Thermal Tolerance Characteristics of Two Honey Bee Races[1]

Hossam F. Abou-Shaara[2,3]

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ABSTRACT Under elevated temperature conditions, Yemeni honey bees exhibited better thermal tolerance than hybrid Carniolan honey bees. Variations between body characteristics, cuticular lipids, cuticle thickness, and total body water content of the two races were investigated. Yemeni bees were smaller than Carniolan bees in all measured body characteristics. The cuticle thickness of Carniolan bees was significantly higher than that of Yemeni honey bees. The cuticular lipid profiles of the two races were similar, and there were no significant differences in the total body water content between them. The results of this study highlight that body size plays a central role in the high thermal tolerance of Yemeni bees over that of Carniolan bees. However, further investigations are required to understand the variations between the two races.

KEY WORDS *Apis mellifera*, cuticular lipids, body weight

Subspecies of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) display remarkable differences in their tolerance to environmental conditions (Ruttner 1988). For example, Yemeni honey bees, *A. m. jemenitica* Ruttner, can tolerate much higher temperatures (Al-Qarni 2006) than European honey bee workers (Ruttner 1988). Such differences in the tolerance ability of honey bees enable them to exist in a wide range of habitats. Two honey bee races are prevalent in Saudi Arabia, Yemeni bees (the indigenous race) and hybrids of Carniolan bees, *A. m. carnica* Pollmann x Egyptian hybrid (the imported race). Al-Qarni (2006) and Abou-Shaara et al. (2012) have found that in Saudi Arabia the indigenous bee exhibits better thermal tolerance to harsh conditions than imported bees.

One reason for differences in heat tolerance could be the variations in morphological characteristics, with body size playing an important role in the thermoregulation process (Henirich 1996). The rate of total heat loss decreases with body size (Dyer & Seeley 1987). Thorax muscles play a key role in generating heat (Roberts & Harrison 1998) used to regulate the colony temperature. In addition, a correlation exists between the morphological and productive characteristics of honey bee colonies (Cobey & Lawrence 1988, Kolmes & Sam 1991, Mostajeran et al. 2006). Although some morphological characteristics of Yemeni honey bees have been previously investigated (Hoppe & Ruttner

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[2] Plant Protection Department, Faculty of Agriculture, Damanhour University, P.O. Box 22516, Damanhour, Egypt. E-mail: entomology_20802000@yahoo.com

[3] Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia.

1990, Abou-Shaara & Al-Ghamdi 2012), morphological characteristics that correlate with the thermoregulation process have not been well elucidated.

Another possible explanation for the difference in heat tolerance among races is the cuticular lipid content of the two races. Insect epicuticular lipids boost water conservation, which reduces water loss through the cuticle (Jones 1954, Gibbs 1998). In addition, cuticle thickness could also be a factor in preserving body water under elevated temperatures. The highest proportion (about 85%) of body water is lost through the cuticle (Nation 2002). Unfortunately, few studies have been conducted on the cuticle thickness of honey bees. Total body water content, in combination with the previously mentioned factors, could play a key role in body tolerance to thermal stress. Therefore, the present study examines variations between morphological characteristics (body and cuticle thickness), cuticular lipids, and total body water content of Yemeni and hybrids of Carniolan honey bees to better understanding factors involved in thermal tolerance.

Materials and Methods

Yemeni honey bees, *A. m. jemenitica*, and hybrids of Carniolan honey bees, *A. m. carnica*, x Egyptian honey bees (Carniolan honey bees throughout this paper) were obtained from the Bee Research Unit, King Saud University (KSU), Riyadh, Saudi Arabia.

Body characteristics. It is known that at least 15 workers per colony are sufficient for morphological studies (Abou-Shaara et al. 2013a). Thus, for each race of honey bees, 30 forager workers were collected from each of ten colonies. Collection containers were weighed to the nearest 0.01 mg (GR 200 balance, A&D Company, Ltd., Tokyo, Japan) before (W1) and after (W2) the collection of 30 bees, and the mean weight per bee was calculated [(W2 − W1)/30] for each honey bee race. Workers were then immobilized in a freezer for up to 15 min. To obtain thorax images, immobilized workers were scanned on their dorsal surface using an HP Scanjet 8300 scanner (Hewlett Packard, Palo Alto, CA) at 236.2 dots/cm [= 600 dots per inch (dpi)]. Subsequently, head, fore wings, and hind wings were separated, mounted onto transparent sticky sheets, and scanned (236.2 dots/cm) to a personal computer. Dimensions were then measured using the ruler tool of Adobe Photoshop® (Adobe Systems Inc., San Jose, CA) according to Abou-Shaara et al. (2011). Such measurements are considered proportional proxies to body size (Waddington & Herbst 1987, Hunt et al. 1998).

