

Original researches

Received: 09.01.2024

Revised: 01.02.2024

Accepted: 16.02.2024

Lviv National University named
after Ivan Franko, University st., 1,
Lviv, 79000, Ukraine.
Tel.: +38-066-654-45-67.
E-mail: 1951nadija@gmail.com

Chernivtsi National University
named after Yuriy Fedkovych,
Kotsyubinsky st., 2,
Chernivtsi, 58012, Ukraine.
Tel.: +38-096-402-14-88.
E-mail: o.cherevatov@chnu.edu.ua

Polissia National University,
Stariy Bulvar, 7, Zhytomyr, 10002,
Ukraine. Tel.: +38-066-757-67-49.
E-mail: olekhalatyuk@gmail.com

Cite this article: Yarovets, V. I.,
Cherevatov, O. V., Galatiuk, O. Y.,
Zastulka, M. V., & Babenko, V. V.
(2024). Features of determining the
subspecies status of honey bees (*Apis
mellifera*) based on morphometric
wing indicators of drones. *Agrology*,
7(1), 3–13. doi: 10.32819/202401

Features of determining the subspecies status of honey bees (*Apis mellifera*) based on morphometric wing indicators of drones

V. I. Yarovets*, O. V. Cherevatov**, O. Y. Galatiuk***, M. V. Zastulka***, V. V. Babenko*

*Lviv National University named after Ivan Franko, Lviv, Ukraine

**Chernivtsi National University named after Yuriy Fedkovych, Chernivtsi, Ukraine

***Polissia National University, Zhytomyr, Ukraine

Abstract. As of today, in Ukraine, there are no opportunities to conduct genetic research on a sufficient scale to identify the composition of the genomes of honey bees. Wing morphometrics of drones is a readily available alternative for obtaining information about the approximate composition of DNA. The goal of the work was to formulate the principles of the main stages of research and illustrate the procedure for obtaining morphometric data, methods of their processing, and possibilities for interpreting the obtained results. In the package of statistical data processing application programs Statistica, the classification of 2,147 drone wings obtained from 6 apiaries of Zhytomyr, Mykolaiv, and Kyiv regions was carried out according to eight indices: Ci, Dbi, Disc.sh, Pci, Ri, Ci.3, Ci.2.1, and Ci.2.2. We propose a method for obtaining morphometric wing standards of bees based on studying a significant number of bee colonies from various apiaries in specific regions of Ukraine. Using a two-stage methodology, three datasets were formed and recommended for use as standards: one for the local population of Ukrainian bees and two for populations of the *A.m.mellifera* subspecies distributed in the Polissia region. We analyzed the reliability of results in determining the taxonomic affiliation of bees was analyzed. Using the example of a single bee colony, a detailed procedure is demonstrated to identify taxonomic affiliation through utilization of the obtained standards. It has been demonstrated that in case of reliable identification of standards belonging to specific subspecies, it is possible to predict the likely composition of chromosomal sets in the genomes of drones, queens, and worker bees partially corresponding to the wing phenotype.

Keywords: classical morphometrics, morphometric standards, drones, subspecies of honeybees, ecotypes, ploidy, hybridization, selection, discriminant analysis.

Introduction

In Ukraine, as well as in other European countries, industrial bee-keeping, which involves keeping a small number of bee lines, is becoming increasingly common. There are instances of using subspecies not native to a given geographic area, leading to the emergence of hybrids that are difficult to control. This situation results in the displacement or complete degradation of local populations of honey bees. Therefore, reserves should be created to preserve the gene pool of autochthonous bee populations. Addressing this issue is closely tied to identifying specific bee colonies and queens that correspond phenotypically, and preferably genetically, to those suitable for breeding and propagating local bee populations. Success in achieving this goal is largely determined by reaching the necessary level of fundamental competitive traits in local populations compared to introduced bee populations. This cannot be resolved without selective breeding, which involves the use of certain methods for evaluating and selecting promising individuals or lines to improve or fix economically beneficial traits and characteristics in the offspring. Regarding bees, such traits may include: productivity, low aggressiveness, disease resistance, varroa tolerance, and so on. It is clear that the required traits are determined by the structure of bee genomes. Therefore, studying the diversity, combinations, and inheritance patterns of specific genes and the corresponding traits they encode is a priority in breeding efforts. Recently, a considerable number of studies have been exploring both mitochondrial and nuclear DNA of bees (Özdil et al., 2012; Evans et al., 2013; Parejo et al., 2018). Some of them pertained to studying the genomes as a whole or their parts in subspecies *A. m. carnica*, *A. m. mellifera*, which is significant as these bees inhabit the territory of Ukraine (Meixner et al., 2007), where such research is limited due to insufficient funding, lack of

modern equipment, and shortage of scientific personnel. Despite these challenges, the Department of Molecular Genetics and Biotechnology at the Chernivtsi National University has been actively conducting research on mitochondrial DNA of bees (Cherevatov et al., 2019, Cherevatov et al., 2020). There are also several sporadic studies that, unfortunately, had not been continued (Metlytska et al., 2010; Metlytska et al., 2012; Hryhorchuk et al., 2020). We are unaware of any other works by Ukrainian researchers on this issue. When studying parts of genomes, molecular markers are obtained, which allow identifying the origin and evolution of the bees (Garnery et al., 1992; Espregueira Themudo et al., 2020; Tihelka et al., 2020; Carr, 2023) and, in some cases, link them to specific economically useful traits (Buttstedt et al., 2013). Thus, a study identified the centre of origin and distribution of honey bees in general, and on the Eurasian continent in particular (Garnery et al., 1992; Tihelka et al., 2020). Based on the available information (On Beekeeping: Law of Ukraine No 184/82), it is considered that there should naturally be at least three significant populations of local bees in Ukraine, namely: Carpathian, Ukrainian steppe, and dark European, likely corresponding to the subspecies *A. m. carnica*, *A. m. macedonica*, and *A. m. mellifera*, respectively.

