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Study of genetic diversity of *Apis mellifera* (Hymenoptera: Apidae) populations in Kurdistan region of Iraq by Morphological markers

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ABSTRACT: Determining the status of genetic Characteristics in living animals and insect is the first step in their breeding. In the current study, a random sample of honey bee workers (400) was taken from 40 colonies in three cities of Sulaymaniyah province in Kurdistan region of Iraq. 12 morphological characters (i.e. the proboscis length, hind leg length, forewing width, forewing length, A4, D7, G18 angles, cubital index, tergit3 + tergit4 length, slenderness index, scutellum color, third & forth tergit color) were measured. Honey bee colonies of three cities have been separated perfectly using morphological characters. The results of study showed that the genetic diversity among and between of honey bee populations in these regions is high. This research provided new information about genetic diversity in selected local honeybee in Kurdistan region of Iraq and will be useful for selection, future local biodiversity conservation and controlled breeding programs.

***Keywords*:** Honey bee, *Apis mellifera meda*, morphological characters, Kurdistan, Iraq

INTRODUCTION

Honey bees (Hymenoptera, Apidae) were naturally distributed in Europe, Africa, and Western Asia before they were spread around the world by human beekeeping activities. The intraspecific taxonomy of *Apis mellifera* L. has been based mainly on morphometric approaches, which subdivide this honeybee subspecies into four different evolutionary lineages: North Mediterranean (C); West Mediterranean (M), African (A); and Oriental (O), located geographically in the Middle East and represented mainly by Caucasian (*Apis mellifera caucasica* Pollmann, 1889), Turkish (*Apis mellifera anatoliaca* Maa, 1953) and Iranian (*Apis mellifera meda*) honeybees (Ruttner, 1988). Honey bees are the most economically valuable pollinators of agricultural crops worldwide (Johnson, 2010). The European honeybee *Apis mellifera* shows a remarkable regional differentiation, as 26 subspecies are known from Africa and the western Palearctic region (Solignac et al., 2003; Sheppard and Meixner, 2003). From morphometric, molecular, ecological, ethological and physiological studies, the 26 subspecies of the honey bee, *A. mellifera*, are grouped into five evolutionary lineages: M from northern and western Europe and northern Africa, A from southern and central Africa, C from the northern Mediterranean region and eastern Europe, O from the eastern Mediterranean and the Near and Middle East region, and Y from the east African country of Ethiopia (Rortais et al., 2011; Ruttner, 1988; Hall and Smith, 1991; Garnery et al., 1992; Arias and Sheppard, 1996; Franck et al., 2000, 2001). From morphological and topographical approach, Iraq is a relatively high

plateau, which is located in the middle part of Alpine-Himalayan fold belt and has climate diversity including dry, semidry, mountainous, cold mountainous and moderate mountainous.
These climatic complexity can be reflected in a considerably diverse flora and fauna, including honey bees (Kauhausen-Keller et al., 2011; Karacaoglu, 1995; Budak, 1995). In Iraq Two species of honey bees, *Apis florea* and *A. mellifera* have been known till now. The distribution place of *A. florea* is just southern east – southern belt of Iraq, but *A. mellifera meda* distributed all around the country (except for deserts). It is the dominant strain of Iraq and unfortunately is neglected in scientific project. It has several sub-communities in Iraq which have different characters, functions and honey production. In fact different climatic conditions have caused biological, behavioral and morphological differences of honey bee in the country which are origin of ecological types of this subspecies. Identification of these ecotypes can help us in the separation of our population and their use in further breeding programs. With regards to the fact that the changes in the honey bee body during the evolution were made to operate the geographical region flowers and the superior strains have the smaller body and long legs, wings and proboscis, So morphometrical studies in division of our honey bee masses have been used in this study. morphological differences in this insect is not only influenced by climatic and geographical conditions, but also other factors including sampling time, pollution by Varroa mite, cell size, larva nutrition, the height of bees habitat. In the study which was conducted on the honey bee masses throughout Iraq in 1989 by Ataollah total Iraqian honey bee community was divided into three sub communities of honeybees in the 1-south, 2-west and western-north and 3-central area. Ataollah (1989) took all of the Kurdistan masses into one population at that time. In this study, we focused on honey bee characters of three cities of Kurdistan province in details (separately) which is done for the first time, so there is no published report concerning the morphological diversity of Kurdistan subcomunities.

MATERIALS AND METHODS

Sampling:

In this study, samples were taken from 40 colonies of four apiaries located in each city (Sulaymaniyah, Kafri and Kalar). 10 colonies from each apiary and 10 honey bee workers from each colony were selected randomly in April 2018. The sampling was done from brood areas on the combs, using the open mouth jar containing the cotton soaked to the ether and poured into Pumpel solution (30 parts distilled water, 6 parts formaldehyde, 15 parts ethanol 65% and 2 parts acetic acid) in the laboratory. 10 samples were selected randomly from every jar for morphometric operations.

