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The influence of geographical area on morphometric parameters of the honey bee in Serbia

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Abstract

In this study, morphological characteristics were measured on worker honey bee samples collected from seven different locations covering the territory of the Republic of Serbia. Three samples were taken from the territory of AP Vojvodina, namely from the Southern Banat District (Vršac; I), the South Bačka District (Bač; II), and the West Bačka District (Crvenka; III). The worker bees from the central Serbia originated from different sites namely, the Rasina District (Aleksandrovac; IV), the Zlatibor District (Lučani; V), Pešter (Sjenica; VI), and the Bor District (Negotin; VII). From each sample, 15 bees were randomly selected, and their front right wings were dissected and mounted on slides in two rows. After wing preparation, they were scanned using an Epson Perfection V600 Photo scanner with a resolution of 4800 dpi. The measurement included a total of 14 parameters, 11 of which were the angles on the forewing, while the other parameters analyzed were the forewing length and width and the cubital index. The results have shown that there is a high statistically significant difference between the localities in the characteristics examined in this study. From a total of 11 angles analyzed, statistical significance ($p<0.01$) of average values between the localities was determined for the following angles: A4, J16, K19, L13, N23, G18, and O26. The analysis of the results of the front wings length and the cubital index has shown a significant difference between the groups of honey bees. The average forewing length across all locations in the study was 9.17mm, while the

average forewing width was 3.43. The difference in terms of the average forewing width was not statistically significant between the locations. The values of the cubital index ranged from 2.28 to 2.79, with an average of 2.49 across all locations.

Key words: honey bee, morphometry, cubital index, Serbia

Introduction

In terms of prerequisites for the development and survival of beekeeping, Serbia has excellent predispositions for the existence and successful implementation of beekeeping production. Serbia is characterized by heterogeneous relief and climatic conditions, as well as diverse bee pastures. According to the area of wild flora, which covers a total of 1.28 million hectares, Serbia is ranked at sixth place in the world, which represents an extremely large nectar potential (www.organic-world.net).

In order to reliably determine honey bee races, study of taxonomy and systematization, measuring and analyzing certain morphometric parameters, as well as a large number of quantitative traits, are used. During the evolutionary development of honey bees, in different parts of the world, and under the influence of different environmental factors, different races with specific biometric traits have emerged (Mladenović & Pešev, 2011). According to Rinderer (1986), there are numerous criteria aimed at determining racial affiliation through morphometric analysis. A large number of characteristics can be monitored, but among them, wing morphometry has proven to be the most reliable.

The main cause of variability that occurs in the process of beekeeping is the use of local ecotypes that are well adapted to the agricultural and ecological environment of a certain area (Jevtić et al., 2007). Certain specificities of relief, terrain exposure, and eco-climatic conditions in the territory of Serbia affect the heterogeneity and variability of the traits of the honey bee. The environment, as a factor, has a significant influence on the body size, as shown by the results of the experiment conducted by Alpatov (1929), cited by Mladenović and Pešev (2011).

Natural populations of honey bees in the territory of the Republic of Serbia have been exposed to negative human influence during the previous decades, primarily through the use of pesticides, herbicides, and other chemical substances used in agriculture, but also due to uncontrolled import and crossing with other races of honey bees (Nedić et al., 2007).

For the preservation of the biological diversity of *Apis mellifera carnica* bees in the territory of the Republic of Serbia, breeding and trade with breeding material of other bee breeds are not allowed (Legislative, 41/2009, 93/2012 and 14/2016).

Basing their study on standard morphometric analysis, while using the forewing parameters, Abou-Sahara and Al-Ghambi (2012) concluded that the length and width of the inner wing were the most successful characteristics in distinguishing between *Apis mellifera jemenitica* and *Apis mellifera carnica*.

Material and Methods

The determination of morphometric characteristics was performed on seven samples of *Apis mellifera* (*carnica*) bees collected in Serbia. Out of the total number of samples, three were collected from the territory of the Autonomous Province of Vojvodina, specifically from the South Banat District (Vršac; I), the South Bačka District (Bač; II), and the West Bačka District (Crvenka; III). The remaining four samples were collected from the central part of Serbia, specifically from the Rasina District (Aleksandrovac; IV), the Zlatibor District (Lučani; V), Pešter (Sjenica; VI), and the Bor District (Negotin; VII). One hundred bees were sampled from each location, which were preserved in 96% ethanol. From each sample, 15 bees were randomly selected, and their front right wings were dissected and mounted on slides in two rows. After wing preparation, they were scanned using an Epson Perfection V600 Photo scanner with a resolution of 4800 dpi. After scanning and image formation, individual measurements of wing characteristics were performed using the ImageJ software. The measurement included a total of 14 parameters, of which 11 were angles on the front wing, while the other parameters were the length (FWL) and width (FWW) of the front wing and the cubital index (CI). The following angles on the front wing were measured: A4, B4, D7, E9, J10, J16, K19, L13, N23, G18, and O26. Univariate (variance) statistical analysis was conducted for 14 morphological characteristics. A descriptive statistical analysis was performed, and comparisons between locations were made using the Duncan's Studentized Multiple Range Test. All statistical analyses were conducted using a standard procedure, and the results of these analyses were interpreted.

