Original Research Paper

# The Impact of Environment on the Morphometric Characteristics of Honeybees Apis Mellifera Carnica in South-East Kazakhstan 

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#### Abstract

Morphometric characteristics of honey bees (length and width of wings, number of roots) depend on the environmental conditions (in particular, the temperature regime) in which individuals develop. The purpose of the study is to determine the distribution of honey bees and a comparative analysis of their morphometric indicators in various climatic zones of South-Eastern Kazakhstan. The main climatic zones of the studied territory were (1) Mountainous and (2) Foothill zones covering the Tien Shan Mountain system and characterized by cold, snowy winters and cool summers, (3) Steppe zone characterized by a continental climate, and (4) Desert zone. The wing data were compared with reference samples from the bee morphometric data bank in Oberursel, Germany. The resolution of the reference images was 1096 DOI. The reference sample for Apis mellifera carnica consisted of 10 colonies originating from Slovenia. The study was conducted on 12 farms bred in various zones of South-Eastern Kazakhstan, including a reference sample, according to 11 main morphometric characteristics of worker bees. The length of the proboscis ranges from $6.21 \pm 0.04 \mathrm{~mm}$ to $6.35 \pm 0.02 \mathrm{~mm}$, while they do not differ from the control ones. The number of hamuli in the main samples averaged 19.6 pcs. compared with Kerbulak, Almaty $20.50 \pm 0.42$ pcs. And the control group was $21.50 \pm 1.3$ pcs. ( $\mathrm{p} \leq 0.05$ ). Differences on this basis between populations reach the first threshold of confidence probability ( $\mathrm{p}>0.95$ ). The range of the cubital index in all the studied groups ranges from $2.27 \pm 0.05,2.74 \pm 0.07$, the Hantel index was also dynamically within the normal range. According to the size of the centroids, a high degree of similarity was found between the entire population of honey bees of SouthEast Kazakhstan, with a degree between 858.40-887.77. The size of the centroid for the control samples was 662.82 , which has too many differences from the population of honey bees in South-East Kazakhstan.


Keywords: Honey Bee, Apis Mellifera Carnica, Biodiversity, Environment, Climatic Zone

## Introduction

Currently, according to morphometric and molecular genetic analysis, the Apis mellifera species includes 30 subspecies grouped into five main evolutionary lines-A, M, C, O, and Y (Meixner et al., 2013). Asia, the Middle East, and Africa are considered possible centers of origin of Apis mellifera, followed by the migration of bees to Eurasia in the western and eastern directions (Kandemir et al., 2011). The morphological Line-C consists of five subspecies of honey bees: Apis mellifera siciliana Grassi, Apis mellifera ligustica Spinola, Apis mellifera cecropia Kiensenwetter, Apis mellifera macedonica Ruttner and Apis mellifera carnica Pollman (Sinacori et al., 1998; Muñoz et al., 2009; Bissembayev et al., 2023).

The natural habitat of Apis mellifera L. is Europe, Africa, and Western Asia (De La Rúa et al., 2009), where many subspecies have adapted to local environmental conditions (Ilyasov et al., 2020).

Currently, there are more than 198,000 bee colonies on the territory of Kazakhstan, which are attributed to evolutionary lineages, mainly C and M (Sheppard and Meixner, 2003).

Beekeeping in Kazakhstan had experienced several downturns and ups and development. After a quartercentury break, work had begun on scientific support for the development of the beekeeping industry in Kazakhstan within the framework of the state budget project. The territory of the South-Eastern zone, where 2 regions of Kazakhstan are located, is represented by four natural and climatic zones from desert to highaltitude, with different climatic conditions that differ from each other. The total area of the two geographical regions of Almaty and Zhetysu region is $224519 \mathrm{~km}^{2}$ (Frisch et al., 2009).

The different climatic conditions of the two regions are due to the fact that the northern part of the territory is mainly a plain with ridges, and sand dunes, which belongs to the steppe and desert zones, and the southern part of the territory is represented by the mountain ranges of the Tien-Shan, which change vertically (Aizen et al., 1997; Sattarov et al., 2022).

In general, the climate is continental, but the foothill and mountain zones have higher moisture content here the winter is mild, and the summer is not too hot (Sheppard and Meixner, 2003).

In the conditions of the plain, the air temperature is not constant and changes daily, not to mention the seasons of the year the coldest temperature is 11-13 in the northern part, and sometimes it reaches-30 degrees or more. In the southern part, winter is mostly dark from $6-11^{\circ} \mathrm{C}$ and in summer the air temperature will warm up to $+25^{\circ} \mathrm{C}$. The duration of the warm period lasts from 220-240 days. The average annual precipitation on the
plain is 125 mm and in mountainous areas, it reaches 900 mm (Salnikov et al., 2023).

The vital activity of the bee family (Apis mellifera) depends on many factors, including environmental conditions. These factors include honeybee species in certain geographical areas (Gazizova et al., 2020; Zemskova et al., 2020).

