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MORPHOMETRIC DIFFERENCES BETWEEN HONEY BEES (*Apis mellifera carpatica*) POPULATIONS FROM TRANSYLVANIAN AREA

DIFERENȚE MORFOMETRICE LA POPULAȚIILE DE ALBINE (*APIS MELLIFERA CARPATICA*) DIN REGIUNEA TRANSILVANIA

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The result of the long evolutionary process formed, by natural selection, variable races and ecotypes which are biological and ecological differentiated. The Romanian, Apis mellifera carpatica *is the most adapted and the most efficient species at the local conditions. Morphometric measurements were the major criteria for classification described by Ruttner (1988) and are the basis of our study. In this way 8 morphometric characters of honeybees from Transylvanian region were measured. The data obtained show that the honeybees' ecotype genes are mixed due to the migratory beekeeping.*
Keywords: *Apis mellifera carpatica, morphometric measurements, geographical analysis, Transylvania area*

Introduction

First classification of honeybee subspecies was purposed by DuPraw (1964, 1965) based on morphometric measurements regarding appreciation of differences in size and color and than improved by Ruttner (1988). The basis of morphometry was established by Cojevnicov in 1898, who realized the first measurements to the Caucasian honeybee proboscis and he proposed the measurement of the chitin parts. According to Ruttner's classification (1992) there are 25 subspecies around the world belonging to honeybee genus. He examined and classified the honeybees from all around the world using 39 morphometric characters. Thanks to Ruttner (1978) the 24 *Apis mellifera* subspecies were grouped in three evolutionary branches based on morphometric measurements: European honeybees (M), African (A), North Mediterranean (C). Beekeeping Institute from Oberursel, Germany, is the Foundation with a collection of over 1400 preserved honeybees samples. All data from here are preserved in a Data

Bank from this Institute (Ruttner, 1987). Moreover, in the selection process new taxonomy appeared (ecotypes, different lines in internal races) which also must be identified (Criţov, 2003).

The Romanian honeybees have longer or shorter body parts according to Fisteag (1937) who established as a rule that the bees from mountain and from the Northern side of country have dark color, short legs and proboscis and big body comparing to the bees from the Southern side which present the opposite characteristics for the same features. The migratory beekeeping mixed this ecotype, so the aim of this research was to distinguish these ecotypes from Transylvanian area. Its purpose was to compare the mountain honeybee ecotype with field ecotype. The comparisons were realized using morphometrical measurements.

Materials and Methods

Materials: Honeybee samples: Honeybee samples *Apis mellifera* were collected from stationary apiaries from different Transylvanian areas: Unirea (county Alba), Tisa (county Maramureş), Săcuieni (county Bihor), Borsec (county Harghita), Făgăraş (county Braşov), Zagoni (county Covasna). Three of this samples were from field regions: Unirea (county Alba), Tisa (county Maramureş), Săcuieni (county Bihor) and three of them from mountain regions: Borsec (county Harghita), Făgăraş (county Braşov), Zagoni (county Covasna). The criteria of apiary selection were considered very important in order to avoid gene mixing between different populations from Transylvania.

It order to have enough samples for a right statistical interpretation, 30 honeybees have been measured from each beehive representative for an apiary. The honeybees chosen for analysis were from inside of the hives (younger bees: 14-21 days old) and not from outside (foragers: over 21 days old).

Collection methods: To achieve such correct measurements of the proboscis the honeybees are killed in Ether vapors. With any other methods of killing, the proboscis remains folded in (Ruttner, 1988).

Morphometrical measurements: Method described by Ruttner in 1988 was applied for our study. Olympus stereomicroscope was used with a camera connected to computer. The magnitude and the clarity of the picture were adjusted, after which the picture was captured with all interested parts of the honeybee. Pictures appeared on the monitor after which the morphometrical measurements were realized with the Program Quick Photo Micro 2.2.

Morphometric Characters:

The **proboscis** was cut from base of proboscis's postmentum and was stretched with a special care on a slide (*Fig. 1*).

The **total length of legs** was determined by summing up of all legs' parts which were measured apart: the length of femur (Fe), length of tibia (Ti) and length of metatarsus (M_L) *(Fig. 2)*. The legs was stretched on a slide and then captured with microscope's camera.