Cuticular lipids. The cuticular lipids of honey bees are known to differ between workers of the same colony as well as between colonies of the same race (Kather et al. 2011). Therefore, a qualitative analysis of the cuticular lipid profiles for the two races was conducted using a GC/MS method adopted from Kather et al. (2011). Three returning forager bees were collected from the entrance of each colony and three colonies per race were used in the analyses (9 workers per race). Schmitt et al. (2007) used five forager worker bees during their study of cuticular hydrocarbons, thus the sample size we used was considered sufficient. Worker bees were then killed by freezing and stored at −20°C until analysis. Each bee was placed in 0.5 ml HPLC-grade hexane for 2 min. Then, 50 µL of the extract was transferred to an open glass vial to evaporate the solvent. Prior to the analysis, each sample was silylated with 30 µL BSTFA at 70°C for 20 min. The samples were then dried and 30 µL hexane was added to each. The

samples were analyzed on an Agilent 7890A GC system (Agilent Technologies Inc., Santa Clara, CA) coupled to a 5975C MS (triple-axis detector). The GC was equipped with an Agilent 19091S column (250 μL ID × 30 m, 0.25 μL film thickness). Helium was used as the carrier gas at a constant flow rate of 1 ml/min. A temperature program of 70°C to 200°C at 40°C/min and 200°C to 320°C at 25°C/min was used in the analysis. Samples were injected in the splitless mode. Detected compounds were considered to be representative of the cuticular lipid profile of the two races. Compounds from the two races were identified using a National Institute of Standards and Technology (NIST) mass spectral (MS) database (NIST 08.L) (NIST 2008), considering only those compounds with a probability above 80%. Also, only the previously detected compounds from honey bees described by Kather et al. (2011) and Schmitt et al. (2007) were considered during the comparison of the two races.

Cuticle thickness. Three forager honey bee workers were collected from each of the Carniolan and Yemeni honey bee colonies. These bees were immersed directly into 70% ethyl alcohol. Elias-Neto et al. (2009) used at least three specimens during their histological study on honey bees, thus the sample size we used was considered sufficient. The thoraxes were then separated and placed in 70% ethyl alcohol until analysis. The separated thoraxes were carefully cut in half along the medial line, and three sites on the second tergite were chosen to measure the cuticle thickness (Figures 1 & 2) using an Inspect S50^{TM} scanning electron microscope (FEI, Eindhoven, The Netherlands). The total of 18 measurements was taken for each of the two races (3 sites per thorax, and 3 thoraxes per race).

Total body water content. The total body water content of the honey bees was determined by first collecting 60 forager worker bees of each race (10 bees per glass container with 6 containers per race). This sample size was considered sufficient because Murylev et al. (2012) used 30 bees of each race during estimation of total body water content. The container weight (W0) was recorded on a Mettler PJ6000 balance (Mettler Toledo AG, Greifensee-Zürich, Switzerland), and the weight of the container with the collected bees was recorded (W1). The wet weight (W3) of the bees was calculated as W1 − W0. These samples were subsequently dried at 100°C for 48 h in accordance with the method of Murylev et al. (2012), and their dry weights were recorded (W4). The percentages of total body water were calculated as follows: [(W1 − W4)/W3] × 100.

Statistical analysis. Means were compared using a *t*-test at a confidence level of 95% with SAS 9.1.3 (SAS Institute 2004).

Results

Yemeni honey bees were smaller than Carniolan honey bees in all measured characteristics (Table 1). In addition, mean body weight of Yemeni honey bees was 28.6% lower than Carniolan honey bee workers (Table 1). Significant differences were found between all measured characteristics ($P < 0.05$) according to a *t*-test. However, there was a high degree of similarity in the cuticular lipid profiles of Yemeni and Carniolan honey bees (Table 2). Fifteen alkanes were detected in Yemeni honey bees, while only 13 alkanes were detected in Carniolan honey bees (dodecane and tricosane were not detected). Four alkenes were detected in Yemeni honey bees while all alkenes except heptadecene were

