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15

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ABSTRACT

Honey bees (*Apis mellifera*) consist of more than 24 different subspecies. Most of these subspecies have been classified according to their morphological characteristics, and morphological characteristics thus have an important role in the classification aspects of honey bees. Different sets of wing and body morphological characteristics have been used to characterize and classify the subspecies by many authors and for various reasons. These characteristics were defined over time and combined from various studies. Wing venation characteristics have been studied more intensely than other body morphological characteristics. Up to now there are no specific review articles focus mainly on body morphological characteristics. Therefore, the available information about sampling method, measuring method, importance and factors affecting these characteristics were reviewed to present essential conclusion and recommendations for researchers.

Key words: *Apis mellifera*, morphometry, body characteristics, honey bees

INTRODUCTION

The honey bee, *Apis mellifera* L., is globally widespread with a wide diversity of subspecies. These subspecies can be classified with morphometric tools (Ruttner et al. 1978). Many studies have been undertaken on honey bees using morphological characteristics (e.g. Abou-Shaara et al. 2012 and Garnery et al. 2004). These characteristics can be divided into three major groups; which are length measurements, color measurements, and wing venation characteristics. Wing venation characteristics were previously reviewed intensively by Abou-Shaara (2013). Here, the major studies that have been done using body morphological characteristics were reviewed to provide recommendations about sampling, measuring method and limitations of body morphological characteristics.

IMPORTANCE OF MORPHOLOGICAL MEASUREMENTS

Body morphological characteristics can be measured for different reasons. A major use is to characterize honey bee races and individuals (Ruttner 1988, Meixner et al. 2007), but also to determine the degree of hybridization with foreign races (Radloff et al. 2003 and Bienefeld et al. 1996). Also, for the discrimination between honey bee subspecies (e.g. Abou-Shaara and Al-Ghamdi 2012, Tofilski 2004). Moreover,

morphological characteristics were measured to investigate the impacts of imported queens on honey bee populations (Guler 2010) or to check populations purity (Miladenovic et al. 2011). Multiple body characteristics, including wing length, wing width and tongue length were used to differentiate between honey bee subspecies (Bucu et al. 1987, Rinderer et al. 1993, Crewe et al. 1994, Ftayeh et al. 1994, Diniz-Filho and Malaspina 1995, Szymula et al. 2010).

Tongue length was found to be an indicator of geographical variation in some studies (Marghitas et al. 2008, Morimoto 1968, Souza et al. 2002). Proboscis length was also found to be the most differentiated characteristics between *A.m.mellifera*, *A.m.carnica* and *A.m.caucasica* (Szymula et al. 2010). In addition, body measurements may show correlations to honey yield. Kolmes and Sam (1991) found that honey production was highly correlated to overall size, corbicular area and wing measurements in Carniolan honey bees. Body characteristics may thus be used for indirect prediction of colony productivity or for selection of productivity where honey bees with bigger legs and wings have higher power flight and could gather more pollen and nectar for brood rearing and consequently colony population (Mostajeran et al. 2006). There is a positive correlation between honey production and corbicular area (Milne and Pries 1984). Szabo and Lefkovich (1988) found that honey production had significant and positive correlations with both fore and hind wing area. Mostajeran et al. (2002) found that honey production was related to tongue length, fore wing length and width, hind wing length, leg length, femur length, tibia

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length and metatarsus width. Waddington (1989) found a correlation between body size and colony productivity. Edriss et al. (2002) indicated that honey production can be improved through selection of the forewing width. Therefore, there is evidence that body morphological characteristics are very important and correlated with colony productive characteristics. However, it must be noted that these relations may attribute to specific conditions rather than indicating general rules.

COLLECTION OF BEE SAMPLES

At least 15 honey bee workers should be collected from each colony during the morphological analysis (Ruttner et al. 2000, Sheppard and Meixner 2003, Meixner et al. 2007, Guler 2010) and at least eight colonies per district can be considered sufficient for morphological study (Abou-Shaara et al. 2012). However, Miguel et al. (2011) used only one honey bee worker per colony for geometric morphometric but generally more is required to obtain reliable results. Samples can be collected in a number of ways (i) directly from brood comb according to Padilla et al. (1992). (ii) shaking bees into a jar, (iii) collecting forager bees (iv) taking one-day old bees by placing sealed brood combs into incubators. Collected bees can be preserved in 95% ethanol until dissection (Arias et al. 2006) or in 70% ethanol (Adl et al. 2007) or killing by a deep-freezer and then dissected (Abou-Shaara et al. 2012). Also, the temporary preparation of the samples can be used (Miladenovic et al. 2011).