There is a very wide biodiversity of honey plants in the South-Eastern beekeeping zone of Kazakhstan, which is located from the mountainous to the desert zone. The South-East Kazakhstan region currently has great potential for intensive reproduction and an increase in the number of honey bee colonies. In southeast Kazakhstan, the structures of sown areas are formed with the inclusion of priority honey-bearing crops, such as sunflower, oil flax, rape, buckwheat, vegetable and melon crops, and safflower, with annual sowing on the area of up to 170 thousand hectares. From the wild vegetation of the lowest strip of mountains in April-June goose onions, willows, tan eremurus, wild onions, sainfoin, thin-leaved peas, mountain cotoneaster, Turkestan motherwort, desert sage, and holo-colored althea are of honey-bearing value. In addition, the rich flora of the region can be rationally used in the preparation of nectar-pollen-bearing conveyors of the beekeeping industry, for forecasting honey collections, and in the preparation of complex breeding and breeding measures to preserve the local population of honey bees (Dimeyeva et al., 2015).

Morphometric characteristics of honey bees (length and width of wings, number of hamuli) depend on the environmental conditions (in particular, the temperature regime) in which individuals develop. Deviation from the temperature optimum leads to a change in the values of morphometric features (Eimanifar et al., 2018).

According to FAO (Faostat, 2022), the number of honey bee colonies in the world had grown by more than a third in 30 years. In 2020, there were 94 million bee colonies in the world- $36 \%$ more than in 1990. The greatest number of bee colonies in 2020 was in Asia (43.5 million). The number of bee colonies increased most seriously in Asia ( $+88 \%$ ), but at that time decreased in Europe ( $-13 \%$ ). In 1990, there were 22.5 million bee colonies in the whole of Europe, 15.1 million were in 2000 , and 19.6 million were in 2020 . At the same time, the number of bee colonies decreased in the Eastern and Western parts of Europe during this period and increased in Southern and Northern Europe.

According to the Bureau of National Statistics of the Republic of Kazakhstan, the beekeeping industry in Kazakhstan was developing dynamically, showed in Fig. 1. In 2021, according to the Bureau, there were 198312 bee colonies in the country, for 8 years the population had doubled.


Fig. 1:19 Landmarks on the right forewing of working honey bees
This study presented comparative morphometric indicators of bees living in different climatic zones of South-East Kazakhstan. The task of this study is selection and breeding work with local bee populations, along with the preservation of the Apis mellifera L. gene pool, also, is the improvement of existing lineages, types, and breeds adapted to certain natural and climatic conditions that were formed in specific natural and climatic conditions under the influence of natural selection and folk breeding, has a high genetic potential of productivity and qualitative originality.

This study was aimed at studying the Apis mellifera carnica honey bee in the environmental conditions of southeastern Kazakhstan, as well as to evaluate the morphometric indicators of the local honey bee populations of Apis mellifera carnica. Knowledge of the honey bee population is important for understanding the general distribution of honey bees.

Thus, full-fledged scientific support for the preservation of valuable gene pools of purebred honey bees, bred breeds, increasing their productivity, winter hardiness, and resistance to diseases is an urgent problem in the world of beekeeping (Chen et al., 2016).

## Aim of Their Search

The purpose of the study is to determine the distribution of honey bees and a comparative analysis of their morphometric indicators in various climatic zones of South-Eastern Kazakhstan.

## Materials and Methods

In the Almaty region, where bees were selected for research, in 2021, the population of bee colonies is 22,973 . For the Almaty region, the context of the country is $12 \%$.

The research was carried out on 12 farms bred in various zones of Southeast Kazakhstan, including reference samples, on 11 main morphometric characteristics of worker bees.

Sample collection. Determination breed affiliation must include samples of interest from the studied apiary or beeline. Samples should be taken from at least three bee families from an apiary or line. If it is necessary to examine the entire population occupying a certain area in order to detect regional differences, then samples are taken from at least 5 families per area so that sampling errors remain within acceptable limits (Radloff et al., 2010).

Samples of worker bees were collected from stationary hives. The collected samples provide different climatic and geographical zones of the South-Eastern Kazakhstan beekeeping zone. Samples of 30-40 working honey bees from a family were considered correct to study intra-family variability (Meixner et al., 2013).

Four-five bee colonies were randomly selected from the apiary, random samples of 50-75 workers per colony were used to create the final data sets from 12 different districts of southeastern Kazakhstan, including about 650 worker honey bees. The samples obtained were preserved in $70 \%$ ethanol. All collected bee farm locations information showed in Table 1.

The Zhanalyk farms located in the desert zone had an altitude of 395 m above sea level and the remaining farms in all zones were much higher than other farms. "Tleukhan" was located at an altitude of 1170 above sea level, which influenced the technology of bee farming.

Table 1: Sampling sites from different districts of South-East Kazakhstan (Almaty and Zhetysu regions)