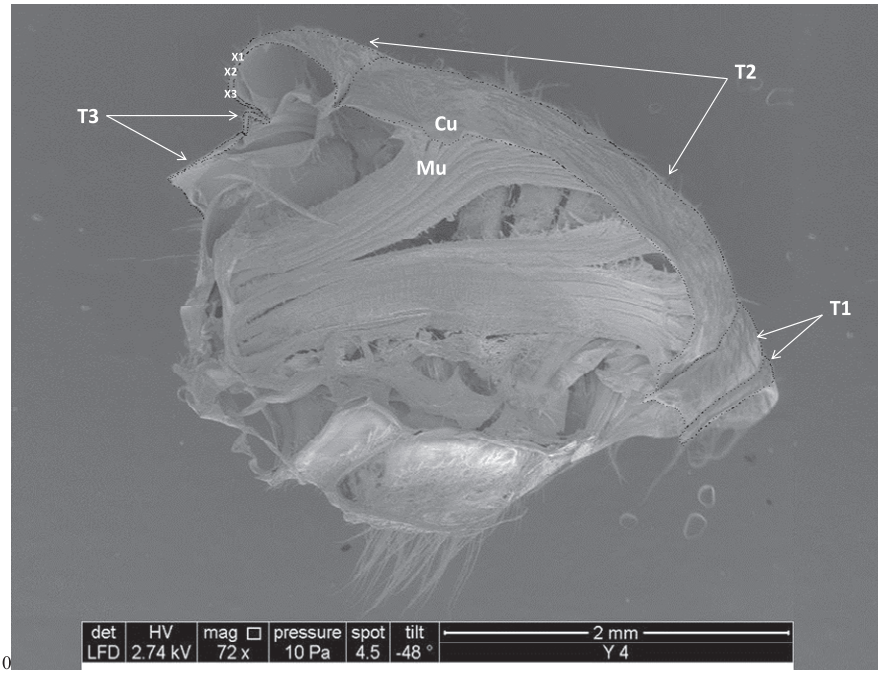

Fig. 1. Half thorax of honey bee worker shows the sites X1, X2, and X3 for measuring cuticle thickness at the 2nd tergite (T1: 1st tergite, T2: 2nd tergite, T3: 3rd tergite, Cu: Cuticle, and Mu: Muscles).

detected in Carniolan honey bees. No differences were found in the two fatty acids observed. Mean cuticle thickness for Carniolan honey bees at 16.0 ± 0.5 µm (mean ± SE) was significantly thicker (t = 5.21, P = 0.0001) than the cuticle thickness mean for Yemeni honey bees at 12.5 ± 0.4 µm. The difference between

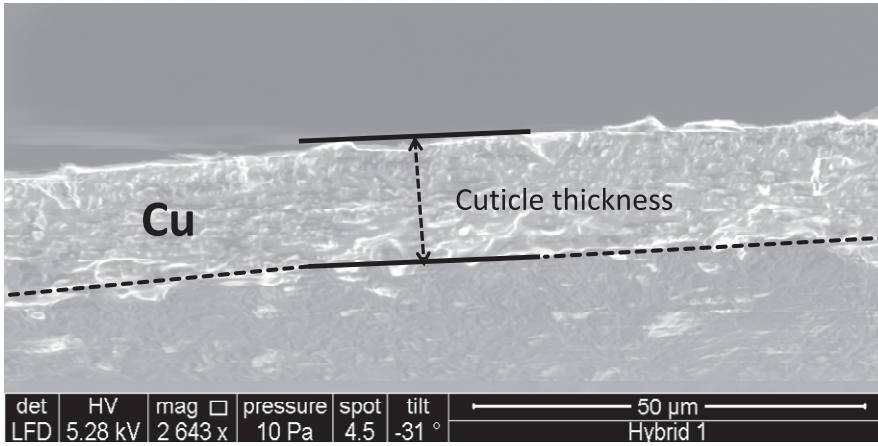

Fig. 2. Honey bee cuticle with measurement of thickness (Cu: Cuticle).

Table 1. Measurements of morphological characteristics (means ± SD) of Yemeni and Carniolan honey bees.

Characteristic	Mean ± SD		Percent difference
	Yemeni honey bees	Carniolan honey bees	
Fore wing length (mm)	7.79 ± 0.21 b	8.78 ± 0.22 a[1]	11.3
Fore wing width (mm)	2.77 ± 0.12 b	3.10 ± 0.13 a	10.6
Hind wing length (mm)	5.46 ± 0.16 b	6.14 ± 0.15 a	11.1
Hind wing width (mm)	1.59 ± 0.12 b	1.82 ± 0.13 a	12.6
Head length (mm)	2.69 ± 0.13 b	3.12 ± 1.50 a	13.8
Head width (mm)	3.31 ± 0.11 b	3.64 ± 0.09 a	9.1
Thorax length (mm)	2.99 ± 0.13 b	3.32 ± 0.16 a	9.9
Thorax width (mm)	2.45 ± 0.20 b	2.94 ± 0.17 a	16.7
Body weight (mg)	71.4 ± 4.8 b	99.9 ± 6.9 a	28.6

[1]According to a *t*-test, means within rows for the two bee races were significantly different ($P < 0.05$) for all characteristics.

the cuticle thicknesses of the two races was approximately 3.5 μm (22.2% less for the Yemeni bees). The total body water content was 70.2 ± 1.4% (mean ± SE) for Carniolan honey bees and 67.0 ± 2.0% for Yemeni honey bees (3.2% less for the Yemeni bees), however this difference was not significant according to a *t*-test ($t = 1.33$, $P = 0.21$).

