Morphometric Characteristics of Carniolan Honeybee Workers in Relation to Age of Comb

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ABSTRACT

This study was conducted at the apiary of the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa, eastern Saudi Arabia, to examine the effect of comb age on some morphological characteristics of the workers of the Carniolan honeybee *Apis mellifera carnica* Pollmann. Comb ages of 1, 2, 3 and 4 years were used in this experiment. Worker body weight decreased in parallel with the increase in comb age. Compared to workers reared in old combs, significant increase in body weights, measured head appendage characteristics, wing characteristics, hind leg characteristics, length and width of the 3rd abdominal sternite, and 1st wax mirror were observed in workers obtained from new combs. Body weight was significantly positively correlated with most of these characteristics. The body size of workers was affected by the age of the comb they were reared in, which directly affected the dimensions of the appendages related to honey and pollen production. Thus, the recommendation is to replace old combs with new ones. Body size of worker can be used as indicator to the morphological characteristics of worker bees related to the activities and productivity of honeybee colonies.

Key Words: Beeswax, Body weight, Comb, Metatarsus, Proboscis

INTRODUCTION

The color of comb wax when first constructed is almost white. Hydrocarbons and ester components of beeswax can absorb several types of material elements (Tulloch, 1980). Storage of pollen and nectar causes combs to acquire a yellowish hue over time because of the accumulation of pollen (Free and Williams, 1974). The rearing of several broods gradually darken comb wax until becoming almost black (Hepburn, 1998), and the cells tend to be smaller (Winston, 1987) because of accumulated fecal material and the cocoons that are deposited by larval and pupal instars developing within the cell (Jay, 1963), in addition to propolis and pollen (Free and Williams, 1974; Taha and El-Sanat, 2007). The darker color may also result from numerous unidentified contaminants that are accumulated and absorbed in the comb wax over time (Taha *et al*., 2010). As a result of the accumulation of larval cocoons and other detritus, the cell walls also thicken (Coggshall and Morse, 1984).

The rearing of several broods in a comb cell decreases cell diameter, leading to a lack of space, and with a relative shortage of food in the cells of an old comb, the workers reared in these cells are consequently smaller. Eventually, worker bees reared in old comb may be smaller and weigh up to 19% less than worker bees reared in a new comb (Buchner, 1955; Alfalah *et al*., 2012).

Furthermore, body weight of workers varies according to honeybee subspecies (Atallah *et al*., 1987; Oldroyd *et al*., 1991), comb cells size (Abdellatif, 1965, Alfalah *et al*., 2012), pollen and nectar flora (Helal *et al*., 2003; Taha, 2005), feeding on proteinaceous diets (Roulston and Cane, 2000; Zheng *et al*., 2014), season (Kunert and Crailsheim, 1988; Ivanov and Spasov, 1990; Helal *et al*., 2003; Taha, 2005), geographical location (Al-Kahtani and Taha, 2014), and infestation with Varroa mite, *Varroa destructor* Anderson and Trueman (Hovec, 1991). Moreover, studies on worker honeybees reveal that environmental factors play a significant role in changing morphometric characteristics (Milne *et al*., 1986; Stanimirovic *et al*., 2008; Ajao *et al*., 2014; Al-Kahtani and Taha, 2014, Charistos *et al*., 2014).

The relationship between comb age and brood rearing activity (El-Dakhakhni, 1995),

honey production (Taha and El-Sanat, 2007), and honey composition (Taha and El-Sanat, 2007; Taha *et al*., 2010) has also been studied. Taha and El-Sanat (2007) found that harvested honey yield decreased as the age of combs increased. The size of comb cells significantly affects the body size of workers (Abdellatif, 1965). Moreover, the productivity of a honeybee colony is highly correlated with the overall size and wing measurements of the honeybee workers (Kolmes and Sam, 1991; Taha, 2005).

Few data are available on the effect of comb age on the morphometrics of honeybee workers. Therefore, the aim of this work was to study the relationships between comb age and morphometric characteristics of Carniolan honeybee workers.