| \# | Geographical zone | Districts | Name of the basic farms | Coordinates | Height above sea level, m |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | Mountain | Alakol | Tleukhan | $45^{\circ} 52^{\prime 25.2 " N ~} 81^{\circ} 07^{\prime} 11.6{ }^{\prime \prime} \mathrm{E}$ | 1170 |
| 2 | Foothill | Tekeli | Myhmet | $44^{\circ} 53^{\prime} 16.8^{\prime \prime} \mathrm{N} 78^{\circ} 45^{\prime} 36.9^{\prime \prime} \mathrm{E}$ | 834 |
| 3 | Foothill | Karatal | Jambo-Tau | $45^{\circ} 23^{\prime} 59.3{ }^{\prime \prime N} 78^{\circ} 00^{\prime} 21.8{ }^{\prime \prime} \mathrm{E}$ | 812 |
| 4 | Foothill | Zhambyl | Pchelka | $43^{\circ} 10^{\prime} 15.1{ }^{\prime \prime} \mathrm{N} 76^{\circ} 02^{\prime} 44.7{ }^{\prime \prime} \mathrm{E}$ | 994 |
| 5 | Foothill | Uygur | Marup | $43^{\circ} 22^{\prime} 55.4{ }^{\prime \prime} \mathrm{N} 79^{\circ} 39^{\prime} 17.7{ }^{\prime \prime} \mathrm{E}$ | 515 |
| 6 | Steppe | Enbekshikazakh | Bee queen | $43^{\circ} 41^{\prime} 07.5^{\prime \prime} \mathrm{N} 77^{\circ} 35^{\prime} 44.3{ }^{\prime \prime} \mathrm{E}$ | 524 |
| 7 | Steppe | Enbekshikazakh2 | Bee queen 2 | $43^{\circ} 41^{\prime} 07.5^{\prime \prime} \mathrm{N} 77^{\circ} 35^{\prime} 44.3{ }^{\prime \prime} \mathrm{E}$ | 524 |
| 8 | Steppe | Talgar | Guldala | $43^{\circ} 20^{\prime} 12.3{ }^{\prime \prime} \mathrm{N} 77^{\circ} 12^{\prime} 08.2^{\prime \prime} \mathrm{E}$ | 620 |
| 9 | Steppe | Kerbulak | Nesterenko | $44^{\circ} 29^{\prime} 38.4{ }^{\prime \prime N}{ }^{\prime} 77^{\circ} 59^{\prime} 02.6^{\prime \prime} \mathrm{E}$ | 630 |
| 10 | Steppe | Almaty | KazNARU | $43^{\circ} 23{ }^{\prime} 33.1{ }^{\prime \prime} \mathrm{N} 77^{\circ} 06^{\prime} 04.8{ }^{\prime \prime} \mathrm{E}$ | 679 |
| 11 | Steppe | Almaty 2 | Kaznaru 2 | $43^{\circ} 23{ }^{\prime} 33.1{ }^{\prime \prime} \mathrm{N} 77^{\circ} 06^{\prime} 04.8{ }^{\prime \prime} \mathrm{E}$ | 679 |
| 12 | Desert | Balkash | Zhanalyk | $44^{\circ} 50^{\prime} 33.1{ }^{\prime \prime} \mathrm{N} 76^{\circ} 17^{\prime} 38.2^{\prime \prime} \mathrm{E}$ | 395 |

The main climatic zones of the studied territory were (1) The mountainous and (2) Foothill zone, encompassing the Tien Shan Mountain system and characterized by cold, snowy winters and cool summers, (3) The steppe zone, characterized by a continental climate and (4) The desert zone.

The wing data were compared with reference samples from the Morphometric Bee Data Bank in Oberursel, Germany. The resolution of the reference images was 1096 dpi. The reference sample for Apis mellifera carnica consisted of 10 colonies originating from Slovenia. Apis mellifera carnica is an indigenous bee subspecies in Central Europe. Apis mellifera carnica was represented as the native subspecies in Croatia and Slovenia (Puškadija et al., 2020).

## Wing Measurement and Analysis

The length of the right forewing showed the greatest variability from all the morphometric indicators. The right forewings were cut out and morphometric parameters were analyzed using a Levenhuk MED D35T LCD microscope. They were scanned using an Epson V600 Photo scanner. The resolution of the images was 3200 dots per inch (image size: $5782 \times 3946$ pixels).

The traditional Alpatov morphometry method was used to identify and classify subspecies of Apis mellifera. 10 morphometric parameters of honey bees were measured using the standard Alpatov method: Proboscis length, tergite length, and width, sternite length and width, wax mirror length and width, and length and width of the right forewing (Alpatov, 1929).

The measurement of the right forewings was marked with 19 characteristic landmarks by Identi Fly software. Figure 1 showed 19 characteristic landmarks marked by the IdentiFly software (Nawrocka et al., 2018).

## Statistical Analysis

The average morphometric values, indexes, and centroid sizes of the colony sample, standard deviation, and standard error were calculated for each colony. Multidimensional statistical data analysis (using 11 morphometric features) consisted of an analysis of the main components to identify possible subclusters. Differences in wing size were based on calculating centroid size and were Analyzed by Analysis of Variance (ANOVA). Discriminant analysis was used to visualize the differences between bee colonies. Statistical analysis of the data was performed using JMP 7 and Statistica 7 (StatSoft).

## Results

## Standard Morphometry

The analysis of the obtained results of exterior morphometric features is shown in Table 2.

The research was carried out on 12 farms bred in various zones of South-East Kazakhstan, including a reference sample, on 11 main morphometric characteristics of worker bees.

According to Table 2, the range of the cubital index in all the studied groups ranges from $2.27 \pm 0.052 .74 \pm 0.07$, the hantel index was also dynamically within the standard. The discoidal shift showed a versatile result, both the low limit and the high limit are fixed. Thus, the colony bees Tekeli had a low discoidal shift of $0.54 \pm 0.33$, probably this was due to the monetization of the Caucasian breed, and hybridization is observed. The maximum limit was fixed in the reference group- $5.72 \pm 0.35$. In other cases, the bees of the Almaty region meet the limits of the norm of Apis mellifera carnica. In the studied groups, $60 \%$ of the body color is gray; yellowness was registered in the first and second tergites.

