). These results are in harmony with those of Berg & Koeniger [39] who stated that, large *A. m. carnica* drones had more spermatozoa (7.08×10^6) than small ones (6.76×10^6) without insignificant difference between both groups. Schlüns et al. [17] proved that small drones (~13% reduced wing size) produced significantly fewer spermatozoa (7.5×10^6) than normally sized drones (11.9×10^6 spermatozoa). Also, Rhodes [1] mentioned that healthy drone can produce $5-10 \times 10^6$ Sperm. Mazeed & Mohanny [10] found that spermatozoa number in all parts of the reproductive organs was lower in older drones than younger ones. Sperm number is basic to the understanding of honey bee mating biology, drone fitness, polyandry and sperm completion [40].

According to the results of this study, it could be concluded that the Yemeni drones of *A. m. jemenitica* were significantly smaller in body size and weight, in reproductive organs measurements, and in sperm numbers. The annual introduction of thousands of *A. m. carnica* hybrid bees colonies may eventually affect the genotyp structure of the Yemeni bees. Studies are needed to explore populations of *A. m. jemenitica* Yemeni bees in order to spot genetic changes. Moreover, other studies such as determining the frequency of diploid males production over time could be carried out to investigate any signs of declining populations.

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