Results and Discussion

Descriptive statistical values of morphological characteristics of the honey bees from different locations in the territory of the Autonomous Province of Vojvodina and from the central region of Serbia are presented in Table 1. The measurement results for the sampled worker bees have shown a wide variation in mean values and standard deviations for the investigated characteristics of the forewing. The average value for angle A4, by locations, ranged from 27.87° (I) to 30.42° (IV). The average value for this angle is in agreement with the results of Mladenović and Simeonova (2010), where the average size of angle A4 for the locality of Lešak was 30.30°. The average values of the angle A4 size for the bees from AP Vojvodina (I) have been statistically highly significantly different ($p<0.01$) from the bees from central Serbia (IV, V and VI). These results differ from those of Nedić et al. (2011), who reported no statistical difference in the size of angle A4 between the bees from the territory of AP Vojvodina and central Serbia. A statistically highly significant difference has been found between locations III and IV, and III and VI. The bee samples were taken from the apiaries where indigenous biological material was raised. The samples from central Serbia were taken from the apiaries that were located in hilly areas and were mostly naturally isolated due to the surrounding terrain. The queens in these apiaries were not introduced from the outside and were grown in the local environment. Due to this fact, this honey bee group seems to remain geographically isolated and possesses certain characteristics of its own. The values of the characteristics monitored in this study are presented in Table 1.

Out of a total of 11 angles, statistical significance has not been determined for angles B4 (109.58°), D7 (97.35°), E9 (23.32°), and J10 (54.74°) (Table 2). The average values for angles B4, D7, and E9 approximately correspond to those reported by Nedić (2009), where the mean values for the angles were 107.59° (B4), 98.33° (D7), and 22.21° (E9). Pihler (2011) notes that the values for angle B4 varied between 100.9° and 110.0° for 5 different races, with an average value of 107.31°. The average values for the other angles (D7, E9, and J10) were 96.73°, 23.13°, and 55.96°, respectively.

Table 1. Means and standard deviation (Sd) of selected measures for the honey bee samples from Serbia. Sizes of angles are given in degree (°), the forewings length and width are given in mm.

Char.	Origin of samples (locations)							Average
	Vršac I	Bač II	Crvenka III	Aleksandrovac IV	Lučani V	Sjenica VI	Negotin VII	
A4	27.87±1.77	28.57±1.83	28.14±2.71	30.42±1.59	29.87±1.12	30.24±1.73	29.76±1.85	29.27±2.05
B4	111.49±5.50	110.40±6.51	109.74±4.23	109.25±4.95	109.33±4.05	106.74±4.54	110.10±6.01	109.58±5.21
D7	95.90±1.82	97.38±2.30	98.09±2.48	99.88±2.23	98.81±2	97.95±3.58	93.42±20.59	97.35±8.12
E9	22.58±0.93	23.10±1.12	23.63±1.17	24.37±1.28	22.75±1.55	22.08±1.38	24.76±7.74	23.32±3.19
J10	54.30±3.16	53.43±2.87	53.81±1.91	55.82±5.78	53.94±4.47	54.76±5.35	57.10±11.60	54.74±5.79
J16	93.91±3.78	97.49±2.33	95.29±2.53	94.78±2.67	97.92±2.76	94.28±1.74	97.45±2.30	95.87±3.02
K19	76.72±1.43	77.46±2.08	77.41±1.74	76.13±2.71	79.40±1.69	78.96±2.36	77.03±2.12	77.59±2.28
L13	13.62±1.04	13.37±1.4	14.62±0.85	14.54±1.15	14.33±1.55	15.24±1.16	15.10±1.31	14.40±1.36
N23	98.91±1.93	98.53±2.57	99.53±2.16	98.37±2.27	96.70±2.50	93.57±2.55	98.51±2.01	97.73±2.92
G18	88.92±2.51	93.97±2.14	92.86±1.67	88.01±2.61	92.09±2.39	92.18±2.31	90.34±3.16	91.05±3.05
O26	37.18±3.05	38.08±2.17	34.20±3.93	33.24±1.86	38.76±3.78	33.98±3.4	38.29±2.80	36.25±71
DPK	9.14±0.14	8.94±0.11	9.30±.010	9.22±.018	9.17±0.13	9.25±0.16	9.20±0.09	9.17±0.17
SPK	3.11±0.06	3.12±0.05	3.26±0.04	3.24±0.05	4.94±7.07	3.20±0.08	3.14±0.05	3.43±2.67
CI	2.57±0.32	2.45±0.32	2.63±0.28	2.37±0.36	2.28±0.22	2.79±0.45	2.38±0.30	2.49±0.36