Thus, the length of the probosci's length from $6.21 \pm 0.04 \mathrm{~mm}$ to $6.35 \pm 0.02 \mathrm{~mm}$, while they do not differ from the control. The number of hamuli in the main samples averaged 19.6 pcs compared to Kerbulak, Almaty $20.50 \pm 0.42 \mathrm{pcs}$ and the control group $21.50 \pm 1.3$ pcs ( $\mathrm{p} \leq 0.05$ ). Differences in this trait between populations reach the first threshold of confidence probability ( $\mathrm{p}>0.95$ ). The maximum value in length and width of tergites is typical for the mountain zone Kerbulak, Almaty, Uygur, and reference $2,20 \pm 0,01$; $4,79 \pm 0,02 ; 2,20 \pm 0,01 ; 4,79 \pm 0,02 ; 2,27 \pm 0,06: 4,61 \pm 0,06$; $2,23 \pm 0,18 ; 4,92 \pm 0,19$ respectively. And the rest of the populations are approximately the same (Table 2).

According to the length of the sternite, the indicators in the reference group were $2.73 \pm 0.23 \mathrm{~mm}$, while in the other colonies, they range from $2.78 \pm 0.03 \mathrm{~mm}$ to $2.9 \pm 0.03$ mm . This can be explained to a greater extent by the process of monetization in recent years. At the same time, the length of the wax mirror is the maximum value recorded in bee colonies Enbekshikazakh-1.28 $\pm 0.01 \mathrm{~mm}$, Kerbulak, Almaty- $1.27 \pm 0.01 \mathrm{~mm}$. Indications of the average width of the wax mirror a high indicator was registered by Enbekshikazakh $2.4 \pm 0.02 \mathrm{~mm}$ corresponds to the standard. In other cases, it corresponds to the reference group. In colony Kerbulak, Almaty, Uygur bees meet the standards of Apis mellifera carnica. It is necessary to note the partial hybridization of bee colonies.

However, the studied groups are characterized by high uniformity, since in almost all indicators the variability is in the range of $1.65-4.1 \%$, with the exception of the length and width of the right wing. It should be noted that when comparing in the zonal aspect, according to the morphometric indicators, the probosci's length of bees bred in four zones was within the permissible norm from 6.25 foothill to 6.29 , not significantly exceeding the desert zone by $0.64 \%$. It was found that bees bred in the steppe zone were significantly different in six indicators
than in the other three zones: By hamulinumber, tergitewidth, sternite length and width, wax mirror length and width, and by proboscis length and the size of the wing were approximately the same with these indicators of bees of the desert and foothill zone. Mountain bees were inferior in all respects to the climate of other zones. Apparently, these different indicators in bees of the Apis mellifera carnica breed bred in different zones are the influence of the habitat.

Cluster analysis is based on the means of all morphometric means and divided into three main groups. The first group contained the bee samples from Alakol, Almaty 2, Tekeli, TalgarZhambyl, Karatal, Enbekshikazakh 2, Balkash. The second group was formed by the samples from districts Enbekshikazakh, Kerbulak, Almaty, and Uygur. The third cluster group contained the reference sample Apis mellifera carnica (Slovenia) Table 3