It needs to be taken into account that there are some factors that can impact on the morphological characteristics. Comb cell size has an impact on morphological characteristics (Ruttner 1988, McMullan and Brown 2006, Gencer and Firati 2005) where workers emerged from large wax cells have larger morphological characteristics. Sample size and time of taking the samples thus could affect comparisons between different data for body morphological characteristics. In general, it is very important to take samples for morphological analysis at the same time for all studied replicates and try taking samples from new combs and under the same condition of feeding as possible.

METHODS OF MEASURING BODY MORPHOLOGICAL CHARACTERISTICS

After the collection of the samples, samples can be mounted on sticky pieces as described by Abou-Shaara and Al-Ghamdi (2013) to facility characteristics measuring. Also, other mounting methods for body parts (e.g. double glass slides) can be used (Abou-Shaara et al. 2011). Several methods have been used to take the body measurements; (i) stereomicroscope with an ocular micrometer (Ruttner et al. 1978, Mattu and Vermam 1984, Edriss et al. 2002, Souza et al. 2002, Sirali et al. 2003, Gencer and Firati 2005, Cakmak et al. 2006, Tan et al. 2006, Mostajeran et al. 2006, Adl et al. 2007, Marghitas et al. 2008). (ii) Photomicroscope (Morrison-Olson 2002). (iii) Projecting mounted slides onto a TV screen

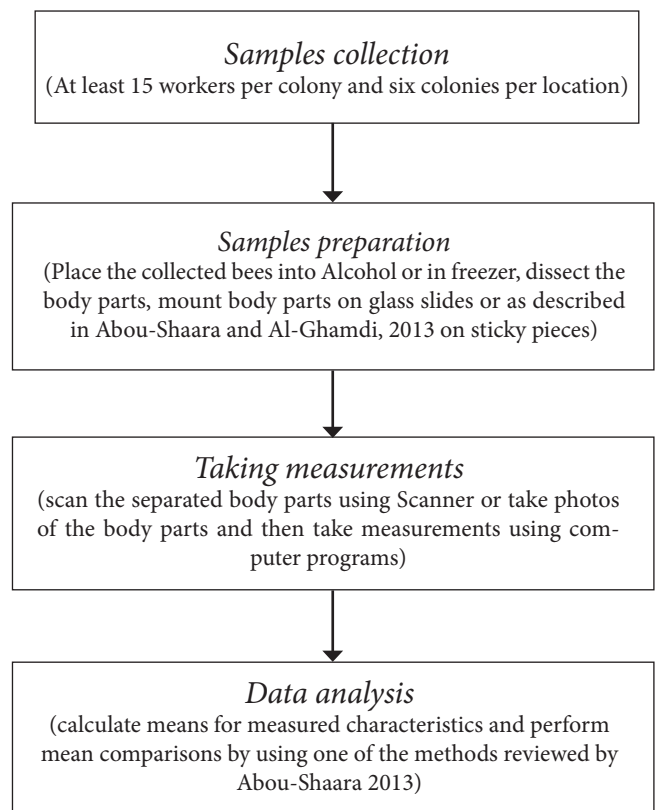


Figure 1: The suggested steps for the morphometric analysis based on body characteristics

(Kandemire et al. 2000). (iv) Using computer-based methods mainly for forewing and some body characteristics (Meixner 1992, Rinderer et al. 1993, Ruttner et al. 2000, May-Itza et al. 2001, Kamel et al. 2003, Schnider et al. 2003, Sheppard and Meixner 2003, Haddad and Fuchs 2004, Meixner et al. 2007, Shaibi et al. 2009, Miladenovic et al. 2011, Abou-Shaara et al. 2012, Abou-Shaara and Al-Ghamdi 2012). (v) Special programs using image analysis systems and the IMAGO program (Padilla et al. 1992). Computer program Object-Image Pre2.11, at a scale of 150:1 (Jones et al. 2005). The image analyzer IMAGEPRO plus version 3.0.1 for Windows 3.1 and Media Cybernetics were also used Andere et al. (2008).