MATERIALS AND METHODS

This study was conducted at the apiary of the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa (25^{O} 25′ 46″ N, 49^{O} 37′ 19″ E), Saudi Arabia, during the spring season in 2016. Sixteen colonies of Carniolan (*Apis mellifera carnica* Pollmann) honeybee of the same population size (each having 8 combs; 3 honey+ pollen and 5 frames covered with bees) headed by young sisters of open-mated queens were obtained on 2015 from the Institute for Bee Research, Hohen Neuendorf, Germany, for use in this experiment. The colonies were divided into four groups (each of 4 colonies). The combs in the hives were replaced by the experimental combs and arranged according to their ages as follows: 1, 2, 3, 4, 1, 2, 3 and 4 years old (group 1); 2, 3, 4, 1, 2, 3, 4 and 1 year old (group 2); 3, 4, 1, 2, 3, 4, 1 and 2 years old (group 3); and 4, 1, 2, 3, 4, 1, 2 and 3 years old (group 4). Comb aged 4, 3, 2 and 1-year old were added as a wax foundation to the colonies in 2011, 2012, 2013 and 2014, respectively.

Experimental workers

Twenty days after queens laid eggs in the combs, a comb of worker-sealed brood of each comb age from the same colony was inserted into a wooden, complete comb cage (45.25 × 25.35 × 9.45 cm) with 2 sides of metal net. The cages were placed under controlled conditions of temperature (32°C) and 60% relative humidity in a Memeret incubator (Germany) until workers emerged (Al-Kahtani and Taha, 2014).

Worker body weight

Forty newly emerged workers (within 6 h of emergence) of each comb were used to determine the fresh body weight (in mg) using an electrical balance after bees were chilled (Hovac, 1991). These workers were treated with hot water and then preserved in 70% ethanol until dissection (Adl *et al*., 2007).

Morphometric characteristics

The following body appendages were removed and placed on glass slides to measure the morphometric characteristics (in mm) using a binocular dissecting microscope equipped with a micrometer lens. The morphometric characteristics of the head appendages that were measured were total antenna length, flagellum length, and proboscis length (El-Aw, 2012; Carreck *et al*., 2013; Meixner *et al*., 2013). The wing morphometric characters included lengths and widths of the right forewing and hind wing and number of hamuli on the right hind wing. The morphometric characters of the hind legs included femur length, tibia length, width of the end of the tibia, and length and width of the metatarsus. The abdominal morphometric characters included lengths of the 3^{rd} and 4^{th} abdominal tergites and sternites, in addition to lengths and widths of the 1^{st} and 4^{th} wax mirrors. Measurements of these characteristics were conducted according to Ruttner (1988).

Statistical analyses

Data were subjected to a one-way analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955). The Pearson correlation between characteristics was determined using

PROC GLM of the SAS statistical software package ver. 9.1 (SAS Institute, 2003).

RESULTS AND DISCUSSION

As shown in Table (1), the body size and 18 of 21 morphometric characteristics of Carniolan honeybee *A. m. carnica* Pollmann workers were significantly affected by the age of the comb in which a worker was reared. Compared with worker bees obtained from old combs, workers reared in new combs had significantly ($p < 0.01$) higher values for body weight, lengths of proboscis, flagellum, and antenna, length of hind wing length, number of hamuli, femur length, tibia width, metatarsus width, 3rd tergite length and 1st wax mirror width.

Body size reflected the sizes of the morphological characteristics of worker bees. The mean body weights of the Carniolan honeybee workers ranged from 95.00 to 112.00 mg and were significantly affected by the age of the comb ($p < 0.01$). The weights of workers decreased with increasing age of the comb in which they were reared. Compared with the body weight of workers obtained from a 4-year-old comb, the body weight increased 17.89%, 14.74% and 6.32% for workers reared in 1, 2 and 3-year-old combs, respectively. These results are consistent with those of Buchner (1955), Abdellatif (1965) and Alfalah *et al*. (2012). The cells become smaller in older combs and the lack of cell space results in a relative shortage of the provided food during larval development. Therefore, the diminished space may force larvae to molt to the non-feeding pre-pupal stage prematurely, causing nurse bees to seal the cells before larvae reach maximum development (Abdellatif, 1965).