Table 2: Morphometric parameter of each district bee colony

|  | Parameters <br> length, mm | Probuscis <br> number | Hamuli <br> length, mm | $3^{\text {rd }}$ tergite <br> width, mm | $3^{\text {rd }}$ sternite <br> length, mm | $3^{\text {rd }}$ sternite <br> width, mm | Wax mirror <br> length, mm | Wax mirror <br> width, mm | forewing <br> length, mm | Right, Zone <br> width, mm | Right, <br> colony |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Steppe | Talgar | $6,24 \pm 0,02$ | $19,80 \pm 0,56$ | $2,14 \pm 0,01$ | $4,63 \pm 0,03$ | $2,82 \pm 0,02$ | $4,40 \pm 0,02$ | $1,20 \pm 0,02$ | $2,16 \pm 0,01$ | $8,96 \pm 0,02$ | $3,19 \pm 0,01$ |
|  | Kerbulak | $6,29 \pm 0,02$ | $20,50 \pm 0,42$ | $2,20 \pm 0,01$ | $4,79 \pm 0,02$ | $2,83 \pm 0,03$ | $4,65 \pm 0,03$ | $1,27 \pm 0,01$ | $2,28 \pm 0,02$ | $9,14 \pm 0,04$ | $3,24 \pm 0,02$ |
|  | Almaty | $6,29 \pm 0,02$ | $20,50 \pm 0,42$ | $2,20 \pm 0,01$ | $4,79 \pm 0,02$ | $2,83 \pm 0,03$ | $4,65 \pm 0,03$ | $1,27 \pm 0,01$ | $2,28 \pm 0,02$ | $9,14 \pm 0,04$ | $3,24 \pm 0,02$ |
|  | Almaty 2 | $6,26 \pm 0,04$ | $19,53 \pm 0,52$ | $2,15 \pm 0,01$ | $4,61 \pm 0,03$ | $2,81 \pm 0,02$ | $4,41 \pm 0,02$ | $1,21 \pm 0,01$ | $2,13 \pm 0,02$ | $8,98 \pm 0,03$ | $3,19 \pm 0,02$ |
|  | Enbekshik | $6,35 \pm 0,02$ | $19,81 \pm 0,90$ | $2,15 \pm 0,02$ | $4,71 \pm 0,03$ | $2,9 \pm 0,03$ | $4,57 \pm 0,03$ | $1,28 \pm 0,01$ | $2,4 \pm 0,02$ | $8,73 \pm 0,7$ | $3,13 \pm 0,02$ |
|  | Kazakh |  |  |  |  |  |  |  |  |  |  |
|  | Enbekshik | $6,30 \pm 0,03$ | $19,03 \pm 0,54$ | $2,14 \pm 0,01$ | $4,65 \pm 0,03$ | $2,81 \pm 0,02$ | $4,41 \pm 0,02$ | $1,21 \pm 0,01$ | $2,13 \pm 0,02$ | $8,96 \pm 0,02$ | $3,19 \pm 0,01$ |
|  | Kazakh 2 |  |  |  |  |  |  |  |  |  |  |
| Desert | Balkash | $6,29 \pm 0,03$ | $19,09 \pm 0,55$ | $2,14 \pm 0,02$ | $4,66 \pm 0,03$ | $2,80 \pm 0,02$ | $4,42 \pm 0,02$ | $1,21 \pm 0,01$ | $2,13 \pm 0,03$ | $8,90 \pm 0,03$ | $3,17 \pm 0,02$ |
|  | Zhambyl | $6,21 \pm 0,04$ | $19,4 \pm 0,55$ | $2,13 \pm 0,01$ | $4,63 \pm 0,03$ | $2,80 \pm 0,02$ | $4,41 \pm 0,02$ | $1,20 \pm 0,01$ | $2,13 \pm 0,03$ | $8,93 \pm 0,03$ | $3,18 \pm 0,01$ |
| Foothill | Tekeli | $6,24 \pm 0,02$ | $19,80 \pm 0,56$ | $2,14 \pm 0,01$ | $4,63 \pm 0,03$ | $2,82 \pm 0,02$ | $4,40 \pm 0,02$ | $1,20 \pm 0,01$ | $2,16 \pm 0,01$ | $8,96 \pm, 02$ | $3,19 \pm 0,01$ |
|  | Karatal | $6,33 \pm 0,03$ | $19,83 \pm 0,52$ | $2,15 \pm 0,01$ | $4,62 \pm 0,03$ | $2,79 \pm 0,02$ | $4,40 \pm 0,02$ | $1,22 \pm 0,01$ | $2,12 \pm 0,02$ | $8,96 \pm 0,03$ | $3,20 \pm 0,01$ |
|  | Uygur | $6,24 \pm 0,05$ | $19,98 \pm 0,76$ | $2,27 \pm 0,06$ | $4,61 \pm 0,06$ | $2,78 \pm 0,03$ | $4,53 \pm 0,03$ | $1,21 \pm 0,01$ | $2,22 \pm 0,02$ | $9,13 \pm 0,01$ | $3,23 \pm 0,01$ |
| Mountain | Alakol | $6,26 \pm 0,03$ | $19,43 \pm 0,53$ | $2,14 \pm 0,01$ | $4,63 \pm 0,03$ | $2,81 \pm 0,02$ | $4,40 \pm 0,02$ | $1,21 \pm 0,01$ | $2,13 \pm 0,02$ | $8,97 \pm 0,03$ | $3,19 \pm 0,01$ |
| Control | Slovenia | $6,35 \pm 0,46$ | $21,50 \pm 1,3$ | $2,23 \pm 0,18$ | $4,92 \pm 0,19$ | $2,73 \pm 0,23$ | $4,31 \pm 0,29$ | $1,22 \pm 0,12$ | $2,11 \pm 0,15$ | $9,02 \pm 0,14$ | $3,12 \pm 0,1$ |

Table 3. Main morphometric indexes for each district bee colony of South-East Kazakhstan