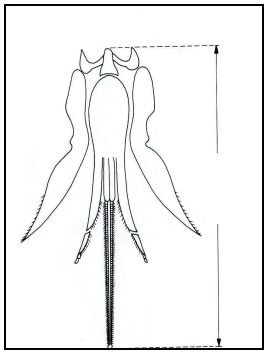

Fig. 1: Length of proboscis (Ruttner, 1988)

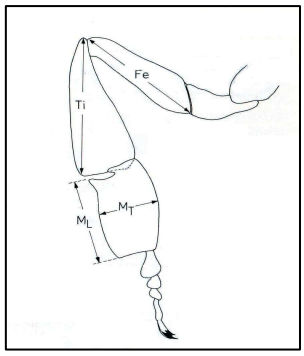

Fig. 2: Length of femur (Fe), tibia (Ti) and metatarsus (M_L), width of metatarsus (M_T) (Ruttner, 1988)

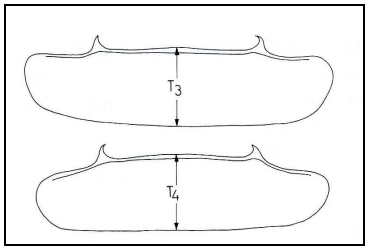

Fig. 3: Longitudinal diameter of tergite 3 (T_3) and 4 (T_4) (Ruttner, 1988)

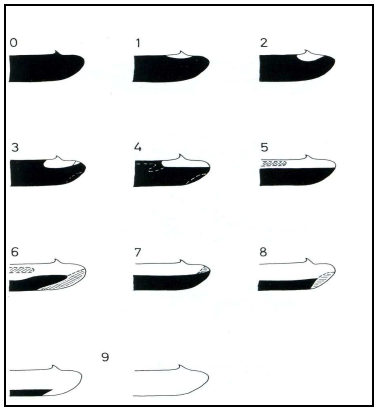

Fig. 4: Classes of pigmentation of tergites 2-4 (Ruttner, 1988)

The **body size index**: The **tergite 3 and 4** were stretched and fixed with arabic gum and then measured across the middle line of the tergite 3 and 4 *(Fig. 3)*. The body size index was obtained from addition of tergite 3 and 4.

Classification of amount of light **pigmentation of tergite 3 and 4** was according to an empirical series of pigmentation patterns *(Fig. 4)* (Ruttner, 1978). This is a subjective empirical classification used to see the differentiation between mountain or field region honeybees. The tergite 3 and 4 were cut in the middle and measured and then compared with *Fig. 4*.

Data analysis: all measurements were transformed into an unique scale, and then the primary data (for statistical processing) were used as criteria to recognize errors of measuring. It was preferred to use operational units like means of samples and not individual values, because the mean represent the biological unit of the colony (of the lot) of bees, while the honeybees are related in different

grades: full or half sisters. Sums were achieved proved to be a valuable instrument for recognizing trends in variation and for quick comparisons.

All data were processed with Origin 7.0 software, using ANOVA significance level 0.05.

Results and Discussions

The length of the proboscis *(Fig. 5)* was considered a very important character because it shows the geographical variability more accurate than all the other characters. Our results showed *(Fig. 5)* that in the mountain regions (Borsec (county Harghita), Făgăraş (county Braşov), Zagoni (county Covasna)) the proboscis were longer (average = 6.21 mm) than in the lower regions (average = 5.99 mm) (Unirea (county Alba), Tisa (county Maramureş), Săcuieni (county Bihor)). This is opposite to Allen's rule (Ruttner, 1988), so that the proboscis decreasing in length in cooling climates. This data shows that the genes were mixed in these regions. An exception can be seen in the North, where the proboscis is longer than in other parts of the country. The same result arouse from data regarding the length of legs. Statistical interpretation of data about proboscis revealed that significant differences were obtained between all analyzed samples (P = 0.00007).

