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RESEARCH ON CHARACTERS CONSERVATION IN MOUNTAIN ECOTYPE QUEEN BEES

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Abstract

One of the objectives of selection programs in bees is the conservation of the gene pool. The mother-daughter replacement induces a decrease in the rate of loss of sex alleles and the viability of the offspring compared to the population in which the replacement is random. The experiment was carried out in the 2022-2023 bee season. Two queen-breeding colonies and ten drone-breeding colonies were selected. Ten queens were reared from each queen rearing colony. From each drone breeding family, the semen obtained from ten mature drones was collected and homogenized. Each queen was inseminated with the semen obtained by homogenization. Queens were introduced into orphaned colonies of equal strength. In the bees resulting from these colonies, morphological and behavioural characters were measured and compared with those of the parental population. According to the statistical results, the difference between the average of the studied characters of the parental population and the descendant population was very significant.

Key words*: breeding, cubital index, homogenized semen, morphometry.*

INTRODUCTION

Within the bee breeding program, emphasis is placed on outlining taxonomic characters valid for bee appreciation at different stages, quantifying the main preselection criterion and the other primary and secondary criteria (Cebotari, 2006; Cebotari & Buzu, 2011; https://www.anarz.eu).

The correct application of the method of breed improvement, formation of hybrids depends on the and economic conditions of the territory (Alpatov, 1929; 1948).

The taxonomic characters preferred by Linnaean systematics are the morphological ones because they are easily visible, are less influenced by the environment, have a higher heritability.

Modern taxonomy also accepts characters according to the degree to which certain populations can be differentiated (Ruttner, 1975; Ruttner et al., 1978).

Ruttner (1986) has analysed and categorized honeybees from all over the world based on morphometric characteristics in: African races of south of the Sahara; West Mediterranean races, and East rases.

Morphological traits of honey bees can be quantified to describe honey bee populations (Louveaux, 1969a; 1969b). To profile honey bee populations, a common approach involves gathering random samples of honey bee workers from various hives and locations (Abou-Shaara et al., 2012).

The study of bee breeds based on morphometry has remained extremely important until today, reflecting also the presence and absence of isolation because in some areas the local bee is hybridized due both to frequent import phenomena of other breeds, but especially to periodic unguided migrations.

This morphometric study is motivated by the need to know and recognize the mountain bee according to measurable criteria, a starting point for its conservation.

MATERIALS AND METHODS

The experiment was conducted during the 2022-2023 beekeeping season. Two queen bee breeding colonies and ten drone breeding colonies were selected from an apiary considered representative of the mountain population. From each queen bee breeding colony, ten queen bees were bred. From each drones breeding colony, semen was collected from a number of ten mature drones. The semen collected was homogenized. Each queen bee was artificially inseminated (Cebotari & Buzu, 2021), with semen obtained by homogenization. The queens were introduced into orphaned colonies of equal strength. In bees resulting from these colonies, morphological and behavioural characters/traits were measured and compared with those in the parental population, to check the degree of variability between parents and offspring (Cornuet et al., 1975). The morphological characters: proboscis (trunk) length, upper wing length, tergite-3 length, and cubital index were individually measured.

The observed behavioural characteristics were: gentleness, quiet behaviour on honeycombs, anecbalia (lack of swarming), honey capping (relative appreciation), degree of nest blockage (% area) (Oztokmak et al., 2023).

The measurements were carried out on ten bees from each colony that received an artificially mated queen (were an artificially mated queen was introduced).

Bee sampling

Bee samples for morphometric examination were taken from the hive and not from bee entrance. Adult bees were generally taken from the last frames with honey. After harvesting, they were sacrificed, and then put in boiling water to soften the chitinous plates (Chauvin, 1968). The bees were dissected using tweezers, scalpels and very fine scissors. After detachment, the chitinous pieces on which the measurements were made were degreased, cleaned and stretched on microscopic slides. Each slide contained 20 pieces. Measurements were made using a microscope and stereoscopic magnifying glass provided with an ocular micrometre.

Morphological measurements and indicators

The *cubital index* (%) - is the ratio of two segments of the wing ribs in bees. This index is used in the analysis of anatomical shape and structure, being a method of distinguishing species and subspecies from living organisms. The pattern of the ribs of the forewing is peculiar to each breed of bees. The measurements were made on the two ribs that form an obtuse angle with the base of the 3rd cubital cell. 40x magnification is used. The cubital index is the ratio of A/B (Figure 1) (Ruttner, 1986).