An available alternative way to identify the approximate composition of bee DNA is morphometric research on the wing phenotype of drones and worker bees. The taxonomic affiliation of bees based on phenotypic features, including wing morphometrics, began to be reliably determined from the second half of the 20th century (DuPraw, 1965; Ruttner et al., 1978). There are several modern computer programs that process primary morphometric data and make certain predictive conclusions regarding the breed affiliation of bees, for example, such as Beemorph (Papp et al., 2015), Morphoj, IdentiFly/DrawWing, Dawino (Bouga et al., 2011). All of those and some other software are

effective tools. However, it seems that Ukrainian beekeepers, in most cases, do not fully grasp the overall picture and the way to use the results obtained through these programs. Sometimes, digital resources have graphical representations of distribution frequencies of individual morphometric traits. These graphics are used to make predictions about the 'breed' and the potential for using queens in breeding programs. The values of feature ranges for certain subspecies are used as evidences. Considering that the ranges for subspecies *A. m. mellifera*, *A. m. caucasica*, or *A. m. carnica*, *A. m. macedonica*, *A. m. ligustica* overlap, predictions are quite dubious. Therefore, it is necessary to clearly formulate the mechanism for conducting the final procedure of using the results of wing phenotype morphometric studies when using the "reverse" method (from phenotype to genotype) to predict the probable genome composition of bees. The main postulate for researchers should be the assertion that breeding is determined not by phenotype but by the genome structure. Efforts of apiarists and breeders should concentrate their efforts in this direction. The procedure to identify the probable composition of bee genomes based on wing phenotype should be clearly regulated and rely on specific indicators that serve as evidences of the correctness of cluster-identification results.

Knowing possible allelic variations allows us to associate them with phenotypic traits and, subsequently, through instrumental insemination, create combinations that are considered optimal. In other words, an additional cost-effective tool emerges, which allows for a targeted selection, as opposed to the traditional method of simple selection of the best individuals based on desired traits, thereby significantly expediting the breeding processes.

Particular attention must be paid to establishing the accuracy of predictions in numerical expressions. At the initial stage of obtaining primary digital information, accuracy is limited by instrumental errors and the quality of wing slide images. Modern digital cameras allow us to take high-quality photos. As for scans, it is necessary to use scanners with the resolution of ≥ 2500 dots per inch.

The reliability of wing classification at the second stage of research is ensured through statistical data-processing methods.

An essential component in addressing the task of preserving indigenous bees is the need for "breeding markers" for local bee populations. Morphometric wing standards of bees serve as one such marker. This idea can be traced in the work of Meixner et al. (2013): "Thus, there is a widely recognised need to encourage regional breeding efforts to preserve local adaptation, and to maintain local strains in isolated conservation apiaries. To attain this goal, it is necessary to have a reference base to identify strains to be used for breeding." To achieve this goal, it is necessary to have a reference base for identifying strains used for selection". The reliability of forming arrays of morphometric standards at the third stage of research requires additional substantiation.

The key challenge arises at the fourth stage of research when it is necessary to identify subspecies affiliation of the obtained standards. This issue was discussed in the work by Babenko et al. (2023a). In this aspect, we should note the studies (Kauhausen-Keller et al., 1997; Kandemir et al., 2011) of taxonomic verification of 24 reference wing samples stored in the database in Oberursel (Hesse, Germany) using classical and geometric morphometrics, respectively. From each bee colony, 15–20 wings were collected in the first study and 30 in the second study, the phenotypic traits of which served as the reference morphometric samples and were used for the purpose of analyzing their conformity to specific subspecies. In other words, correctness of compliance was verified with the concept of standardization. We are unaware of publications by other researchers on a similar topic. However, it is crucial to emphasize that our approach to obtaining morphometric standards fundamentally differs from how it was done in those works. This is due to limited access to the wing database in Oberursel and other foreign databases that meet reliability conditions. Another reason is absence of a database of standards for local bee populations in Ukraine that underwent intensive selection. Therefore, we propose an approach where large datasets of feature values from different bee colonies of local bee populations are accepted as standards. We found no similar studies. The procedure for obtaining such standards involves using as many bee colonies as possible, classifying wing phenotypes through discriminant analysis, creating arrays of similar phenotypic standard data based on this, and identifying the latter as belonging to specific bee taxa. The final evidence of interpreting the obtained standard data, i.e., belonging to specific subspecies, populati-

ons, ecotypes, or lines, is carried out by comparing them to the previously obtained morphometric standards of wing phenotypes using Mahalanobis distances (MD) or data confirmed by genetic research.

The objective of the study was formulating the principles for conducting the main stages of morphometric studies of bee and drone wings, demonstrating methods of obtaining morphometric standards, and presenting the possibilities of interpreting the obtained results.

Materials and methods

To conduct the research, we used 2,147 drone wings from 28 bee colonies, collected in 2021 from 6 apiaries. The apiaries belonged to the beekeepers: Stoliar I. (Glybochok village, Zhytomyr Oblast), Halatiuk O. (Chudniv village, Veresny village, Zhytomyr city, Zhytomyr Oblast), Strilchuk M. (Shtukar village, Mykolaiv Oblast), and Kryvchenko O. (Kaharlyk city, Kyiv Oblast). The initial processing of wings was carried out using the TpsDig2 program, following the methodology described in our previous work (Yarovets et al., 2023a). The classification of wings was conducted based on eight indices: Ci, Dbi, Disc.sh, Pci, Ri, Ci.3, Ci.2.1, Ci.2.2 using the STATISTICA software package for statistical data processing. The study was performed in the following order:

- 1) obtaining primary data such as index values, angles, lengths of segments, etc., in cases of using classical morphometrics (Bouga et al., 2011), or values of coordinates of landmarks on the wings in cases of using geometric morphometrics (Tofilski, 2008);

- 2) classification of wings based on the received raw data using methods of statistical data processing (Yerina et al., 2014). Wing classification should be performed even in cases involving so-called "purebred" queens. Only after obtaining reliably formed wing clusters can they be used to identify the wing phenotype;

- 3) based on the classification of wings, arrays of feature values of primary local standards were obtained, as described in the study (Yarovets et al., 2023b). These may relate to individual bee colonies, lines, apiaries, small geographic groupings, or significant areas of local bee populations, depending on task at hand. The relationships between the benchmarks were explored using Mahalanobis distances (Babenko et al., 2023b);