Significant differences ($p<0.01$) between sites have been found for the size of angle J16. The average size of this angle ranged from 93.91° (I) to 97.92° (V). The bees from site I differed significantly in the size of angle J16 from those from sites II, V, and VII. The overall average value for angle J16 (95.87°) for the bees from all sites was lower compared to the value (100.75°) reported by Bouga et al. (2011) for the *Apis mellifera carnica* bee colonies from Portugal.

Kauhausen-Keller and Keller (1994) reported mean values of 79°, 12.3°, 93.9°, 92.4°, and 37.8° for angles K19, L13, N23, G18, and O26, respectively, in *A. m. carnica*. The mean values for the same angles determined in our study were consistent with the results of Kauhausen-Keller and Keller (1994).

Taking into account the differences in the average values for angle K19, as shown in Table 2, the honey bees from site I differed significantly from those from sites V and VI. The bees from Rasina district (IV) differed significantly ($p<0.01$) in the K19 angle from those from Zlatibor district (V and VI). A significant difference has also been found between the honey bees from sites V and VII.

A highly significant difference was found for the honey bees from site I and site II compared to those from sites VI and VII for angle L13. These differences can be explained by the development of locally adapted ecotypes of the *A. m. carnica* subspecies over time and isolated breeding on those sites.

The honey bees bred in the Sjenica region (VI) differed significantly in the size of angle N23 compared to those from other sites. The average value of angle G18 for the honey bees from the Southern Banat region (I) did not differ from the honey bees from the sites in the Rasina district (IV) and Bor district (VII), while this value differed significantly ($p<0.01$) from the samples collected in the other regions. The average size of angle O26 for the bees from sites V, VII, II, and I (38.76°, 38.29°, 38.08°, and 37.18°, respectively) was significantly higher ($p<0.01$) compared to the samples from sites III, VI, and IV (34.20°, 33.98°, and 33.26°, respectively).

Mladenovic et al. (2011) analyzed morphometric parameters of bees from 6 locations in southern Serbia and found the average wing length to be 8.96 mm, which is lower than the results of our research (9.17mm). Georgijeva (2014) reported that the wing length of selected lines of honeybees (*A. m. carnica Poll*.) ranged from 9.19 to 9.55 mm (with an average of 9.40 mm across all lines). In our research, the honey bees from the Bač (II) location had the shortest wing length (8.94 mm), and were significantly different from the bees from other locations ($p<0.01$). There was no statistically significant difference in this characteristic between the remaining locations.

Table 2. Comparison of the significance of the differences between the average values. Angle sizes are given in degrees (°), the forewings length and width are given in mm.

Char.	Group							Average
	I	II	III	IV	V	VI	VII	
A4	27.87	28.57	28.14	30.42	29.87	30.24	29.76	29.27
	bd	acd	bcd	a*	ac	a	acd	
B4	111.49	110.40	109.74	109.25	109.33	106.74	110.10	109.58
	a	a	a	a	a	a	a	
D7	95.90	97.38	98.09	99.88	98.81	97.95	93.42	97.35
	a	a	a	a	a	a	a	
E9	22.58	23.10	23.63	24.37	22.75	22.08	24.76	23.32
	a	a	a	a	a	a	a	
J10	54.30	53.43	53.81	55.82	53.94	54.76	57.10	54.74
	a	a	a	a	a	a	a	
J16	93.91	97.49	95.29	94.78	97.92	94.28	97.45	95.87
	bc	a	ac	bc	a	bc	a	
K19	76.72	77.46	77.41	76.13	79.40	78.96	77.03	77.59
	b	ab	ab	b	a	a	b	
L13	13.62	13.37	14.62	14.54	14.33	15.24	15.10	14.40
	b	b	ab	ab	ab	a	a	
N23	98.91	98.53	99.53	98.37	96.70	93.57	98.51	97.73
	ac	ac	a	ac	c	b	ac	
G18	88.92	93.97	91.86	88.01	92.09	92.18	90.34	91.05
	b	a	ac	b	ac	ac	bc	
O26	37.18	38.08	34.20	33.24	38.76	33.98	38.29	36.25
	a	a	b	b	a	b	a	
FWL	9.14	8.94	9.30	9.22	9.17	9.25	9.20	9.17
	c	b	a	ac	ac	ac	ac	
FWW	3.11	3.12	3.26	3.24	4.94	3.20	3.14	3.43
	a	a	a	a	a	a	a	
CI	2.57	2.45	2.63	2.37	2.28	2.79	2.38	2.49
	ab	b	ab	b	b	a	b	