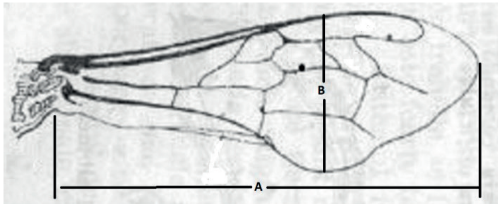

Figure 1. Upper wing width length in bees
(after Ruttner, 1986)

Proboscis length (trunk) (mm) - measurement was made from the extremity of the prementum to the extremity of the gloss and the spoon (la bellum). The measurements were made with a magnification of 16x (Ruttner, 1986).

Upper wing length (mm) - the length of the forewing was measured with a magnification of 16x (Ruttner, 1986).

Tergit-3 length (mm) - was measured to establish the degree of hybridization of a bee population. It is transmitted hereditarily to the lineage, being a character with high heritability (0.7-0.8) (Ruttner, 1986).

The measurement of behavioural indices was achieved by awarding points for the characters as gentleness, quiet behavior on the honeycombs and anecbalia, indicating the type of ticking (wet dry or mixed), and percentage for the degree of blocking of the nest, the bee colonies from which the samples originated (Ruttner, 1975; Ruttner et al., 1978) (Table 1).

Table 1. Score for establishing Behavioural indices

Crt. No.	Behavioural aspect	Unit of measurement
1.	Gentleness	Max. 4 points
2.	Quiet behavior on the honeycombs	Max. 4 points
3.	Anecbalia (lack of swarming)	2-4 points (minimum 3)
4.	Honey caulking (relative appreciation)	Type of wet, wet or mixed cap (%)
5.	The degree of blockage of the nest	% area with brood (minimum 40%)

The results of the measurements were statistically processed to establish differences between the parental population and the bee population obtained from artificial insemination with homogenized semen. Data analysis was performed using Fisher Test for check the homogeneity of the variances and the modified Student Test, Behrens-Fisher variant, for the case of heterogeneous variances.

RESULTS AND DISCUSSIONS

A number of 200 bees were taken both from the parental population - queen breeding colonies and drone breeders - and from bee colonies obtained by introducing artificially inseminated queens with homogenized semen.

The samples were collected from bee colonies with high honey yields. The yields obtained were generally over 20-25 kg annually.

The sampled colonies had uniform bees, even though it included yellow corners on the abdominal tergites. All samples were collected during the active season (summer) so that there were no differences between samples.

Cubital Index

The values measured at the cubital index in the parental and offspring of the mountain bee population have values lose to those of the specific values found in the specialized literature (Table 2).

The measurements performed on bees from the Republic of Moldova and Ukraine, revealed higher values of the cubital index (40.00% and 52.80%) (Eremia & Cataraga, 2021), compared to the mean values determined in the present study for the bee mountain ecotype (37.20%, and 39.04%).

Table 2. Values of statistical parameters of the cubital index

Specification	Parental population (PP)	Descending population (DP)
Mean	0.390	0.372
Variance	0.00315	0.00301
Standard deviation	0.0560	0.0547
Maximum	0.5333	0.5133
Minimum	0.25	0.25

After applying the Fisher test, it was found that the variances of the two populations are heterogeneous.

Thus, the Fisher test, obtained by reporting the larger variant to the smaller one, led to the value of 1.0465.

The critical value of the Fisher test obtained at 398 DF and the significance level of 0.1% has the value 1.

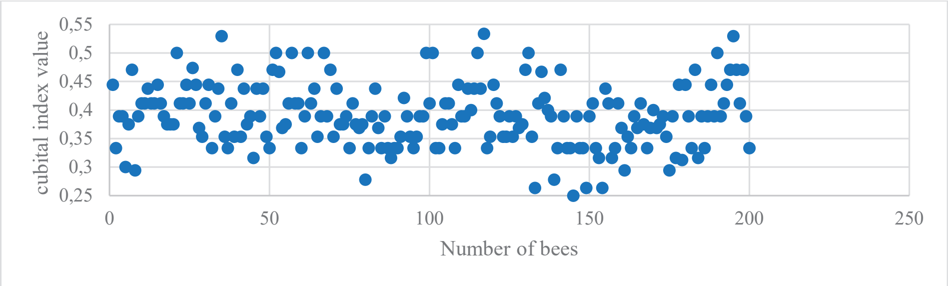

Figure 2. Cubital index values for the parental population (PP)