The structures that were measured play prominent roles in the activities of bees. For the head characteristics, the lengths of the proboscis, antenna, and flagellum of the Carniolan honeybee workers were significantly affected by the age of the comb ($p < 0.01$). Compared with the proboscis length of a worker obtained from a 4-year-old comb, the proboscis length increased by 14.02%, 11.03% and 3.74% for a worker reared in 1, 2 and 3-year-old combs, respectively. These results confirmed those obtained by Alfalah *et al*. (2012). Compared with the flagellum length of a worker obtained from a 4-year-old comb, the flagellum length increased by 20.00%, 16.00% and 6.00% for a worker reared in 1, 2 and 3-year-old combs, respectively. These variations were likely related to body size, because body weight was significantly positively correlated with proboscis length ($r = 0.74$) and antenna length ($r = 0.49$) (Table 2). Al-Kahtani and Taha (2014) reported similar relationships for the dwarf honeybee (*A. florea* F.), and workers reared in 2, 3 or 4-year-old combs had reductions in proboscis length of 2.62%, 9.07% or 12.30%, respectively, compared with workers from a 1 year old comb.

Table (1): Body weights (mg) and average measurements of 21 morphometric characteristics (mm) of Carniolan honeybee (*Apis m. carnica*) workers in relation to age of comb.

Character		Age of comb (years)				Sig.
		1	2	3	4	
Body weight		112.00±3.44 a	109.00±4.01a	101.00±4.44 b	95.00±4.64c	**
Proboscis length		6.10±0.14a	5.94±0.25 b	5.55±0.33 c	5.3±0.42 d	**
Flagellum length		3.00±0.03a	2.90±0.04 a	2.65±0.02b	2.50±0.05 c	**
Total length of antenna		4.20±0.03a	4.15±0.04a	4.03±0.05 b	3.90±0.14 c	**
Forewing	Length	9.05±0.15	9.03±0.14	9.00±0.18	9.00±0.20	NS
	Width	3.50±0.01a	3.50±0.02a	3.48±0.04 b	3.48±0.03 b	*
Hind wing	Length	6.70±0.14a	6.60±0.12b	6.52±0.18 c	6.45±0.20 d	**
	Width	2.20±0.04a	2.16±0.04 a	2.10±0.06 b	2.05±0.03 c	*
	No. hamuli	22.00±1.04a	22.00±1.15a	21.40±1.64 b	21.50±2.01b	*

Table 1 (cont):

Character		Age of comb (years)				Sig
		1	2	3	4	
Femur length		2.80±0.03[a]	2.70±0.05[b]	2.65±0.04[b]	2.55±0.08[c]	**
Tibia	Length	3.00±0.09[a]	3.00±0.14[a]	2.94±0.07[b]	2.90±0.14[b]	*
	Width	1.20±0.02[a]	1.17±0.04[a]	1.10±0.01[b]	1.05±0.02[c]	**
Metatarsus	Length	2.50±0.02[a]	2.48±0.03[a]	2.45±0.04[b]	2.45±0.04[b]	*
	Width	1.20±0.01[a]	1.19±0.01[a]	1.15±0.02[b]	1.10±0.01[c]	**
3rd Tergite length		2.30±0.02[a]	2.25±0.04[a]	2.10±0.01[b]	2.05±0.02[c]	**
3rd Sternite length		2.20±0.02[a]	2.16±0.01[a]	2.12±0.02[b]	2.10±0.0[b]	*
4th Tergite length		2.30±0.01[a]	2.25±0.02[a]	2.21±0.04[b]	2.20±0.04[b]	*
4th Sternite length		2.20±0.06	2.20±0.05	2.14±0.08	2.10±0.06	NS
1st Wax mirror	Length	1.55±0.02[a]	1.55±0.04[a]	1.51±0.03[b]	1.50±0.02[b]	*
	Width	2.50±0.01[a]	2.48±0.02[a]	2.30±0.03[b]	2.20±0.02[c]	**
4th Wax mirror	Length	1.45±0.01[a]	1.44±0.03[a]	1.41±0.02[b]	1.42±0.02[b]	*
	Width	1.42±0.04	1.42±0.07	1.40±0.04	1.40±0.02	NS

Values are the mean ± S.D. Means in each row followed by a different letter are significantly different. ** $p < 0.01$.