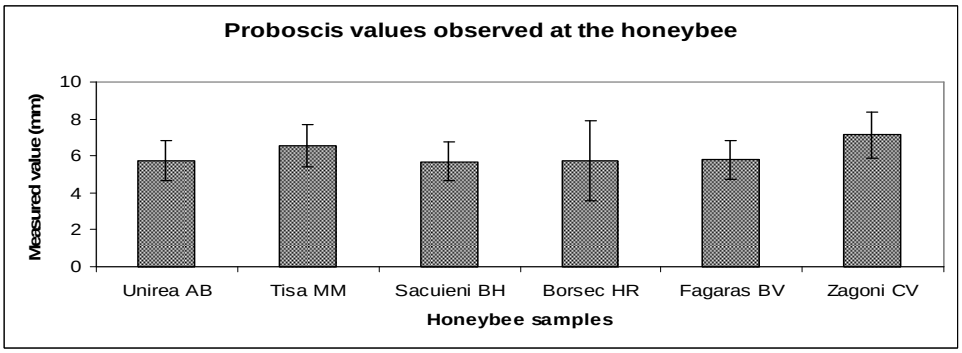

Fig. 5: Length of the proboscis of Apis mellifera honeybee

Data about the length of the leg *(Fig. 6)* present the minimal values were obtained at samples from lower regions (average = 8.99 mm) Săcuieni (county Bihor) < Tisa (county Maramureş) < (Unirea (county Alba) and the maximum values were obtained for mountain regions (average = 9.22 mm) Borsec (county Harghita) > Zagoni (county Covasna) > Făgăraş (county Braşov). This is contrary to Allen's rule (Ruttner, 1988): the length of proboscis and leg are decreasing in warmer climates. This data shows that the genes were mixed in these regions.

There were no significant differences between the total leg length of honeybees (P = 0.53746), but there were recorded significant differences between

all legs' segments: femur (P = 0,00527), tibia (P = 0,0006), metatarsus (P = 0,00229).

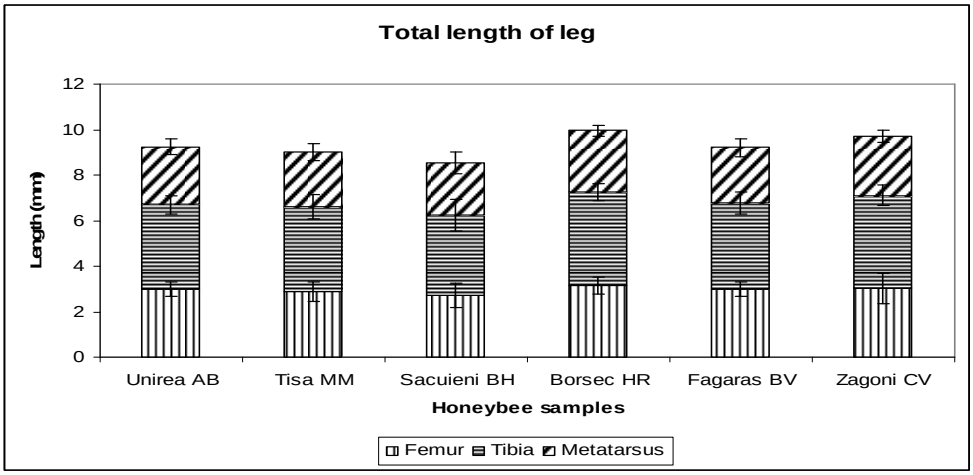

Fig. 6. Total length of the leg (Femur + tibia + metatarsus)

Bergmann's rule about the differences between geographic races is stating that the ecotypes are larger in the North or in cool climate than in the South (Ruttner, 1988). In our results the body size of honeybees *(Fig. 7)* were not significant different (P = 0.60775) regardless the area the samples are coming from. It is now a certainty that the previously identified ecotype can not be distinguished in the present. Insignificant difference were observed between T3 length of all samples (P = 0,43154) and for T4 (P = 0,68815).

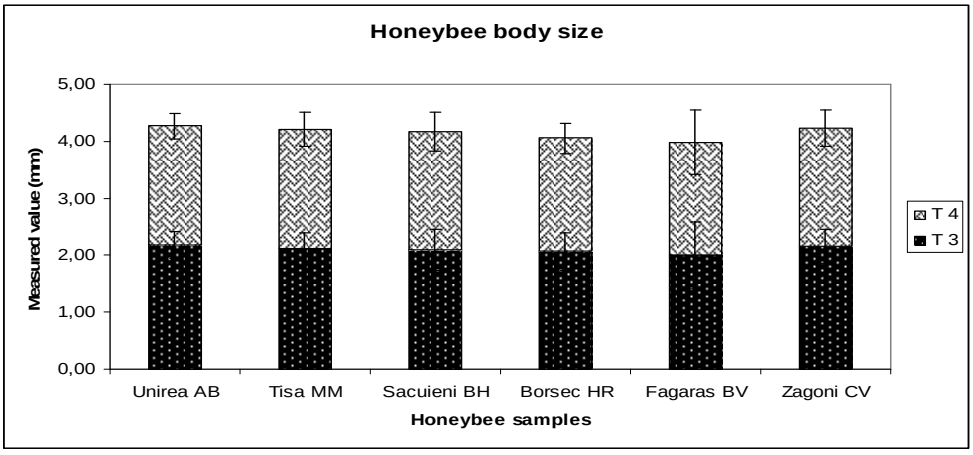

Fig. 7. Size of the body (tergit 3 + tergit 4)

Fisteag's rule was that the honeybee color was darker in cool climates. On a pigmentation scale from 0 to 9 of tergit 3 and 4, Transylvanian honeysbees were mostly below 5 (*Fig. 7*) and our data confirm that in Northern and mountainous places the colour of honeybees is darker. This is mostly evident at tergit 4 in samples from Tisa (the Northest specimen sampled, found in Maramureş county) and at specimen from Borsec and Zagoni (both being mountainous samples). The pigmentation character was not very constant, but was a reliable guide to differentiate the ecotypes. From our data the pigmentation of analyzed honeybees was very significant different (P = 0.0000).