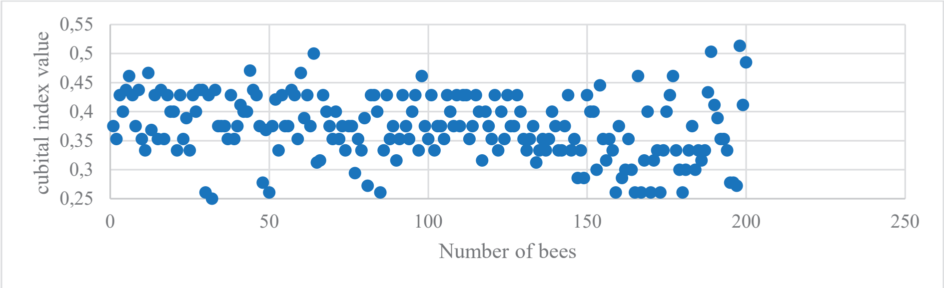

Figure 3. Cubital index values for descending population (DP)

Figures 2 and 3 indicate the distribution of determined values in the parental population compared to the descending population.
Since the variances are heterogeneous, the Behrens-Fisher Student's Test was used to verify the equality of the means of the two populations (Table 3).

Table 3. Student Test values of the two populations for the cubital index

Groups	Sample size	t calculated	t critical, $\alpha = 0.001$
PP	200	3.315***	3.290
DP	200		

As a result, the difference of cubital index values in the two populations studied was very significant.

Proboscis Length

Regarding the proboscis length, differences were noted between the parental and descendant populations. A heterogeneous distribution of the measured values, with a lower standard deviation was noted (Table 4).

Table 4. Values of the statistical parameters of proboscis lengths

Specification	Parental population (PP)	Descending population (DP)
Mean	6.355	6.447
Variance	0.0441	0.0289
Standard deviation	0.2101	0.1701
Maximum	6.8	6.8
Minimum	5.5	5.7

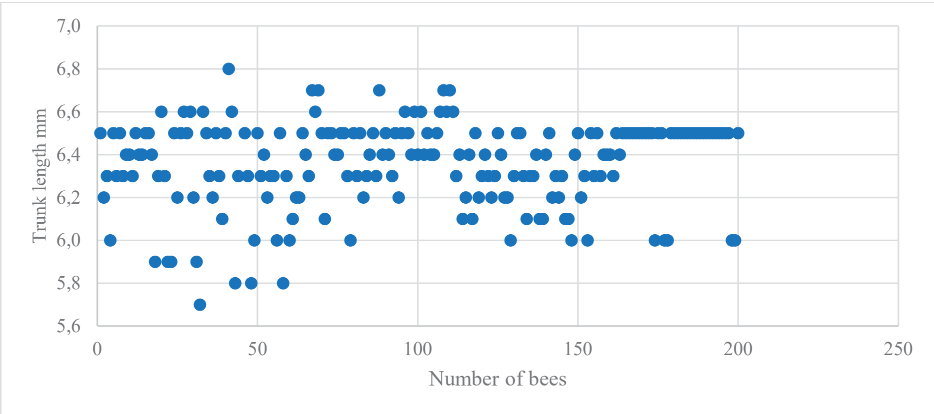

Figure 4. Proboscis length values for the parental population (PP)

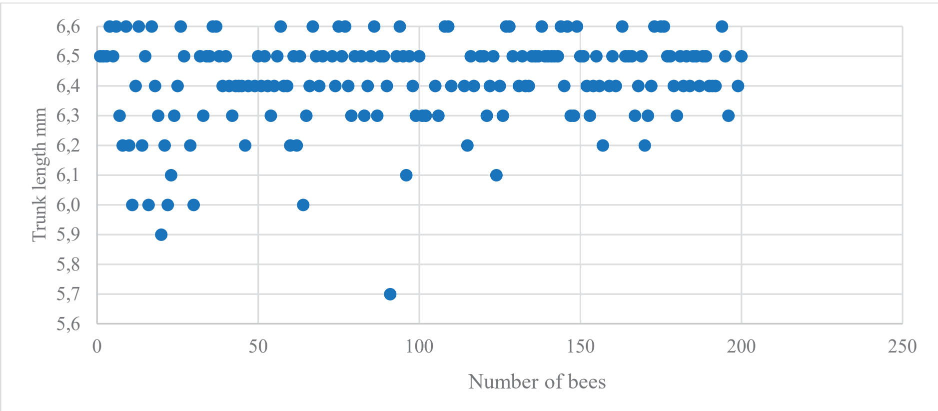

Figure 5. Proboscis length values for descending population (DP)