The thorax is the center of movement, and the wings are essential for the collection of nectar, pollen, propolis and/or water, in addition to a major role in the thermal regulation of combs (Ajao *et al*., 2014). The dimensions measured for the forewing and hind wing and the numbers of hamuli were significantly different ($p < 0.01$) among comb ages. Workers from new combs had the highest values of length and width of the two wings and number of hamuli on the hind wing. Compared with the hind wing length of a worker obtained from a 4-year-old comb, the hind wing length increased by 3.88%, 2.33% and 1.09% for a worker reared in 1, 2 and 3-year-old combs, respectively. Compared with the femur length of a worker obtained from a 4-year-old comb, the femur length increased by 9.80%, 5.88% and 3.92% for a worker reared in 1, 2 and 3-year-old combs, respectively. Compared with the tibia width of a worker obtained from a 4-year-old comb, the tibia width increased by 14.29%, 11.43% and 4.76% for a worker reared in 1, 2 and 3-year-old combs, respectively. Compared with the metatarsus width of a worker reared in a 4-year-old comb, the metatarsus width increased by 9.09%, 8.18% and 4.55% for a worker reared in 1, 2 and 3-year-old combs, respectively. These results are consistent with those obtained by Alfalah *et al*. (2012) for tibia length. The variations in the measured characteristics of the thorax were likely a result of the significant ($p < 0.01$) variation in body size. Body weight was significantly and positively correlated with forewing length ($r = 0.75$) and width ($r = 0.77$), hind wing length ($r = 0.57$), number of hamuli ($r = 0.56$), femur length ($r = 0.47$), tibia length ($r = 0.70$), tibia width ($r = 0.60$), and metatarsus length ($r = 0.45$). These results confirmed those of Al-Kahtani and Taha (2014) for the dwarf honeybee.

Significant differences were found for the abdominal characteristics among workers obtained from combs of different ages. Compared with the 3rd tergite length of a worker obtained from a 4-year-old comb, the 3rd tergite length increased by 12.20%, 9.76% and 2.44% for a worker reared in 1, 2 and 3-year-old combs, respectively. Compared with the 4th tergite length of a worker from a 4-year-old comb, the 4th tergite length was increased by 4.55%, 2.27% and 0.45% for a worker reared in 1, 2 and 3-year-old combs, respectively. Compared with the 1st wax mirror width of a worker from a 4-year-old comb, the 1st wax mirror width increased by 13.64%, 12.73% and 4.55% for a worker reared in 1, 2 and 3-year-old

combs, respectively. These increases were likely related to the body size of workers. Body weight was significantly positively correlated with the 3rd tergite width, 1st wax mirror length, 1st wax mirror width and 4th wax mirror length (Table 2). Al-Kahtani and Taha (2014) obtained similar results for workers of the dwarf honeybee.

Forewing length was significantly and positively correlated with proboscis length, antenna length, forewing width, hind wing length, number of hamuli, tibia length and width, 3rd sternite width, 1st wax mirror length and width, and 4th wax mirror length. Third sternite length was significantly positively correlated with all determined characteristics, except for proboscis length, hind wing width, tibia length, 4th sternite length, and 1st and 4th wax mirrors lengths and widths. The 1st wax mirror length and width were significantly positively correlated with proboscis length, forewing length and width, hind wing length, and tibia length (Table 2). Al-Kahtani and Taha (2014) found similar results for workers of the dwarf honeybee. Fourth sternite length was negatively correlated with hind wing length and 1st wax mirror length. Additionally, the correlation between 3rd sternite length and hind wing width was negative.

Table 2. Pearson correlation coefficients for body weight and 19 characteristics of Carniolan honeybee (*A. m. carnica*) workers.

Character-istic	BW	PL	FL	AL	FrL	FrW	HnL	HnW	NH	FmL	TL	TW	ML	3rd TL	3rd SL	4th TL	4th SL	1st WML	1st WMW
BW																			
PL	0.74**																		
FL	0.33	0.32																	
AL	0.49*	0.36	0.75**																
FrL	0.75**	0.66**	0.32	0.48*															
FrW	0.77**	0.57**	0.48*	0.66**	0.79**														
HnL	0.57**	0.37	0.34	0.44*	0.65**	0.66**													
HnW	0.34	0.40*	0.16	0.20	0.28	0.23	0.33												
NH	0.56**	0.46*	0.75**	0.70**	0.40*	0.33	0.46*	0.37											
FmL	0.47*	0.35	0.33	0.44*	0.22	0.44*	0.49*	0.11	0.17										
TL	0.70**	0.44*	0.37	0.28	0.48*	0.29	0.55**	0.43*	0.33	0.40*									
TW	0.60**	0.33	0.40*	0.37	0.45*	0.60**	0.45*	0.33	0.36	0.66**	0.71**								
ML	0.45*	0.50*	0.46*	0.38	0.36	0.64**	0.60**	0.27	0.32	0.56**	0.48*	0.35							
3rd TL	0.34	0.19	0.49*	0.47*	0.12	0.34	0.30	-0.08	0.44*	0.56**	0.30	0.58**	0.38						
3rd SL	0.73**	0.35	0.50**	0.73**	0.48*	0.70**	0.55**	0.10	0.50**	0.55**	0.29	0.57**	0.59**	0.73**					
4th TL	0.32	0.10	0.55**	0.30	0.18	0.31	-0.10	0.12	0.48*	0.01	0.26	0.40*	0.41*	0.49*	0.60**				
4th SL	0.28	0.17	0.30	0.36	0.27	0.40*	0.44*	0.31	0.46*	0.38	0.50**	0.63**	0.60**	0.38	0.28	0.19			
1st WML	060**	0.47*	0.17	0.28	0.50**	0.60**	0.56**	0.37	0.45*	0.39	0.60**	0.34	0.33	0.14	0.22	-0.10	0.64**		
1st WMW	0.61**	0.59**	0.34	0.43*	0.47*	0.65**	0.50**	0.40*	0.26	0.47*	0.70**	0.50**	0.46*	0.29	0.37	0.12	0.37	0.47*	
4th WML	063**	0.44*	0.16	0.27	0.55**	0.63**	0.55**	0.33	0.48*	0.37	0.62**	0.32	0.32	0.15	0.22	0.05	0.59**	0.65**	0.35