Morphological measurement:

Bees were transformed into bottle contain of pample solution. Measuring the apparent characteristics was done on 10 worker bees from each colony. 400 workers were dissected totally and 12 characteristics (i.e. the proboscis length, hind leg length, forewing width, forewing length, A4, D7, G18 angles, cubital index, tergit3 + tergit4 length, slenderness index, scutellum color, third & forth tergit color) were measured according to Ruttner et al. (1978).

Data analysis:

Analysis were done by statistical software, SAS software (2002), Minitab 14 and SPSS v. 11.5.

RESULTS

In this study, in order to investigation genetic diversity in honey bee populations of Kurdistan region of Iraq morphlogical marker were used. Results obtained from the measurement of twelve external characteristics are presented in the table 1(the Kurdistan results of Ataollah study (1998) provided in this table too).

Table1. The measurement of external characteristics of honey bee worker in Kurdistan province (total colonies) (Mean ±SEM)

Variable	Mean± SEM	Min	Max	Ataollah results
Forewing length (mm)	0.00307± 9.023	8.7	9.6	9.1489± 0.0136
Forewing width (mm)	0.00219± 3.021	2.9	3.4	3.1202±0.00651
Angle A4	0.044± 30.121	27	36	0.1322±30.082
Angle D7	0.137± 97.222	84	107	0.2112±100.63
Angle G18	0.120± 94.116	77	103	0.1619±94.059
Cubital index	0.0116± 2.366	1.7	3.4	0.0319±2.3812
Hind leg length (mm)	0.00712± 7.446	6.3	9	0.0128±7.7283
Proboscis length (mm)	0.00737± 6.084	4	6.8	0.0184±6.3499
Tergit3+ tergit4 length (mm)	0.0046± 4.079	3.7	4.5	0.009135±4.3887
Slenderness Index	0.00334± 0.765	0.64	0.99	0.0111±0.8109
Scutellum color	0.134± 7.366	0	9	0.1367±7.8137
3rd abdominal tergit color	0.0777± 6.886	0	9	0.1467±6

The average measurement of characteristics and correlation amounts among measured characteristics in studied cities are reported in the table 2 and 3. Also, the comparisons of characteristics in the different cities are presented in the table 4.

Table 2: The average measurement of characteristics in Sulaymaniyah, Kafri and Kalar (Mean±SD).

Variable	Sulaymaniyah	Kafri	Kalar
Forewing length (mm)	9.07 ± 0.07	9.13 ± 0.04	9.06 ± 0.05
Forewing width (mm)	2.78 ± 0.06	3.03 ± 0.014	3.04 ± 0.05
Angle A4	30.05 ± 0.46	30.13 ± 0.43	30.15 ± 0.15
Angle D7	98.17 ± 1.36	97.94 ± 1.04	98.54 ± 0.96
Angle G18	92.67 ± 1.26	92.74 ± 0.14	93.95 ± 0.67
Cubital index	2.46 ± 0.07	2.43 ± 0.15	2.44 ± 0.05
Hind leg length (mm)	7.27 ± 0.06	7.34 ± 0.06	7.34 ± 0.06
Proboscis length (mm)	6.06 ± 0.03	6.03 ± 0.05	6.14 ± 0.07
Tergit3+ tergit4 length (mm)	4.06 ± 0.054	4.13 ± 0.04	4.05 ± 0.03
Slenderness Index	0.77 ± 0.03	0.74 ± 0.04	0.75 ± 0.03
Scutellum color	7.26 ± 0.23	7.73 ± 0.54	6.54 ± 0.26
3rd abdominal tergit color	7.07 ± 0.14	7.54 ± 0.15	5.94 ± 0.23

Table 3: The correlation amounts among measured characteristics (**: meaningful correlation in the level 99%, *: meaningful correlation in the level 95%).