The average values obtained for the proboscis length (6.355 mm, respectively 6.447 mm) are slightly higher than those in the specialized literature, where the values are in the range of 5.950-6.340 mm (El-Aw et al., 2012; Rahimi & Mirmoayedi, 2013; Mohammed et al., 2017; Eremia & Cataraga, 2021).
The proboscis length character also shows a more uneven distribution of measured values in the parental population (Figure 4) compared to the descending population (Figure 5).
The Behrens-Fisher Student's Test was used to test the significance of the differences between the means of the two populations (Tables 5).

Table 5. Student Test values of the two populations for the proboscis length character

Groups	Sample size	t calculated	t critical, α = 0.001
PP	200	4.799***	3.290
DP	200		

As a consequence, the difference between the two populations regrading proboscis length character was very significant.

Upper Wing Length

The distribution of upper wing length character values has the same characteristic as previous characters.
The average values obtained of the upper wing length (9.563 mm, respectively 9.478 mm) are higher than those in the specialized literature, where the values are in the range of 8.70-9.25 mm (El-Aw et al., 2012; Rahimi & Mirmoayedi, 2013; Mohammed et al., 2017; Eremia & Cataraga, 2021).
The variances of the two populations, the descending population and the parental population, are heterogeneous (Table 6).

Table 6. Values of upper wing length statistical parameters

Specification	Parental population (PP)	Descending population (DP)
Mean	9.563	9.478
Variance	0.0628	0.0560
Standard deviation	0.2506	0.2368
Maximum	10.0	10.0
Minimum	9.0	9.0

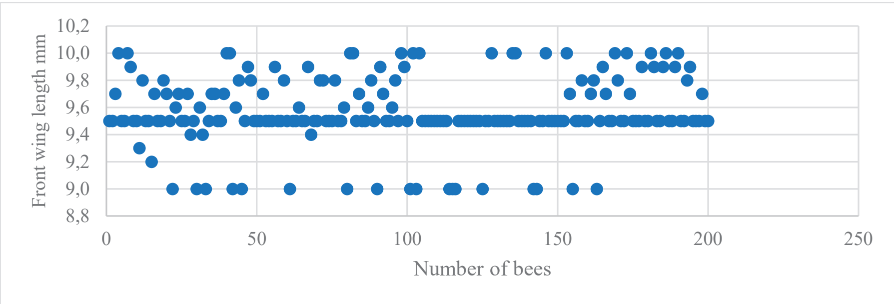

Figure 6. Upper wing length values for the parent population (PP)

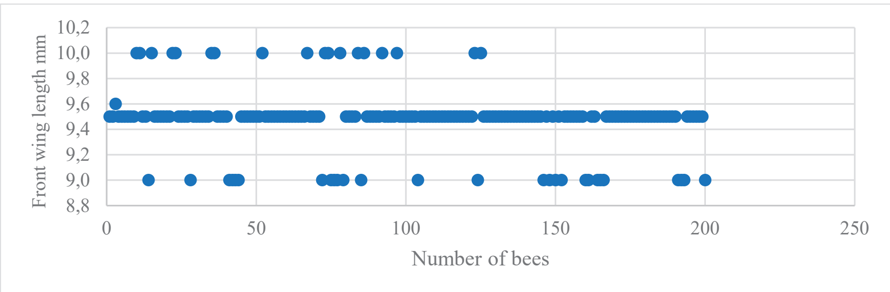

Figure 7. Upper wing length values for descending population (DP)

For the upper wing length character, a more uneven distribution of measured values is observed in the parent population (Figure 6) compared to the descending population, where measured values are distributed over three levels (Figure 7).

The measured character values are comparable in the two populations. Applying the Fisher test revealed that the variances of the two populations are heterogeneous (Table 7).

Table 7. Student Test values of the two populations for the for the upper wing length character

Groups	Sample size	t calculated	t critical, α = 0.001
PP	200	3.485***	3.290
DP	200		

To test the significance of differences in the means of the two populations, Behrens-Fisher Student's Test was applied. The difference of the values of the upper wing length character in the two populations was very significant.

Tergit-3 length

For the tergit-3 length character, a heterogeneity of measured traits mean values it was observed in both populations (Table 8).