| Bee colony | area | Cubital index |  |  | Hantel index |  |  | Discoidal shift$\qquad$$\mathrm{X} \pm \mathrm{m}$ | Tarsal index $\qquad$ <br> $\mathrm{X} \pm \mathrm{m}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\mathrm{X} \pm \mathrm{m}$ | $\sigma$ | $\mathrm{C}_{\mathrm{v}}, \%$ | $\mathrm{X} \pm \mathrm{m}$ | $\sigma$ | $\mathrm{C}_{\mathrm{v}}, \%$ |  |  |
| Steppe | Talgar | 2,57 $\pm 0,05$ | 0,32 | 12,30 | 1,00 $\pm 0,01$ | 0,07 | 6,97 | 1,78 $\pm 0,46$ | 49,29 $\pm 0,58$ |
|  | Kerbulak | $2,72 \pm 0,30$ | 0,46 | 16,87 | $1,03 \pm 0,01$ | 0,08 | 7,70 | 2,96 $\pm 0,28$ | 47,52 $\pm 0,55$ |
|  | Almaty | 2,62 $\pm 0,06$ | 0,39 | 14,80 | $1,03 \pm 0,01$ | 0,08 | 7,67 | 3,52 $\pm 0,35$ | 47,52 $\pm 0,55$ |
|  | Almaty 2 | $2,74 \pm 0,07$ | 0,48 | 17,70 | $1,03 \pm 0,01$ | 0,09 | 8,40 | 3,35 $\pm 0,33$ | 48,67 $\pm 0,61$ |
|  | Enbekshikazakh | $2,48 \pm 0,04$ | 0,30 | 12,22 | 0,98 $\pm 0,01$ | 0,09 | 8,82 | 1,28 $\pm 0,31$ | 47,07 $\pm 1,05$ |
|  | Enbekshikazakh | 22,63 $\pm 0,07$ | 0,37 | 14,11 | 1,08 $\pm 0,01$ | 0,08 | 7,43 | 4,71 $\pm 0,36$ | 48,76 $\pm 0,53$ |
| Desert | Balkash | $2,51 \pm 0,07$ | 0,33 | 10,55 | $0,99 \pm 0,01$ | 0,08 | 8,74 | 2,88 $\pm 0,66$ | $48,77 \pm 0,56$ |
| Foothill | Zhambyl | 2,60 $\pm 0,09$ | 0,44 | 15,66 | 1,09 $\pm 0,02$ | 0,09 | 8,77 | 2,81 $\pm 0,36$ | 48,99 $\pm 0,69$ |
|  | Tekeli | 2,4 $\pm 0,05$ | 0,32 | 14,50 | 0,92 $\pm 0,01$ | 0,11 | 11,47 | 0,54 $\pm 0,33$ | 49,29 $\pm 0,58$ |
|  | Karatal | 2,27 $\pm 0,05$ | 0,41 | 18,47 | 0,93 $\pm 0,01$ | 0,08 | 8,37 | 1,81 $\pm 0,34$ | 48,61 $\pm 0,58$ |
|  | Uygur | $2,53 \pm 0,06$ | 0,38 | 15,80 | 0,96 $\pm 0,01$ | 0,09 | 9,13 | 1,27 $\pm 0,28$ | 47,87 $\pm 0,59$ |
| Mountain | Alakol | 2,61 $\pm 0,08$ | 0,43 | 16,67 | 1,11 $\pm 0,02$ | 0,09 | 7,87 | 3,81 $\pm 0,31$ | $48,77 \pm 0,57$ |
| Reference | Slovenia | $2,63 \pm 0,09$ | 0,19 | 10,8 | 1,08 $\pm 0,03$ | 0,08 | 8 | $5,72 \pm 0,35$ | 45,56 $\pm 2,25$ |

Table 4: Mean, standard deviation, and standard error values for wing centroid size

| Level | Mean | Std Dev | Std Err Mean |
| :--- | :---: | ---: | :--- |
| control | 662,825 | 2,9418 | 1,6985 |
| desert | 866,263 | 7,6601 | 4,4226 |
| foothill | 866,744 | 17,2641 | 4,4576 |
| mountain | 887,778 | 12,7952 | 5,7222 |
| steppe | 858,407 | 9,1878 | 1,7363 |

Table 5: Compared the centroid size between bee colonies each district of South-East Kazakhstan ANOVA Tukey HSD test

|  | mountain | foothill | desert | steppe | control |
| :--- | :---: | ---: | ---: | ---: | ---: |
| Mountain | 0,00 | 21,03 | 21,51 | 29,37 | 224,95 |
| foothill | $* * *$ | 0,00 | 0,48 | 8,34 | 203,92 |
| desert | $* *$ | NS | 0,00 | 7,86 | 203,44 |
| steppe | $*$ | $* * *$ | NS | 0,00 | 195,58 |
| control | $*$ | $*$ | $*$ | $*$ | 0,00 |

*p $<0.0001, * * p<0.001,{ }^{* * *} \mathrm{p}<0.05$, NS-not significant

## Wing Size

Centroid size was calculated from the square root of the sum of the squared distances between the center of the polygon and each landmark. Wing size shown by centroid size, differed significantly between districts and reference samples (ANOVA: F ratio $=25.56, \mathrm{p}<0.001$ ). The reference wing showed the smallest size. According to the mountain located, Alakol samples showed the largest, then dessert samples Uygur and Balkash, decrease towards steppe-located samples. The means, standard deviations, and standard errors for each bee colony wing were given in Table 4.

Mean wing centroid size was smaller in reference samples and samples from districts Alakol, and Zhambyl showed higher sizes, 887.78 , and 885.67 , respectively.

According to centroid sizes, a high degree of similarity was found between all South-East Kazakhstan honey bee populations, with degrees between $858,40-887,77$. The centroid size for control samples was 662,82 , which has too many differences from South-East Kazakhstan honey bee population Table 4.

According to morphometric parameters, 5 colonies from foothill zone populations had discrepancies in the coefficient of variation, shown in Fig. 3.

Comparing the centroid size between bee colonies in each district of South-East Kazakhstan by ANOVA Tukey HSD test Table 5.

According to Identifly software (Nawrocka et al., 2018), 58 of 59 bee colonies from districts of South-East Kazakhstan showed C lineage. Only one colony (bee colony \#31) showed similarity to the O lineage sample from the Tekeli district shown in Fig. 2.

Investigation between researched bee colonies and reference samples did not show differences between districts Fig. 2. But the results of the discriminant analysis showed strong differences between zones. Control honey bee samples had no differences with steppe and foothill honey bee populations. Despite this, the foothill populations were scattered in different directions Fig. 2.


Fig. 2: Discriminant analysis of honeybee wings from each region based on canonical variate analysis of wings


Fig. 3: Discriminant analysis of honeybee wings from each region based on factorial analysis of wings

According to morphometric parameters, 5 colonies from foothill zone populations had discrepancies in the coefficient of variation, shown in Fig. 3.