*Means for the same characteristics followed by different letters within locations are significantly different ($p<0.01$) according to variance analysis followed by the Duncan´s Studentized multiple range tests.

Compared to the results reported by Jevtic et al. (2007), where the wing length for the bees from the Banat district was 9.82 mm, there is a clear difference, as the average wing length for the bees from the South Banat district in our research was 9.14 mm.

One of the most commonly analyzed morphometric characteristics used to determine the race is the cubital index. The highest cubital index value of 2.79 has been found in the bees from Sjenica (VI). They were significantly different from the bees from Bač (II) (2.45), Negotin (VII) (2.38), Aleksandrovac (IV) (2.37), and Lučani (V) (2.28) in terms of this characteristic. Kauhausen-Keller and Keller (1994) reported that the cubital index size for the reference sample of *A. m. carnica* bees was 2.7. El-Aw et al. (2012) reported an average cubital index value of 2.89 in the parent generation of *A. mellifera L*.

Conclusion

Based on the results of the research, significant variability of honey bees has been found among different locations in Serbia. The analysis of variance results has shown high statistical significance of differences between the mean values for angles: A4, J16, K19, L13, N23, G18, and O26. There was also a statistically highly significant difference in the values of the forewing length and cubital index between the honey bee samples from different locations. The honey bees bred in the area of Sjenica (VI) were statistically significantly different in terms of the size of angle N23 compared to those from the other locations. The average length of the front wing for all samples analyzed was 9.17 mm. The Bač location significantly differed in terms of the wing length compared to other locations. As regards the average values of the wing width, no statistically significant difference was observed between the locations studied. The values of the cubital index ranged from 2.28 to 2.79, with an average of 2.49 across all locations. In terms of the average values of the cubital index, a statistically highly significant difference has been observed between the locations. The Sjenica location exhibited a significant difference compared to Bač, Aleksandrovac, Lučani, and Negotin. The analysis of morphological characteristics indicates the existence of local subpopulations of honey bees, which may be a consequence of their isolated breeding in specific environmental conditions.

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Утицај географског подручја на морфометријске параметре медоносне пчеле у Србији

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Сажетак

У овом истраживању мерене су морфометријске карактеристике на узорцима радилица медоносне пчеле прикупљених са седам различитих локалитета на територији Републике Србије. Узета су три узорка са територије АП Војводине и то из Јужнобанатског округа (Вршац; I), Јужнобачког округа (Бач; II) и Западнобачког округа (Црвенка; III)). Из централне Србије пчеле радилице су потицале са различитих локалитета и то из Расинског округа (Александровац; IV), Златиборског округа (Лучани; V), Пештерског (Сјеничког; VI) и Борског округа (Неготин; VII). Из сваког узорка насумично је одабрано 15 пчела, а њихова предња десна крила су сецирана и постављена на слајдове у два реда. Након припреме крила, скенирана су помоћу Epson Perfection V600 Photo скенера са резолуцијом од 4800 dpi. Мерење је обухватило укупно 14 параметара, од којих су 11 били углови на предњем крилу, док су остали анализирани параметри били дужина и ширина предњег крила и кубитални индекс. Резултати су показали да постоји високо статистички значајна разлика између локалитета у испитиваним карактеристикама. Од укупно 11 анализираних углова, статистичка значајност ($p<0,01$) просечних вредности између локалитета утврђена је за следеће углове: A4, J16, K19, L13, N23, G18 i O26. Анализа резултата дужине предњих крила и кубиталног индекса показала је значајну разлику између група медоносних пчела. Просечна дужина предњег крила на свим локацијама у студији била је 9,17 mm, док је просечна ширина предњег крила била 3,43 mm. Разлика у просечној ширини предњег крила није била статистички значајна између локалитета. Вредности кубиталног индекса у оквиру анализираних локалитета су се кретале од 2,28 до 2,79, са просеком од 2,49.

Кључне ријечи: медоносна пчела, морфометрија, кубитални индекс, Србија

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