- 4) interpretation of the probable subspecies affiliation of the obtained primary standards using the local standards for the subspecies *A.m.caucasica* obtained in previous studies (designated as "Cc307.1" and "Cc307.2") (Halatiuk et al., 2023a) (<https://drive>) and a small area of the Carpathian region (designated as "LocalCarpatia") (Yarovets et al., 2023b). The second prototype was not precisely attributed to a specific subspecies but has the status of a prototype of a local micropopulation. In the future, they can be used to determine the subspecies affiliation of other studied bee colonies;

- 5) addition of the information about the obtained standards to the electronic database (bank), which may contain information: - a list of standard names; - arrays of numerical feature values; - scans of wings; - data on the origin of wings used to form the respective standard; - references to the source of publication; - data from the "BeeBreed" website of the State Beekeeping Institute "Hohen Neuendorf e.V. (LIB)" (Germany) (<https://www2.hu-berlin.de/bienenkunde2/>) or "Buckfast Pedigree" ([https://perso.unamur.be/~jvandyck/homage/](https://perso.unamur.be/~jvandyck/homage/elver/archiv.html) elver/archiv.html), or other; additional data that expand the knowledge about the standard. In fact, this is the ultimate goal and ultimate task of bee wing morphometry. Subsequently, the use of a bank of morphometric standards allows the subspecies affiliation of bees of individual bee colonies to be established without the need to perform steps 3–5;

- 6) prediction of the probable subspecies affiliation of the haploid chromosomal sets of drones and the diploid genome set of the queen based on the analysis of morphometric data of drone wings from one colony.

Results

The application of discriminant analysis involves a preliminary check of the normality of the distribution of the obtained experimental data. To check the investigated features for normal distribution, we used the Pearson χ^2 -test. For small samples, the Shapiro-Wilk criterion can also be applied (Fetisov, 2018).

The Histogram of Disc.sh. illustrates good agreement of the empirical distribution of the feature with the normal, while the histogram of

Ci demonstrates the difference of the empirical distribution from the normal. In this case, it is advisable to use nonlinear dependencies to create a classification model. For example, logarithmizing the data for the values of Ci index normalizes the data.

Normally distributed datasets were analyzed to detect differences between the group means using the Student's t-test and analysis of variance (ANOVA). In cases where the data were not normally distributed, differences between groups were analyzed using non-parametric tests (Mann-Whitney or Kruskal-Wallis).

The total of 2,147 drone wings was classified into four clusters using 8 indices. The accuracy of the classification was 95.4%. Thus, the wings of each bee colony were initially distributed among four clusters. Further, wings that likely belong to drones from other bee colonies were discarded, and classification into two clusters was performed for

each bee colony separately. Afterwards, four distinct groups of wings were formed from the clusters of wings of the investigated bee colonies. They were labeled as No. 1, No. 2, No. 3, and No. 4. The numbers of clusters used in the groups were 4, 7, 4, and 4, respectively. The selection criteria for the groups were the average values of features (indices) for clusters and the proximity of Euclidean distances between them, as well as the Mahalanobis distances between the centroids of individual wing clusters in cases where the values of the latter were ≤ 2.6 .

A distinctive feature of these groups was the low values of cubital index Ci (< 1.5) and Disc.sh. (< -2.5). The term used for such clusters is "low-cubital." Based on the mean values of features for clusters of individual bee colonies, the mean values of features for the obtained wing groups were calculated (Table 1).

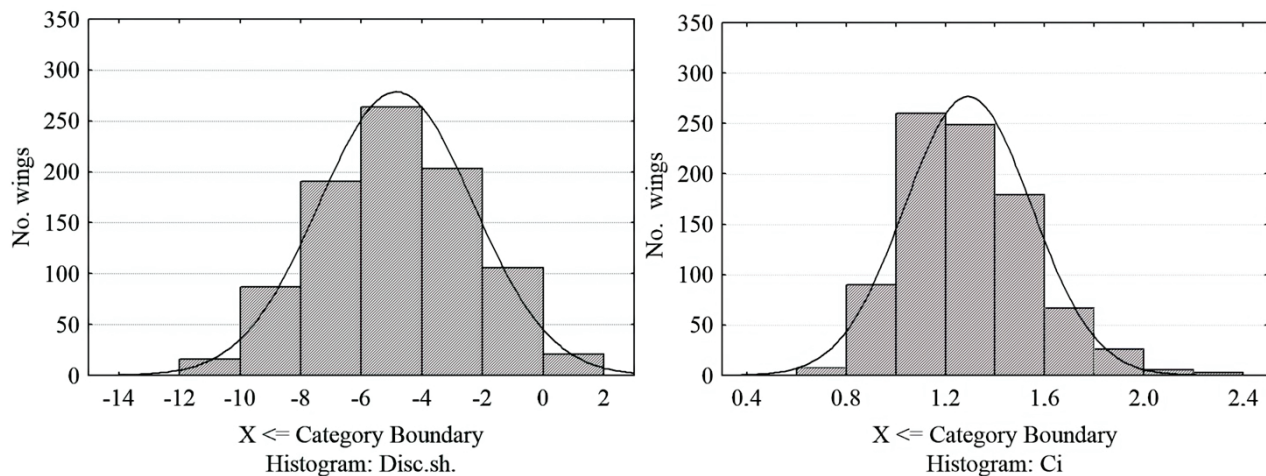


Fig. 1. Histograms of the distributions of two classification features of drone wings (n = 888) overlaid on curves of normal distribution

Table 1

Mean values and coefficients of variation of features for initially formed groups (number of wings of drones in groups: No. 1 (n = 209), No. 2 (n = 345), No. 3 (n = 145), No. 4 (n = 189))

Indexes*	Group No.1, Mean	Group No.1, Cv** %	Group No.2, Mean	Group No.2, Cv** %	Group No.3, Mean	Group No.3, Cv** %	Group No.4, Mean	Group No.4, Cv** %
Ci	1.137	4.1	1.258	5.9	1.455	2.8	1.396	3.5
Dbi	0.699	4.3	0.796	7.7	0.807	6.1	0.87	3.1
Pci	3.145	1.1	2.936	5.7	2.918	3.3	2.947	3.3
Disc.sh	-7.782	6.2	-4.965	13.2	-3.754	19.3	-2.25	8.1
Ri	1.226	3.9	1.345	3.4	1.349	3.4	1.464	3.0
Ci.3	1.657	3.7	1.595	6.7	1.58	7.4	1.706	4.6
Ci.2.1	1.526	2.9	1.647	6.4	1.709	4.9	1.706	1.2
Ci.2.2	3.886	11.7	4.106	8.0	3.769	5.9	3.984	6.3

Note: * – classification indices Ci, Dbi, Disc.sh, Pci, Ri according to the DAWINO protocol (Bouga et al., 2011), Ci.3, Ci.3, Ci.2.1 are proposed in the work (Yarovets et al., 2023a); ** – coefficients of variation (%).