	Wing width	Wing length	Angle A4	Angle G18	Angle D7	Cubital index	Probos cis length	Hind leg length	Tergit3+ tergit4 length	Slender nes Index	Scutellum color
Wing length	**0.529	-	-	-	-	-	-	-	-	-	-
Angle A4	0.055	*0.045-	-	-	-	-	-	-	-	-	-
Angle G18	0.015	0.052-	**0.063	-	-	-	-	-	-	-	-
Angle D7	0.043	0.031	0.053	**0.434	-	-	-	-	-	-	-
Cubital index	**0.142	**0.061	0.072-	0.020-	0.014	-	-	-	-	-	-
Proboscis length	**0.151	**0.168	0.050-	0.035	0.019	0.026-	-	-	-	-	-
Hind leg length	**0.114	**0.199	**0.079-	0.047	0.047-	0.031	**0.130	-	-	-	-
Tergit3+tergit4length(mm	**0.142	**0.126	0.079-	0.052-	0.026	0.018	**0.048	*0.060	-	-	-
Slenderness Index	0.0155-	0.039	0.080-	0.077	0.047	0.053-	0.021-	0.042-	0.011	-	-
Scutellum color	0.340	0.053	0.052-	0.051-	0.042-	0.035	0.045-	0.046	0.040-	0.048-	-
3rd abdominal tergit color	*0.0437	**0.063	0.048	**0.094-	*0.071-	0.024	0.003-	0.051-	0.051	0.023-	**0.563

Table 4: Coefficient of principle components in different external characteristics in differentiations of three cities.

variable	Second Principle Component	First Principle Component
Fore wing length (mm)	-0.321	-0.084
Forewing width (mm)	-0.440	-0.060
Angle A4	-0.214	0.347
Angle D7	0.1677	0.363
Angle G18	-0.057	0.338
Cubital index	-0.440	-0.021
Hind leg length (mm)	-0.145	0.377
Proboscis length (mm)	-0.331	-0.271
Tergit3+ tergit4 length (mm)	-0.284	-0.186
Slenderness Index	-0.414	0.150
Scutellum color	-0.113	-0.341
3rd tergit color	-0.125	-0.393

Table 5. Value and eigenvalue of correlation matrix in every one of principle component in different citation of three cities.

		Component amount	Density diversity of component (%)	Relative diversity of component (%)
First Component	Principle	5.58	54.70	54.70
Second Component	Principle	4.31	100	55.30

Table 6: The characteristics related to the differentiating studied cities in the main compilations.

City	Second Principle Component	First Principle Component
Sulaymaniyah	2.48	-1.24
Kafri	-2.42	-1.54
Kalar	-0.25	2.88

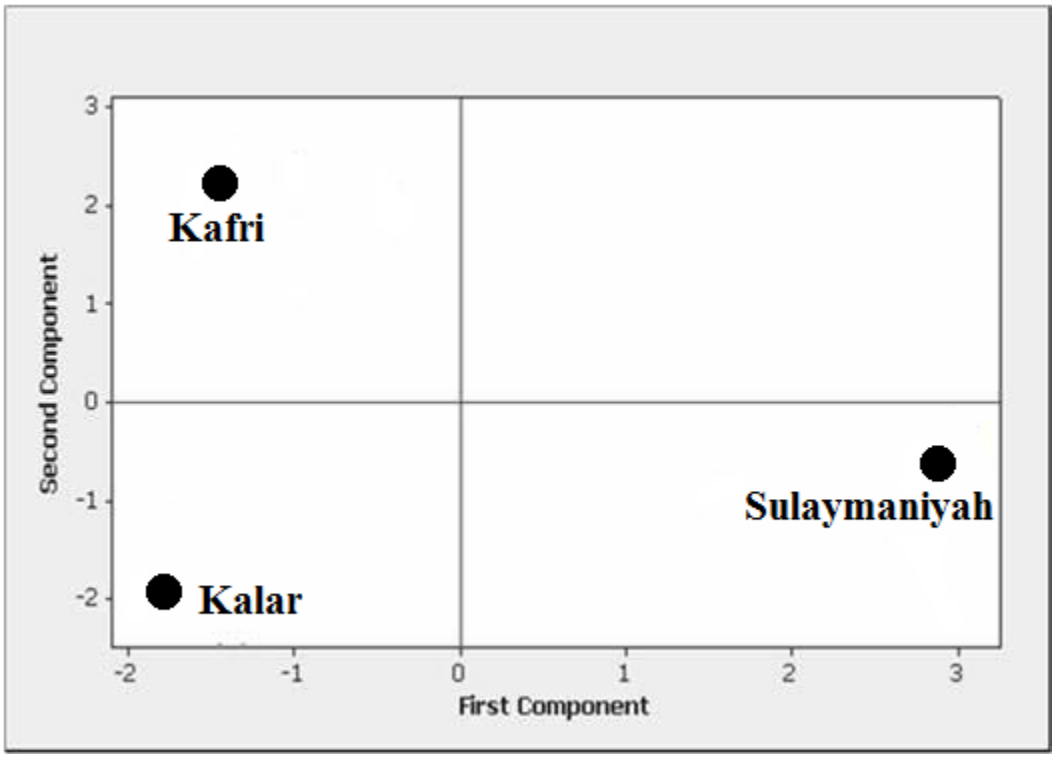

Figure 1. Comparison and separation of studied cities using the analysis to the principle components

Various morphological character of honey bee measured during the current investigation indicate that the greatest portion in the first original component is related to the color of third tergum and also scutellm and posterior leg length. The greatest portion in the second original component is related to the cubital index, forewing wide and forewing length, therefore, the cities having darker bees are placed in left side of Figure 1 and the cities having the bees with larger posterior leg are placed in right side of the figure. Also the city, having the bees with the larger cubital index, wide and height of forewing is placed in lower parts of the figure.