## Discussion

The data presented morphometric indicators of honey bee samples from South-East Kazakhstan. After comprehensive studies of morphometric indicators of Apis mellifera carnica in South-East Kazakhstan, a trend was revealed to change the taxonomic affiliation of local bees in the apiaries of the region based on the assessment of morphometric characteristics. These changes were the result of the climatic diversity of the region, where there were different climatic and geographical zones (Aglagane et al., 2022).

Only one colony from Tekeli from the foothill zone was classified as lineage $O$. Other colonies were identified as lineage C, by subspecies as Apis mellifera carnica with high possibilities. However, local beekeepers have no information about bee queens.

In the present study, the mean of right forewing length and width were 8.98-3.19, respectively. And right forewing length and width of the control sample from Slovenia were 9.02-3.19, respectively.

The average probosci's length results of our study and control samples were 6.27-6.35, respectively.

Since morphometric features depend on the geographical location of bees, their age, and nutrition and vary widely even within the same breed or population, there was a need to develop approaches based on the analysis of the genotype of honey bees.

It is established that all the South-Eastern zone of Kazakhstan has, basically, a single origin and belongs to the Apis mellifera carnica breed. Nevertheless, they differ in some indicators under the influence of the environment of natural and climatic zones.

Thus, each of the initial populations is valuable and interesting in its own way, but the bees of the mountainous zone turned out to be more unique in origin and morphological characteristics. Bees isolated by high mountains and various ridges, with a lack of food, had to repeatedly cross obstacles in search of food to ensure the safety of offspring and procreation. In this regard, these bees had an increase in the honey goiter, the length and width of the wings, which allow them to carry more food with the least weight of the individual worker bee. The population of the mountain belt of the republic is extremely valuable for the mass reproduction of bee colonies of the Apis mellifera carnica population.

Obtained data in this research will be carried out to breed zonal ecological types within one breed, which will expand the gene pool wealth of the Apis mellifera carnica breed. It is possible that the genes of local populations of native bees on the territory of various zones of Kazakhstan will be used.

## Conclusion

The present study established the morphometric method has been used as a tool to research differences in honey bees in southeast Kazakhstan. There were compared main morphometric parameters between Apis mellifera carnica species from various climatic zones. All these obtained parameters showed insignificant differences between analyzed bee samples. Parameters can be particularly emphasized such as hamuli number, tergite width, sternite length and width, wax mirror length and width, and by proboscis length. These indicators showed especially in the steppe zone honey bee samples. Also, the results showed slight differences in the cubital index and discoidal shift between all samples. At the same time, there weren't any differences by the hantel and tarsal indexes. The wing centroid sizeы showed varying values, especially in the mountain bee samples showed the highest value-887.77, the other climatic zones showed similar results 858.4-866.74. All analyzed colonies showed C lineage by Identifly software. Investigation between colonies and reference samples did not show differences. All canonical variation results were obtained by discriminant analysis.

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## Author's Contributions

Ulzhan Auezkhanovna Nuralieva: The author of the idea, head of the event, generalization.

Nuradil Nurbekuly Spatay and Ainur Malikovna Davletova: Share of implementation and contribution to the preparation of the article.

Maxat Risbekovich Toishimanov: Share of implementation and contribution to the preparation of the article, revision of the article.

Gaukhar Abikenovna Moldakhmetova: Responsible executor, preparation of the manuscript, revision of the article.

Zhanar Abikenovna Kussainova: Responsible executor, preparation of the manuscript.

Akmal Abduvaitovich Khudaiberdiev and Svetlana Nikolaevna Khrapova: Performer, analysis of research results.

Dastanbek Asylbekovich Baimukanov: Responsible executor, preparation of the manuscript, final revision of the article.

## Ethics

There is no conflict of interest. Scientific ethics is observed when setting up experimental works.

## References

Aglagane, A., Tofilski, A., Er-Rguibi, O., Laghzaoui, E. M., Kimdil, L., El Mouden, E. H., ... \& Aourir, M. (2022). Geographical Variation of Honey Bee (Apis mellifera L. 1758) Populations in SouthEastern Morocco: A Geometric Morphometric Analysis. Insects, 13(3), 288. https://doi.org/10.3390/insects 13030288
Aizen, V. B., Aizen, E. M., Melack, J. M., \& Dozier, J. (1997). Climatic and hydrologic changes in the Tien Shan, central Asia. Journal of Climate, 10(6), 1393-1404. https://doi.org/10.1175/15200442(1997)010<1393:CAHCIT>2.0.CO;2
Alpatov, W. W. (1929). Biometrical studies on variation and races of the honey bee (Apis mellifera L.). The Quarterly Review of Biology, 4(1), 1-58. https://doi.org/10.1086/394322
Bissembayev, A.T., Zemskova N.E., Sattarov V.N., Semenov V.G., Kargayeva M.T. \& Baimukanov A.D. (2023). Eidonomy of Apis mellifera Workers and Drones in Apiaries. OnLine Journal of Biological Sciences. 23(2). P. 170-176.
https://doi.org/10.3844/ojbsci.2023.170.176