To understand the relationships between the four specified groups, we carried out an analysis of similarity among them based on Mahalanobis distances (MD) values (Table 2).

Table 2

The Mahalanobis distances between the centroids of four groups of drone wings (number of wings in groups: No. 1 (n = 209), No. 2 (n = 345), No. 3 (n = 145), No. 4 (n = 189))

Groups wings, names	No.1	No.2	No.3	No.4
No.1	0	–	–	–
No.2	2.40	0	–	–
No.3	3.31	1.43	0	–
No.4	4.40	2.42	2.18	0

In our previous works, we developed an empirical similarity scale as follows: ranges 0–2.0 indicate high similarity, 2.0–2.6 – moderate similarity, 2.6–3.5 – low similarity, > 3.5 – no similarity. Based on this scale, we observed that wing group No. 3 showed a high degree of similarity to group No. 2. Furthermore, data on the distribution of individual wings among groups confirm this similarity and indicate that a significant number of wings in group No. 3 actually belonged to groups No. 2 and No. 4 (Table 3). Similar deviations were also observed for the other three wing groups, but they were significantly smaller.

The ambiguous nature of the information presented (Tables 2 and 3) necessitates reclassifying wings into 3 clusters. In other words, from the initial four groups, three new groups were formed, primarily due to the redistribution of wings from group No. 3 among the other three. The new groups consisted of the following number of wings: No. 1.nov – 307, No. 2.nov – 274, No. 3.nov – 307. The accuracy of classification significantly increased to 97.4%.

The data of descriptive statistics significantly facilitate the ability to determine the belonging of an object to a respective cluster based on the values of classification features (Table 4–6). The results of one-way analysis of variance of refined classification of bee wings (n = 888) into three clusters for indices Ci, Dbi, Disc.sh., Pci, Ri, Ci.3, Ci.2.1, and Ci.2.2 indicate a statistically significant difference ($P < 0.001$) between intergroup (between-cluster) (MS Effect) and intragroup (within-cluster) (MS Error) variances. In other words, the means of the listed indices for each group are significantly different from each other, providing substantial grounds to consider the division into three clusters reliable. The importance of using these features for wing classification is evidenced by the summary results of discriminant analysis of bee wings (n = 888). The P-value for all features was < 0.05 , indicating the significance of the applied features in the classification model into three clusters. The order of the location of the indices, according to increase

of their influence ("partial λ ") on the classification is as follows: Ci.2.2, Ci.2.1, Dbi, Ri, Ci, Disc.sh., Ci.3, Pci. Therefore, indices C.2.2 and C.2.1 had the least influence on the classification of this array of drone wings, and indices Ci.3 and Pci had the greatest influence. Cubital index Ci and index Disc.sh., although they were not decisive, also had a significant effect on distinguishing the wings of drones.

Table 3

The distribution of individual drone wings ($n = 888$) among the centroids of four groups of wings (number of wings in groups: No.1 ($n = 209$), No. 2 ($n = 345$), No. 3 ($n = 145$), No. 4 ($n = 189$))

Studied groups, names	Number of wings belonging to comparison groups	Percentage of wings belonging to comparison groups, %	Comparison groups, names
Group No.1	186	89.0	No. 1
	23	11.0	No. 2
	0	0.0	No. 3
	0	0.0	No. 4
Group No.2	38	11.0	No. 1
	257	74.5	No. 2
	26	7.5	No. 3
	24	7.0	No. 4
Group No.3	5	3.4	No. 1
	52	35.9	No. 2
	71	49.0	No. 3
	17	11.7	No. 4
Group No.4	0	0.0	No. 1
	26	13.8	No. 2
	13	6.9	No. 3
	150	79.4	No. 4

Table 5

Confidence intervals for the mean values of features for three clusters (final classification of drone wings, $n = 888$)

Classification features *	Cluster 1. Values of confidence interval bounds (95 %), means, min–max	Cluster 2. Values of confidence interval bounds (95 %), means, min–max	Cluster 3. Values of confidence interval bounds (95 %), means, min–max
Ci	1.140–1.185	1.431–1.492	1.244–1.292
Dbi	0.690–0.702	0.849–0.867	0.818–0.833
Pci	3.141–3.173	2.929–2.968	2.830–2.861
Disc.sh.	-7.239–-6.829	-2.372–-2.015	-5.214–-4.878
Ri	1.243–1.259	1.439–1.456	1.335–1.349
Ci.3	1.66–1.661	1.673–1.710	1.515–1.545
Ci.2.1	1.531–1.553	1.668–1.697	1.688–1.721
Ci.2.2	3.894–4.021	3.989–4.142	3.848–3.970

Note: * – indices see Table 1.