Moreover, Abou-Shaara et al. (2011) presented a simple method for measuring body and wing morphological characteristics by using photoshop program; this method was called Scan Photo method while Miladenovic et al. (2011) used AutoCAD program to take the measurements. In general, all the previous methods can be used but it is preferable to use computer-based method to save time where microscopic methods were found to be time-consuming (Szymula et al. 2010). Moreover, any suitable software can be used in combination with camera or scanner to take the measurements (Fig. 1).

BODY CHARACTERISTICS

Various body characteristics of honey bees were measured by many authors (e.g. Meixner et al. 2007, Shaibi et al. 2009, Miladenovic et al. 2011, Abou-Shaara et al. 2012, Abou-Shaara

and Al-Ghamdi 2012). Commonly, body characteristics are normally measured according to Ruttner et al. (1978) and Ruttner (1988). These characteristics can be divided according to body parts into; head, thorax and abdomen characteristics. (i) head characteristics include; head capsule width (HCW) and length (HCL), antenna length (AL) and number of segments (ANS), compound eyes length (CEL) and width (CEW), and tongue length (TonL). Some authors also studied the mandible length (ML) beside some other characteristics. (ii) thorax characteristics; fore wing length (FWL) and width (FWW), hind wing length (HWL) and width (HWW), number of hooks (NH), thorax width (ThW), femur length (FL), tibia length (TL), basitarsus length (BL) and width (BW), and pollen basket size (PBS), brush hair rows number (HN). (iii) abdomen characteristics; lengths of tergite 3 (T3) and 4 (T4), body size (T3+T4), length of hairs on tergite 5 (HLT5), pigmentation of tergite 2-4, length of sternite 3 (LS3), wax mirror length (WML) and transversal (WMT) and sting shaft length (StL). In general, measurements have to be taken as the maximum distance and in units of millimeters (mm) except the number of hooks. Moreover, there are some indexes were revealed from these characteristics (e.g. forewing index = length /width of fore wing).

FACTORS AFFECTING BODY MEASUREMENT VARIATION

Previous works on honey bee workers showed that environmental factors have a major impact on morphological characteristics (Eischen et al. 1982, Milne and Pries 1984, Milne et al. 1986, Stanimirovic et al. 2008). Marghitas et al. (2008) found that in the mountain regions of Transylvania for example worker proboscis were longer 6.21 mm than that in lower regions 5.99 mm. The importation of honey bee subspecies into different areas might induce high levels of hybridization within populations (Garnery et al. 1998, Rortais et al. 2004, Alqarni et al. 2011) and produce subspecies admixtures (Arias et al. 2006). Also, migratory beekeeping may play a key role in forming differences (Marghitas et al. 2008). Morphological characteristics for uncontrolled honey bee populations showed low stability through time (Abou-Shaara et al. 2012). Thus to characterize uncontrolled populations, taking the characteristics mean for two successive years is highly recommended. Some other factors that may impact on wing and body morphological characteristics were reviewed by Abou-Shaara (2013).

CONCLUSIONS

Various methods were used in taking morphological measurements. However, computer-based methods using programs (e.g., Photoshop, image tool and AutoCAD) could be recommended to save time and obtain accurate measurements. It is worth noting that the sample size, sampling season, sampling technique and measuring method differ from author to another and from country to another and should be better harmonized. Therefore, it

is recommended to use standard methods for measuring these characteristics to facility comparing results of different subspecies and countries. Figure 1 shows the recommended steps for the morphometric analysis based on body characteristics. Fifteen workers per colony and six colonies per district should be sufficient for sample size. Taking samples from colony combs are easier than forager bees. Ongoing evaluation of morphological characteristics could help in understanding racial fluctuations due to beekeeping, hybridization and environmental factors. In addition, morphological characteristics are also correlated with colony productive characteristics. As a result, body morphological characteristics can be used as a simple indicator for estimating fluctuations in genetic and productive characteristics of honey bee colonies. It is apparent that still more work is required to provide insights into the seasonal impacts on body morphological characteristics.

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