Chen, C., Liu, Z., Pan, Q., Chen, X., Wang, H., Guo, H., ... \& Shi, W. (2016). Genomic analyses reveal demographic history and temperate adaptation of the newly discovered honey bee subspecies Apis mellifera Sini Xinyuan n. ssp. Molecular Biology and Evolution, 33(5), 1337-1348. https://doi.org/10.1093/molbev/msw017
De la Rúa, P., Jaffé, R., Dall'Olio, R., Muñoz, I., \& Serrano, J. (2009). Biodiversität, Naturschutz und aktuelle Bedrohungen der europäischen Honigbienen. Apidologie, 40, 263-284. https://doi.org/10.1051/apido/2009027
Dimeyeva, L. A., Sitpayeva, G. T., Sultanova, B. M., Ussen, K., \& Islamgulova, A. F. (2015). Highaltitude flora and vegetation of Kazakhstan and climate change impacts. Climate Change Impacts on High-Altitude Ecosystems, 1-48. https://doi.org/10.1007/978-3-319-12859-7_1
Eimanifar, A., Brooks, S. A., Bustamante, T., \& Ellis, J. D. (2018). Population genomics and morphometric assignment of western honey bees (Apis mellifera L.) in the Republic of South Africa. BMC Genomics, 19, 1-26. https://doi.org/10.5061/dryad.98jh446
Faostat, (2022). The FAO's crops and livestock products statistics (FAOSTAT). Crops and livestock products. https://www.fao.org/faostat/en/\#data/QCL
Frisch, K., Voigt, S., Voigt, T., Hellwig, A., Verestek, V., \& Weber, Y. (2009). Extreme aridity prior to lake expansion deciphered from facies evolution in the Miocene Ili Basin, south-east Kazakhstan. Sedimentology, 66(5), 1716-1745. https://doi.org/10.1111/sed. 12556
Gazizova, N. R., Mannapov, A. G., Sattarov, V. N., Semenov, V. G., Skvortsov, A. I., \& Madeybekin, I. N. (2020, November). Morphological characterization of the Apis mellifera drones in the Southern Urals. In IOP Conference Series: Earth and Environmental Science (Vol. 604, No. 1, p. 012030). IOP Publishing. https://doi.org/10.1088/1755-1315/604/1/012030
Ilyasov, R. A., Lee, M. L., Takahashi, J. I., Kwon, H. W., \& Nikolenko, A. G. (2020). A revision of subspecies structure of western honey bee Apis mellifera. Saudi Journal of Biological Sciences, 27(12), 3615-3621. https://doi.org/10.1016/j.sjbs.2020.08.001
Kandemir, I., Özkan, A., \& Fuchs, S. (2011). Reevaluation of honeybee (Apis mellifera) microtaxonomy: A geometric morphometric approach. Apidologie, 42, 618-627. https://doi.org/10.1007/s13592-011-0063-3

Meixner, M. D., Pinto, M. A., Bouga, M., Kryger, P., Ivanova, E., \& Fuchs, S. (2013). Standard methods for characterising subspecies and ecotypes of Apis mellifera. Journal of Apicultural Research, 52(4), 1-28. https://doi.org/10.3896/IBRA.1.52.4.05
Muñoz, I., Dall'Olio, R., Lodesani, M., \& De la Rúa, P. (2009). Population genetic structure of coastal Croatian honeybees (Apis mellifera carnica). Apidologie, 40(6), 617-626. https://doi.org/10.1051/apido/2009041
Puškadija, Z., Kovačić, M., Raguž, N., Lukić, B., Prešern, J., \& Tofilski, A. (2020). Morphological diversity of Carniolan honey bee (Apis mellifera carnica) in Croatia and Slovenia. Journal of Apicultural Research, 60(2), 326-336. https://doi.org/10.1080/00218839.2020.1843847
Salnikov, V., Talanov, Y., Polyakova, S., Assylbekova, A., Kauazov, A., Bultekov, N., ... \& Beldeubayev, Y. (2023). An Assessment of the Present Trends in Temperature and Precipitation Extremes in Kazakhstan. Climate, 11(2), 33. https://doi.org/10.3390/cli11020033
Sattarov, V. N., Sabirjonova, M. R., Semenov, V. G., Kargaeyeva, M. T., \& Baimukanov, A. D. (2022). logico-a meaningful model of Apis mellifera conservation as a basis for the development of new strategies in animals.
Sheppard, W. S., \& Meixner, M. D. (2003). Apis mellifera pomonella, a new honey bee subspecies from Central Asia. Apidologie, 34(4), 367-375. https://doi.org/10.1051/apido:2003037
Sinacori, A., Rinderer, T. E., Lancaster, V., \& Sheppard, W. S. (1998). A morphological and mitochondrial assessment of Apis mellifera from Palermo, Italy. Apidologie, 29(6), 481-490. https://doi.org/10.1051/apido:19980601
Nawrocka, A., Kandemir, İ., Fuchs, S., \& Tofilski, A. (2018). Computer software for identification of honey bee subspecies and evolutionary lineages. Apidologie, 49, 172-184. https://doi.org/10.1007/s13592-017-0538-y
Radloff, S. E., Hepburn, C., Hepburn, H. R., Fuchs, S., Hadisoesilo, S., Tan, K., ... \& Kuznetsov, V. (2010). Population structure and classification of Apis cerana. Apidologie, 41(6), 589-601. https://doi.org/10.1051/apido/2010008
Zemskova, N. E., Sattarov, V. N., Skvortsov, A. I., \& Semenov, V. G. (2020). Morphological characteristics of honey bees of the Volga region. In BIO Web of Conferences (Vol. 17, p. 00035). EDP Sciences. https://doi.org/10.1051/bioconf/20201700035