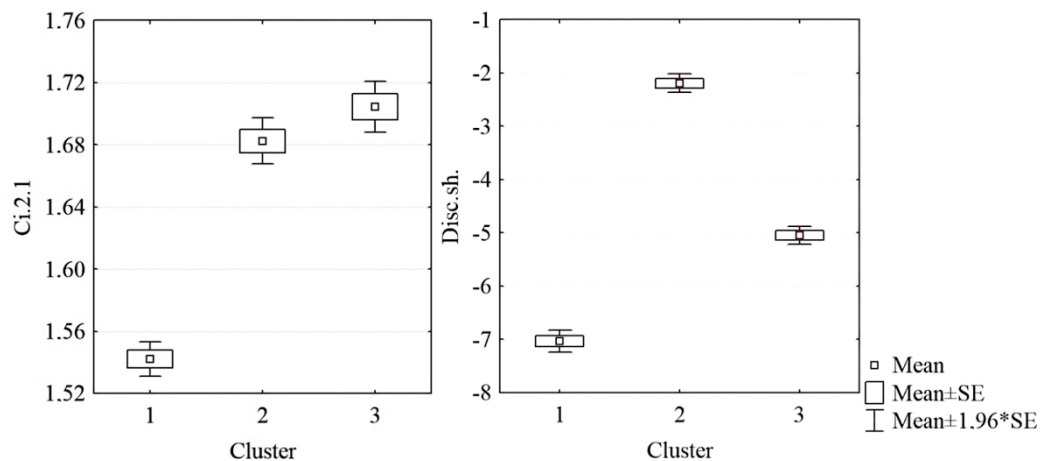


Fig. 2. Confidence intervals for the mean values of the indices Ci.2.1 and Disc.sh.: mean – average value of the index, SE – standard error of the mean; Mean $\pm$ 1.96SE – 95% confidence interval for the mean value

The importance of the information provided in Table 5 lies in the fact that the values of confidence intervals can serve as limits for typical values of wing classification features for the three formed datasets. However, it is essential to understand that this information needs to be used in a comprehensive manner. That is, relying on the values of one or two crucial indices, according to beekeepers, one cannot identify the affiliation of wings to a specific standard using the provided typical ranges. Otherwise, it leads to insufficient reliability in the breeding interpretation of the phenotype of the examined wings. Regrettably, this practice does exist; however, it cannot be deemed acceptable. To obtain

Table 4

The average values of indices obtained as a result of the classification of drone wings ($n = 888$) into three clusters (CV – coefficients of variation, %)

Classification features*	Cluster 1 Mean	Cluster 1 CV**, %	Cluster 2 Mean	Cluster 2 CV**, %	Cluster 3 Mean	Cluster 3 CV**, %
Ci	1.163	17.4	1.461	17.7	1.268	17.0
Dbi	0.696	7.6	0.858	8.6	0.825	7.9
Pci	3.157	4.4	2.949	5.5	2.846	4.9
Disc.sh.	-7.034	26.0	-2.193	68.4	-5.046	29.6
Ri	1.251	5.7	1.448	5.0	1.342	4.8
Ci.3	1.678	8.9	1.691	9.2	1.53	8.7
Ci.2.1	1.542	6.4	1.682	7.4	1.704	8.6
C.2.2	3.957	14.3	4.065	15.8	3.909	13.9

Note: * – for indices see Table 1.

Differences in the coefficients of variation (CV) of values of the indices between the data in Table 1 and Table 4 are due to the fact that CV values in Table 4 were calculated across all wings, unlike Table 1 where they were calculated based on the means for individual clusters. Particularly large CV values for the Disc.sh. index are explained by significant experimental errors in establishing the coordinates of individual landmarks on the wing, which are used to calculate the values of this index. Despite this fact, the average values of the Disc.sh. index for individual clusters had a significant effect on the classification overall, as previously indicated by the discriminant analysis data.

It is also necessary to note the dependence between the means of the Ci and Disc.sh. indices: increase in one was accompanied by increase in the other, and vice versa.

accurate information in this regard, it is necessary to involve at least eight indices. For this purpose, we used the coefficient values to calculate linear classification functions (Table 6).

The object of the study (the wing) was assigned to the cluster for which the classification function attains the highest value.

The refined repeated classification into three clusters requires verification, as illustrated by the data in Tables 7 and 8.

The Mahalanobis distances (MD) between the groups of wings No.1.nov, No.2.nov, and No.3.nov were greater than 2.6, indicating the dissimilarity between them. This distinguishes the current classification

from the initial one, where the MD values between the groups of wings in pairs ranged 1.43 to 2.42 (see Table 2).

Table 6

Classification model obtained as a result of discriminant analysis of drone wings (n = 888)

Variables	Coefficients of classification function, Cluster 1	Coefficients of classification function, Cluster 2	Coefficients of classification function, Cluster 3
Ci	76.05	82.61	77.51
Dbi	387.78	414.42	408.73
Pci	208.6	201.55	195.21
Disc.sh.	-15.26	-14.12	-14.86
Ri	230.22	255.94	237.97
Ci.3	146.22	149.1	140.02
Ci.2.1	198.86	206.65	210.68
Ci.2.2	48.27	49.17	49.17
Constant	-1078.67	-1137.03	-1076.6

The data presented in Table 8 indicate that the composition of groups No. 1.nov, No. 2.nov, No. 3.nov practically coincides with clusters 1, 2, 3 (Table 4), confirming the significant difference between