*Correlation is significant at the level of 0.05 (2-tailed). **Correlation is significant at the level of 0.01 (2-tailed). Body weight = BW, Proboscis length = PL, Flagellum length = FL, Antenna length = AL, Forewing length = FrL, Forewing width = FrW, Hind wing length = HnL, Hind wing width = HnW, No. hamuli = NH, Femur length = FmL, Tibia length = TL, Tibia width = TW, Metatarsus length = ML, 3rd Tergite length = 3rd TL, 3rd Sternite width = 3rd SL, 4th Tergite length = 4th TL, 4th Sternite width = 4th SL, 1st Wax mirror length = 1st WML, 1st Wax mirror width = 1st WMW, 4th Wax mirror length = 4th WML.

CONCLUSIONS

The age of the comb affected the body size of reared workers, which directly affected the sizes of the morphological characteristics related to honey and pollen production and

therefore likely affected the productivity of the colony. Finally, we can use body size of worker as indicator to the morphological characteristics of worker bees related to the activities and productivity of honeybee colonies. Further analyses will be required to reveal the character of honey bee in another subspecies and other area.

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العلاقة بين الصفات المظهرية لشغالات النحل الكرنيولي وعمر القرص الشمعي

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الملخص

أجريت هذه الدراسة في منحل محطة التدريب والأبحاث الزراعية والبيطرية، جامعة الملك فيصل، الأحساء، السعودية؛ وذلك لاختبار تأثير عمر القرص الشمعي على بعض الصفات المورفولوجية لشغالات نحل العسل (سلالة النحل الكرنيولي). بالمقارنة بالأقراص الحديثة، وجد أن حجم العيون السداسية كانت أصغر في الأقراص القديمة. كذلك وجد أن وزن جسم الشغالة ينخفض بالتوازي مع زيادة عمر القرص الشمعي. وبالمقارنة بالشغالات التي رُبيت في الأقراص القديمة، أظهرت الشغالات التي أُنتجت في الأقراص الحديثة تفوقًا ملحوظًا في وزن الجسم، وصفات زوائد الرأس محل الدراسة، وصفات الأجنحة، وخصائص الأرجل الخلفية، وطول وعرض الناحية البطنية لحلقة البطن الثالثة، ومرآة الشمع الأولى. وقد أظهر وزن جسم الشغالة علاقة ارتباط معنوي موجب مع معظم الصفات محل الدراسة. وبذلك يمكن استنتاج أن حجم العيون السداسية في الأقراص الشمعية يمكن أن يؤثر على حجم الشغالات المرباة فيها، ولذلك يمكن التوصية باستبدال الأقراص القديمة والتي تؤثر بشكل مباشر على حجم الزوائد المرتبطة بإنتاج العسل وحبوب اللقاح بأخرى جديدة. كما يمكن استخدام حجم الجسم كعامل ومؤشر للخصائص المورفولوجية لشغالات نحل العسل المتعلقة بأنشطة وإنتاجية طوائف النحل.

الكلمات المفتاحية: الأقراص، الخرطوم، الرسغ القاعدي، شمع النحل، وزن الجسم.