Discussion

Body characteristics. Yemeni honey bees were smaller than Carniolan honey bees in all measured characters, and the differences in wing lengths and widths between the two races are similar to previous findings of Abou-Shaara & Al-Ghamdi (2012). The smaller size of the Yemeni bees may be an adaptation for living in hot and arid environments. It has been reported that Yemeni honey bees have a higher tolerance to hot conditions than do Carniolan honey bees (Al-Qarni 2006, Abou-Shaara et al. 2012). Accelerated body cooling could help explain the adaptation of small body size in hot conditions (Dyer & Seeley 1987, Heinrich 1996). On the contrary, the larger size of Carniolan honey bees may enable them to adapt to moderate and cold conditions by generating more heat, thus keeping their bodies warmer.

Cuticular lipids. It is known that the insects living in hot and arid environments have an abundance of alkanes (Hadley & Schultz 1987). Alkanes have a high melting point, which helps reduce water loss through the cuticle and protects insects from desiccation (Gibbs 1998). Yemeni bees had two more alkanes than Carniolan bees, which had a lipid profile similar to that reported by Abou-Shaara et al. (2013b). However, this minor variation in alkane profiles between the two races probably doesn't fully explain the differences in thermal tolerance, and other factors may play a role.

Few difference in alkenes and fatty acids were detected between the two races. Similarly, Kather et al. (2011) found no differences between honey bee colonies in

Table 2. Cuticular lipids of Yemeni and Carniolan honey bees.

Retention time (min.)	Chemical class	Component	Presence (+) or absence (–) of component	
			Yemeni honey bees	Carniolan honey bees
3.507	Alkane	Dodecane	+	–
3.851	Alkane	Tetradecane	+	+
4.209	Alkane	Tridecane	+	+
4.983	Alkane	Octacosane	+	+
5.727	Fatty acid	Hexadecanoic acid	+	+
6.328	Alkane	Heneicosane	+	+
6.455	Fatty acid	Octadecanoic acid	+	+
6.671	Alkane	Docosane	+	+
6.755	Alkene	Docosene	+	+
7.004	Alkane	Heptadecane	+	+
7.273	Alkane	Tricosane	+	–
7.669	Alkane	Pentacosane	+	+
8.028	Alkane	Hexacosane	+	+
8.433	Alkane	Heptacosane	+	+
9.395	Alkane	Nonacosane	+	+
9.567	Alkane	Hexadecane	+	+
9.988	Alkane	Triacontane	+	+
10.718	Alkane	Tetracosane	+	+
10.597	Alkene	1-Nonadecene	+	+
12.385	Alkene	9-Triosene	+	+
12.585	Alkene	Heptadecene	+	–

alkenes and fatty acids. These two groups of chemicals have a role in nestmate recognition as they are easily recognized and discerned (Chaline et al. 2005), but have no clear role in reducing body water losses.

Cuitcle thickness. In this study, Carniolan honey bees had a significantly thicker cuticle than Yemeni honey bees, but this does not help explain the difference in the thermal tolerance between the two races. Even though Yemeni honey bees have a thinner cuticle, they have more thermal tolerance than Carniolan honey bees as found by Abou-Shaara et al. (2012). They found that Yemeni and Carniolan honey bees began to display intolerance at 61°C and 57.5°C, respectively, when they were exposed to temperature program from 30°C to 70°C, and Yemeni honey bees survived longer at 40°C and low relative humidity of 15% compared with Carniolan bees. Additionally, layers within the cuticle may be very thin, with the epicuticle layer being only 1 to 4 μm (Nation 2002), and the wax layer of honey bees is only 0.13 μm (Lockey 1959).

Total body water content. No significant differences were found in the total body water content of Yemeni and Carniolan honey bees, and the means for the two races are in accordance with those obtained by Murylev et al. (2012) for *A. m. mellifera* and *A. m. carpathica*. They found that body water content ranged from 63.2% to 72.4% and from 67.3% to 74.2% for *A. m. mellifera* and *A. m.*

carpathica, respectively during different seasons. In general, the high body water content could be explained by the nectar diet of the honey bees (Nicolson 2008).

The results of this study highlight that cuticular lipids, cuticle thickness, and body water content have no clear role in the higher thermal tolerance of Yemeni honey bees over that of Carniolan honey bees. However, this study provides evidence that Yemeni honey bees may be more tolerant to thermal stress than Carniolan honey bees due to their smaller size. Moreover, further investigations on genetic differences and heat shock proteins are required to understand the variations between the two races in their tolerances of thermal stress.

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