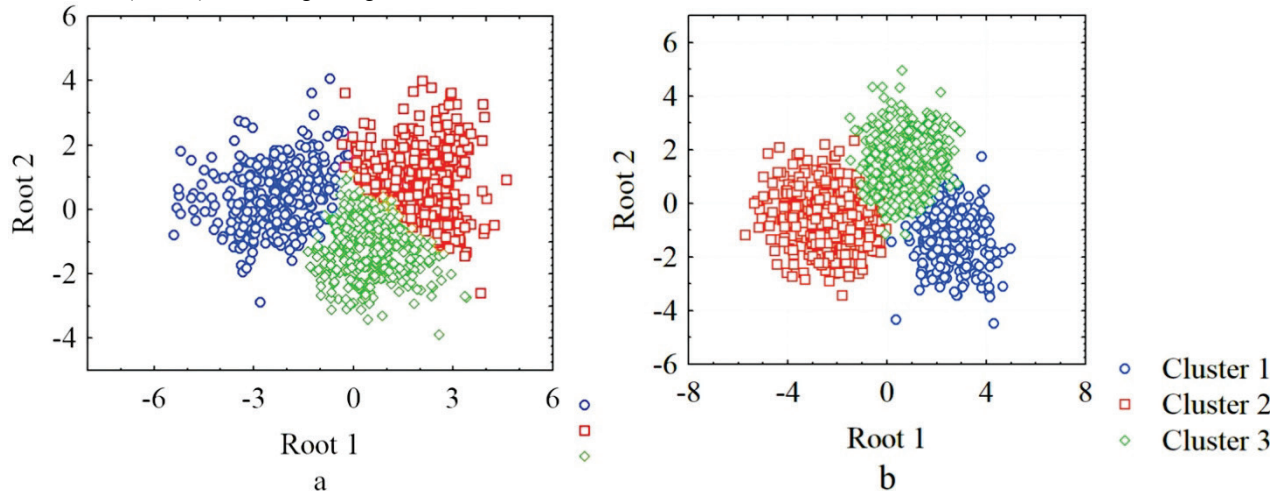


Fig. 3. Examples of wing distribution in the space of canonical variables between three clusters: *a* – total number of wings (N = 888), the number of wings in groups: No. 1.nov (n = 307), No. 2.nov (n = 274), No. 3.nov (n = 307) according to clusters 1, 2, and 3; *b* – data from another study, total number of wings (N = 1095), distribution of wings: n = 293, n = 436, n = 366 corresponding to clusters 1, 2, and 3

Table 8

Distribution of individual wings (N = 888) among the centroids of the new three groups of wings, the number of wings in the groups: n = 307, n = 274, n = 307 respectively

Studied groups, names	Number of wings belonging to comparison groups	Percentage of wings belonging to comparison groups, %	Comparison groups, names
Group No. 1.nov	301	98.0	No. 1.nov
	4	1.3	No. 2.nov
	2	0.7	No. 3.nov
Group No. 2.nov	2	0.7	No. 1.nov
	265	96.7	No. 2.nov
	7	2.6	No. 3.nov
Group No. 3.nov	4	1.3	No. 1.nov
	4	1.3	No. 2.nov
	299	97.4	No. 3.nov

In our study, we used the "Cc307.1", "Cc307.2" local prototypes for the *A. m. caucasica* subspecies and "LocalCarpathic" for the Carpathian region. The comparison results are presented in Tables 9 and 10.

Table 9

Mahalanobis distances between centroids of three new wing groups with a local standard from the Carpathian region

Groups and standards, names	No. 1.nov	No. 3.nov	No. 2.nov	"Lokal Carpathic"*
No. 1.nov	0	–	–	–
No. 3.nov	3.22	0	–	–
No. 2.nov	4.08	2.52	0	–
"LokalCarpathic"*	3.84	2.59	1.93	0

Note: * – group of wings used as sample.

the reformatted groups and indicating high reliability of the results of the refined repeated classification. This fact allowed us to accept the obtained arrays of feature values of the specified three groups as reference.

The final stage of the work needed breed identification of the formed local standards, which was carried out by comparing previously obtained standards using bee wings, for example, from the database in Oberursel, or by conducting genetic analysis for at least one colony belonging to a particular standard. After this, it is possible to establish the affiliation of the wing phenotype of the entire standard to one of the subspecies.

Table 7

The Mahalanobis distances between the centroids of the new three groups of wings

Groups wings, names	No.1.nov	No.2.nov	No.3.nov
No. 1.nov	0	4.44	3.38
No. 2.nov	4.44	0	2.66
No. 3.nov	3.38	2.66	0

Table 10

Mahalanobis distances between centroids of three new wing groups with two local standards of the *A.m.caucasica* subspecies

Groups and standards, names	No.1.nov	No.3.nov	"Cc307.1"***	"Cc307.2"***	No.2.nov
No. 1.nov	0	–	–	–	–
No. 3.nov	3.31	0	–	–	–
"Cc307.1"***	2.62	2.90	0	–	–
"Cc307.2"***	4.19	3.23	2.02	0	–
No. 2.nov	4.24	2.61	3.38	2.30	0

Note: * – groups of wings used as samples.

The data provided in Table 10 confirm the high similarity between the two wing samples of the *A. m. caucasica* subspecies of drones and indicate the moderate similarity between the "Cc307.2" sample and the No. 2.nov group. To further explore the nature of this phenomenon, we conducted an additional classification of wing groups No. 1.nov, No. 2.nov, No. 3.nov, and reference standards "Cc307.1" and "Cc307.2". The result of the classification into four clusters, with an accuracy of 95.2%, proved to be the best (Table 11).

The degree of similarity (DS) of wing groups No. 1.nov, No. 2.nov, No. 3.nov to the subspecies *A. m. caucasica* was the ratio of the number of wings significantly similar to the comparison standard (NS) to the total number of wings in the group (NO). To assess the degree of similarity of parts of the wings of standards No. 1.nov, No. 2.nov, No. 3.nov to the subspecies *A. m. caucasica*, we will also use the values of MD (Table 12).

The capability of classical morphometrics to obtain high-resolution standards is demonstrated through the classification of 73 wings of drones of the *A.m.caucasica* subspecies (Table 13).

Table 11

The distribution of drone wings (N = 961) in the classification model into four clusters (the number of wings in clusters 1, 2, 3, 4: n = 244; n = 198; n = 279; n = 240 respectively; DS – degree of similarity of the wings of the studied groups to the corresponding cluster, expressed as a percentage)

Groups and standards, names	Number wings, No, pieces	Cluster 1 Ds, %	Cluster 2 Ds, %	Cluster 3 Ds, %	Cluster 4 Ds, %
No. 3.nov	307	1.0	0.0	88.9	10.1
No. 1.nov	307	76.9	3.6	0.0	19.5
"Cc307.1"*	41	4.9	0.0	14.6	80.5
"Cc307.2"*	32	0.0	0.0	0.0	100.0
No. 2.nov	274	1.1	68.25	0.0	30.7

Note: * – groups of wings used as samples.

Table 12

The Mahalanobis distances between the centroids of wing portions of the three groups with the centroid of the local standard of the subspecies *A. m. caucasica* (n – the number of wings in the groups being studied)

Groups and standards, names	No. 3.nov (n = 31)	No. 2.nov (n = 84)	No. 1.nov (n = 60)	Cc307* (n = 65)
No.3.nov	0	–	–	–
No.2.nov	2.81	0	–	–
No.1.nov	2.17	2.18	0	–
Cc307*	2.08	1.83	2.27	0

Note: * – group of wings used as sample.