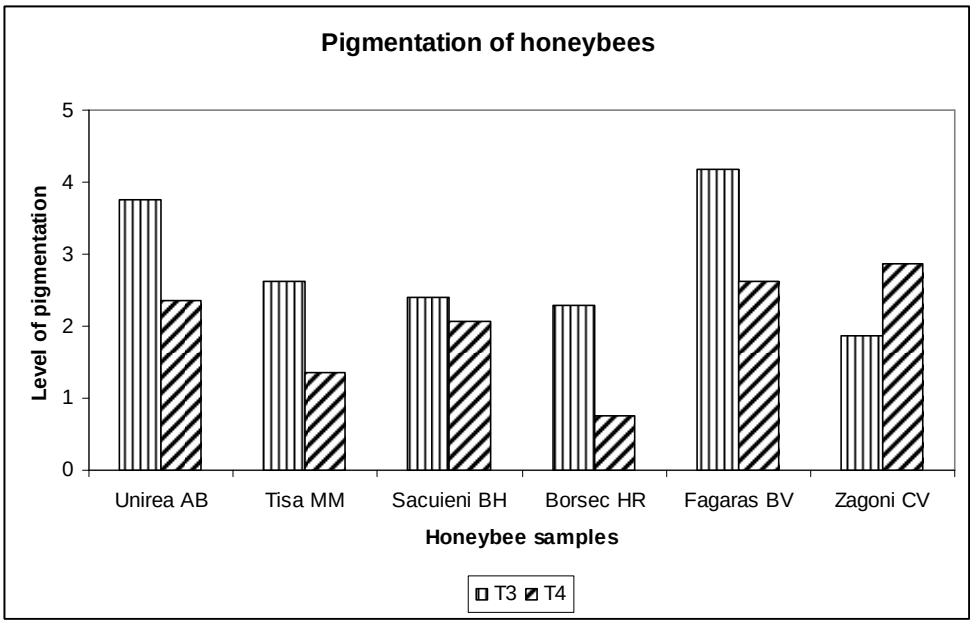

Fig. 8: Level of pigmentation of tergit 3 and 4

Unfortunately, from the data observed at the samples from Sibiu area it was concluded that the most used honeybee is Buckfast hybrid. As a consequence of Romania adhesion to EU the borders disappeared and European beekeepers (which may have other bee breeds or hybrids different form ours) are free to enter into our space in order to practice migratory beekeeping. This is unfortunately not convenient for Romanian genetic inheritance and it is altering the biodiversity ecotype of Romanian honeybees. This fact involves many risks and any underestimation of this problem is dangerous for economy, for importer beekeepers and also for global durable development.

Yet, it is certain the fact that the Romanian honeybee *A.m. carpatica* is very well adapted to local conditions, implicitly the most productive.

Conclusions

The data obtained show that the honeybees ecotype genes are mixed due to the migratory beekeeping. It is the purpose of the authors to continue the research regarding the morphometric measurements and to develop new studies on *Apis mellifera carpatica* with genetic analysis at DNA microsatellite level in order to see if there are more relevant differences between honeybee populations.

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Bibliography

1. **DuPraw E. J.** (1964) - *Non-Linnean taxonomy and the systematics of honeybees*, Syst. Yool. 14, 1-24.
2. **DuPraw E. J.** (1965) - *The recognition and handling of honeybee specimens in non-Linnean taxonomy*, J. Apic. Res. 4, 71-84.
3. **Fisteag J. N.** (1937) - *Cercetări Biometrice la Albinele Româneşti*, Diss. Nr. 606. d. vet. med. Fac. d. Universitiit Bukarest.
4. **Ruttner F., Tassencourt L., Louveaux J.** (1978), - *Biometrical-statistical analysis of the georaphic variability of* Apis mellifera L., Apidologie 9, 363-381.
5. **Ruttner F.** (1988) - *Biogeography and Taxonomy of Honeybees*, Springer-Verlang, Berlin.
6. **Ruttner F.** (1992) - *Naturgesichte der Honigbiene, Munchen*, Ehrenwirth.