It can be concluded from the figure 1 that the length of posterior leg in sari honeybees is larger and their color is dark yellow because it is placed in the right side of the figure 1, also the second principle component is close to zero, so they are equal with the honey bees of Kafri and Kalar in view of cubital index, the length and wide of wing. The other cities are darker than Sulaymaniyah bees and the Kafri honey bees are different from the Kalar ones in view of second principle component, and their distance is larger along the components axis, the honeybees of Kafri have smaller cubital index, fore wing length and wide.

DISCUSSION

The present study is the first study in the last 5 years for evaluating the honey bee morphological characteristics in northeast Iraq (Kurdistan region). According to the obtained results and statistical analysis of data and in comparison with the results of Ataollah (1989), there is partial reduction throughout the measured characteristics, in other words: honey bees of studied cities are fatter, smaller and yellowish but proportional to the results **Amssalu** ET AL.(2004), there is increase in color of third tergum and the length of proboscis and in cubital index of Kurdistan honey bees. With regards to the table 3, we can concluded that there is positive and meaningful correlation between the color of scutellum and third tergum (in the level 99%) in addition , the color of third tergum has meaningful correlation with the length of forewing, the wide of forewing, the angle G18 and D7.There is also meaningful correlation in the angle G18 with the angle D7 and A4 (in the level 99%).There is meaningful and positive correlation between characteristic of forewing length and other characteristics such as the wide of forewing, the length of third and fourth tergum, the length of posterior leg and the proboscis length (in the level 99%). There is also positive and meaningful correlation between the proboscis length and the length and wide of forewing, posterior leg and bee length (in the level 99%).

There is positive and meaningful correlation between 3rd and 4th abdominal tergit length, forewing length, proboscis length, and cubital index. Also a positive and meaningful correlation between posterior leg length and forewing length and wide and proboscis length and bee length is considered. Ataollah (1989) indicated that five characteristics of forewing length, the wide of forewing, the proboscis length, the bee length and the length of posterior leg have positive correlation with each other and among them; there is no correlation between the wide of forewing and the proboscis length.

The comparison of three cities indicates that the angle of D7 is larger in the worker bees of Kafri and than other two while the length of front length, the wide of forewing, cubital index, the length of third and fourth tergum, sixth tergum index, the color of scutellum, the color of third tergum is more in the worker bees of Kalar than other cities. Finally, the angle A4, G18, the length of posterior leg and proboscis length is larger in Sulaymaniyah bees.

For explaining our results we had a look on apicultural industry in these three cities. There are some differences between cities which can help us for explanation of the results. According to Daly et al. (1988) and Dejong & De Jong, (1982) the Varroa mites can affect the morphological characters such as forewing sizes, weigh, and make some deformations. So we comprised their contamination to Varroa mites. These cities are different in their contamination density to Varroa mites. The percentage of Varroasis is the highest for Kafri. The reason is that beekeepers in Kafri departure their hives at least 3 times a year to different areas such as Horaman plain. Kafri doesn't have enough plant vegetation, pollen and nectar resources, so beekeepers had to departure their hives to find food sources. Beekeepers in Sulaymaniyah don't departure their bees, because there are big jungles around the city which provide them enough food resources. Kalar has a middle condition. In addition to Varoasis, it seems that climate condition play a role here (even minor); with regards to the different latitude and weather of the cities we can conclude that this agent also affects the separation of populations. In Kurdistan province Sulaymaniyah has an average climate, but it is somewhat sunnier and has more spring rain. So we conclude that weather condition, Varoasis percentage and hive migration have effects on morphological characters. According to this fact that mating in the honey bees is uncontrollable naturally, and northern cities of the country are host of many beekeepers from different parts of Iraq, the genetic diversity is high in honey bees populations of this region, but since most crosses in our country is inbred, it is necessary to know all of masses and their characteristics, so the target goals can be obtained in short time in the breeding of best honeybees in framework of a national project. With regards to the stability of honeybees in northern region of the Iraq, it is necessary to plan on the honeybees in northern cities. In finally, the results of study provided new information about genetic diversity in selected local honeybee in Kurdistan region of Iraq and will be useful for selection, future local biodiversity conservation and controlled breeding programs.

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