Table 13

Data of repeated classification of drone wings (n = 73) of subspecies *A. m. caucasica* using 12 features into two clusters (the number of wings in clusters 1 and 2: n = 34, n = 39 respectively, mean ± SE)

Classification features *	Cluster 1	Cluster 2	Cluster 3
Ci	1.709 ± 0.046	1.547 ± 0.046	1.622 ± 0.034
Dbi	0.782 ± 0.013	0.719 ± 0.007	0.749 ± 0.008
Disc.sh.	-2.416 ± 0.327	-5.195 ± 0.190	-3.901 ± 0.244
Ri	1.387 ± 0.019	1.274 ± 0.008	1.327 ± 0.012
Ci.3	1.599 ± 0.020	1.631 ± 0.020	1.616 ± 0.014
Ci.2.1	1.580 ± 0.017	1.505 ± 0.013	1.540 ± 0.011
A1	2.709 ± 0.006	2.721 ± 0.005	2.715 ± 0.004
A4	0.515 ± 0.005	0.566 ± 0.005	0.542 ± 0.005
B3	1.503 ± 0.008	1.535 ± 0.007	1.520 ± 0.005
B4	1.935 ± 0.015	1.821 ± 0.014	1.874 ± 0.012
E9	0.338 ± 0.004	0.316 ± 0.003	0.326 ± 0.003
J10	1.071 ± 0.010	1.088 ± 0.012	1.080 ± 0.008

Note: * – indexes: Ci, Dbi, Disc.sh., Ri, Ci.3, Ci.2.1 (see Table 1); angles: A1, A4, B3, B4, E9, J10 (according to the DAWINO protocol (Bouga et al., 2011)); ** – angles are provided in radians.

Using the example of studying the wings of drones from bee colony Krv.27, we will demonstrate how the taxonomic affiliation of drone wing phenotypes is established in practice.

Samples of drone wings were obtained in 2021 from the apiary located in the town of Kaharlyk, Kyiv Oblast. However, a reliable interpretation of the breed phenotype of the wings was not obtained. The beekeeper provided 100 drone wings for the study. Five wings were found to be defective and were discarded. Importantly, the bee colony was kept under conditions that practically prevented the migration of drones. Despite this, to check the effectiveness of isolation measures for drones, the next step was classification of 95 wings into three clusters using the model. In this case, the majority of wings (92 pieces) concentrated in two clusters. The other 3 wings were considered as wings of "wandering" drones and were not further considered. Thus, it can be concluded that the migration of drones between bee colonies was minimal. The next step involved a refined classification of wings into two clusters labeled as Krv.27.1 and Krv.27.2, as the queen bee, based on the analyzed features, producing two main types of distinct drones (Table 14).

Thus, two small-sized datasets with eight features each were further used to determine the affiliation of their phenotypes to a specific "breed". Taking into account the data of the average values of the cubital index of wings from worker bees of the predecessor queen and the queen herself, the bee colony was tentatively assigned to Ukrainian

steppe bees. Therefore, three sets of reference data, No.1.nov, No.2.nov, and No.3.nov obtained in this study were used for the identification of drone wings. All the standards characterized the phenotype of drone wings in bee populations present in the territory of Ukraine (Table 15, 16).

Table 14

Classification data for the wings of drones (n = 92, mean ± SE) from the bee colony Krv.27 into two clusters (the number of wings in each cluster n = 46)

Classification features*	Cluster Krv.27.1	Cluster Krv.27.2
Ci	1.285 ± 0.037	1.486 ± 0.031
Dbi	0.795 ± 0.008	0.870 ± 0.010
Pci	2.961 ± 0.015	2.937 ± 0.020
Disc.sh	-2.204 ± 0.214	-0.120 ± 0.185
Ri	1.452 ± 0.010	1.548 ± 0.009
Ci.3	1.735 ± 0.017	1.635 ± 0.016
Ci.2.1	1.550 ± 0.020	1.663 ± 0.010
C.2.2	4.038 ± 0.068	3.970 ± 0.063

Note: * – classification features (see Table 1).

Table 15

The Mahalanobis distances between the centroids of two investigated clusters of colony Krv.27 and three centroids of drone standards obtained in this study

Groups wings, names	No. 1.nov*	No. 2.nov*	No. 3.nov*	Krv.27.1	Krv.27.2
No. 1.nov*	0	–	–	–	–
No. 2.nov*	4.27	0	–	–	–
No. 3.nov*	3.33	2.65	0	–	–
Krv.27.1	4.10	2.18	3.51	0	–
Krv.27.2	5.87	2.09	4.22	2.28	0

Note: * – groups of wings used as samples.

Table 16

The distribution of individual wings for two clusters of colony Krv.27 among the three centroids of the drone wing standards, (the number of wings in clusters Krv.27 and Krv.27: n = 46, n = 46)

Studied colony, names	Number of wings belonging to comparison standards	Percentage of wings belonging to comparison standards, %	Comparison standards*, names
Cluster Krv.27.1	1 39 6	2.2 84.8 13.0	No. 1.nov No. 2.nov No. 3.nov
Cluster Krv.27.2	0 46 0	0.0 100.0 0.0	No. 1.nov No. 2.nov No. 3.nov

Note: * – groups of wings used as samples.

Discussion

Preparation for research. Every beekeeper or researcher, before starting the work on obtaining primary morphometric data, should thoroughly sample and generalize the task that needs to be solved, outlining the scope of the required experimental material.

Our practice of studying drone wings in the territory of Ukraine has revealed the need to make certain adjustments to the research methodology. This was associated with several circumstances, namely: a) the simultaneous presence of bees of different subspecies from evolutionary lines C, M, and O; b) the absence of "purebred" bee colonies at apiaries or in microgeographic areas, the absence of "purebred" bee colonies in apiaries or microgeographic zones, the presence of which must be confirmed by documents. As a result of the above, predominantly hybrids were subject to the study. In this context, it should be noted that in the case of studying a small apiary with 50–100 bee colonies, it is recommended to examine 15–25 of them. If the subject of the study is several apiaries in a small region, community, region, or several regions, it is recommended to study 5–10 bee colonies from each apiary. The number of wings from each bee colony should be at least 100. These conditions apply to bee colonies with queens that have been naturally inseminated. Practice shows that in most cases, the number of clusters of wings of worker bees after classification is 4–5. This means that on average, each cluster will

consist of 20–25 wings. This number of wings in each cluster allows checking the normality of the distribution of the investigated features and, therefore, proper identification of confidence intervals for the mean values.