The average values obtained for tergit-3 length (2.936 mm, respectively 2.862 mm) fall within the range found in the specialized literature, where the values are in the range 1.98-4.37 mm (Rahimi & Mirmoayedi, 2013; Eremia & Cataraga, 2021).

Table 8. Statistical parameter values for tergit-3 length

Specify	Parental population (PP)	Descending population (DP)
Mean	2.936	2.862
Variance	0.0166	0.0068
Standard deviation	0.1288	0.0830
Maximum	3.3	3.1
Minimum	2.6	2.6

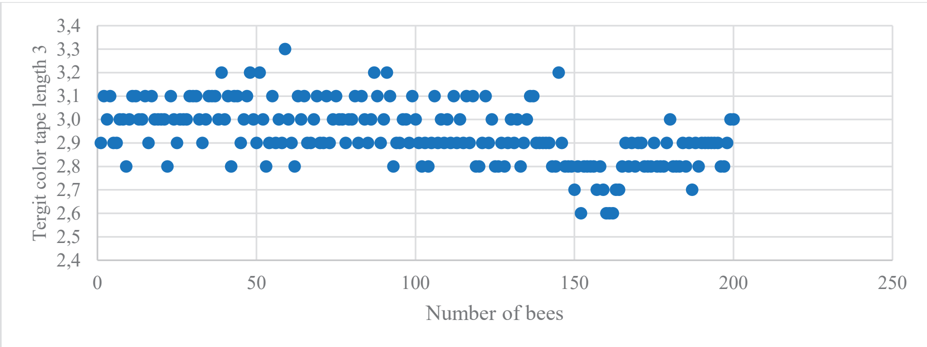

Figure 8. Tergit-3 length values for the parent population (PP)

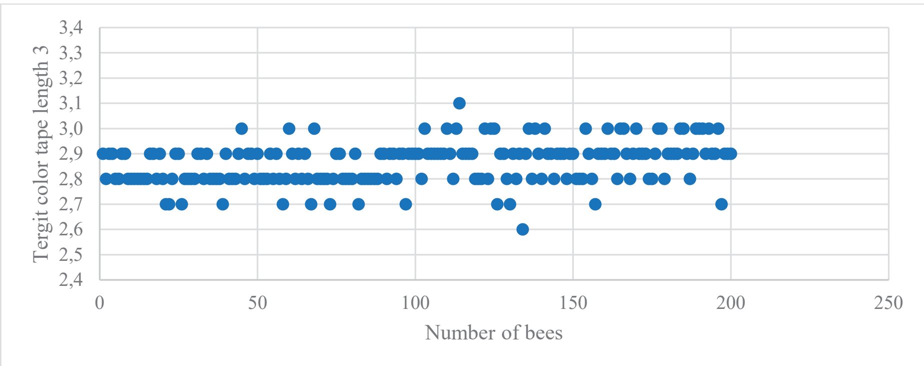

Figure 9. Tergit-3 length values for descendant population (PD)

For the tergit-3 length character, it is observed that the distribution of measured values in the parental population is more homogeneous (Figure 8) compared to the descending population (Figure 9).

Table 9. Student Test values of the two populations for the tergit-3 length character

Groups	Sample size	t calculated	t critical, α = 0.001
PP	200	6.874***	3.290
DP	200		

The difference of values of the tergit-3 length character in the two populations is very significant ($P < 0.001$) (Table 9).

Behaviour Characters

Behavioural character measurements show small differences between the parental and descendant populations (Table 10).

This highlights the fact that the descendant population has productive behaviour quite similar to that of the parental population.

Table 10. Behaviour analysis

Specification	Parental population	Descendant population
Gentleness	3.4	3.5
Quiet behavior on the honeycombs	3.7	3.4
Anecbalia (lack of swarming)	2.8	3.0
Honey caulking (relative appreciation)	64.4% dry caulking	60.2% dry caulking
The degree of blockage of the nest	54.3%	55.2%

CONCLUSIONS

Morphological character values measured in *Apis mellifera carpatica* bees, mountain ecotype, are within or close to the average morphological character values identified by other studies.

For all the characters studied, the statistical analysis highlighted the fact that there are very significant differences between the means of the two populations. These results are valid for the samples taken in the study. In other conditions, for other samples, the results could be different.

Conservation studies of the mountain ecotype of the *Apis mellifera carpatica* breed have not been carried out until now in our country.

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