Regarding drone wings, it is known that the queen produces two types of drones, so the classification of wings is carried out into two clusters. It can be assumed that it is sufficient to use 50–60 wings. This holds true when bee colonies are kept under special conditions, without migration of drones between colonies. If these conditions are not met, additional research may be needed to exclude "wandering" drones from the analysis. To do this, we first apply a general classification to the entire array of drone wings from the studied bee colonies into 3–5 clusters. Based on the obtained data, we can see that in most cases, there will be clusters with a small number of wings. These were the ones that differed in characteristics from the main group of wings and were excluded from further consideration. However, it is not uncommon for "wandering" drones to closely resemble drones from other bee colonies in terms of phenotype. In this case, the methodology of excluding "wandering" drone wings from the study does not work. The presented arguments indicate the need to use $\Rightarrow$ 100 wings of drones from each bee colony under study.

Many researchers overlook the clear fact that queens, in the vast majority of cases, produce multiple main types of worker bees significantly different from each other in phenotype, determined by the queen's genome composition and the origin of sperm in the spermatheca. However, when processing primary morphometric data, average feature values are established, based on which conclusions are subsequently drawn regarding the breed composition of bees, which is a fundamentally erroneous approach. Only in specific cases, primarily concerning bee colonies that are reliably classified into certain subspecies, the differences between bee phenotypes are such that they can be considered identical, allowing the use of averaged feature values. In other cases, it is necessary to first classify wings, and then, as a second step, establish the average feature values for individual wing clusters obtained as a result of classification. In case of studying a specific bee colony, wing classification is carried out using previously obtained classification functions or existing benchmarks. The difficulty lies in the fact that such data are not usually published, so independent wing classification is mostly performed.

When forming wing benchmarks, an important criterion for including certain wing clusters in each benchmark was the values of coefficients of variation of classification-feature mean values (CV) (Table 1), features for which $CV = <20$ were considered acceptable. In some cases, for example, for the Disc.sh. feature, this rule may be ignored due to the peculiarities in establishing this feature, as discussed earlier in the text. As seen, most of the final CV values, calculated based on the mean feature values for individual clusters included in a specific benchmark, follow the $CV = <20$ rule (Table 1, 4).

In summary, it can be stated that the formation of primary arrays of standard data using Euclidean distances cannot guarantee the required reliability of using arrays of phenotype feature data as local standards. This necessitates additional verification and reclassification of the primary groups of wings. A similar two-step methodology for obtaining primary and final arrays of reference data is recommended for creating local standards for small ecotypes or individual local populations.

The methodology of morphometric studies "from scratch" applies to situations where there are no existing electronic databases of morphometric data for the vast majority of bee populations present in Ukraine. If such databases appear in the future, there will be no need to perform all the outlined stages of research except for the last one, where a comparison of the morphometric data of a specific researched bee colony with available benchmark data is carried out. This will allow obtaining a reliable interpretation of bee wing phenotypes.

The final procedure, indicated as stage 6, involves using the obtained values of drone wing phenotype features to predict the probable breeding affiliation of haploid chromosome sets of drones. It is essential to note that data obtained in this way are significantly less accurate compared to genetic analysis data because they only concern the part of DNA responsible solely for the wing phenotype. However, it is necessary to consider that the wing phenotype, established based on quantitative morphometric features, is determined not by an individual gene but by a polygene. That is, a portion of the genome is, in fact, involved in shaping the wing phenotype of bees. Therefore, skepticism regarding the limited informativeness of morphometric data when

establishing the probable breeding of the genome is reasonably moderate. In this context, it is also necessary to point out that the morphometry of drone wings has significant advantages over the morphometry of worker bee wings. The latter has an auxiliary nature and allows, in specific cases, assessing the composition and character of sperm in the spermatheca of the queen.

Reliability of bee wing morphometric studies. The reliability of wing classification and identification of bees according to the accepted taxonomic gradation is a distinct issue that requires detailed discussion. The reliability of the obtained results is closely linked to the issue of choosing the optimal set of features that can be used for the classification of bee wings. Meixner et al. (2013) provides a comprehensive characterization of standard morphometric methods used for identifying the taxonomy of bees. In case of classical wing morphometry, a detailed list of features is provided, and the methodology for identifying phenotypes is described. Analyses of studies by various authors and the drawn conclusions are predominantly based on morphometric data from worker bees. Using the full set of features according to the Dawino protocol is not productive, as a significant portion of them has minimal impact on the final classification result due to low informativeness. Additionally, it was demonstrated that the quantitative and qualitative composition of important features for the classification of specific datasets varies (Cherevatov et al., 2023). The analysis of classification results for more than 20 cases has shown that, in order to achieve an acceptable level of classification accuracy, it is sufficient to use eight features listed in the work (Yarovets et al., 2022a). The authors believe that such an approach allows standardizing the data for the created reference database, thus significantly simplifying their utilization in subsequent research. However, they do not object using the full spectrum of features if necessary.

Criteria for assessing the significance of features and the reliability of classification overall. Various criteria for assessing the reliability of the obtained results are employed in classical or geometric morphometrics through the use of statistical analysis software. This work focused only on the criteria used for classical wing morphometrics. Most of them have numerical expressions, such as mean feature values, mean errors, coefficients of variation, percentage of prior classification correctness, Wilks' statistics, and Student's t-statistics. However, some criteria have a more qualitative nature, although they use specific numerical data. For example, graphical representation of confidence intervals for the mean values of classification features illustrates the qualitative picture of the significance and reliability of each individual feature used for classification without the need to delve into the content of numerical data (Fig. 2). The visual analysis of Figure 2 dispels any doubts regarding the significance of the Disc.sh. feature. Doubts arise when considering the high CV value for this feature (Table 4). However, the cluster arrangement in Figure 2 unequivocally demonstrates the reliability of wing division into three clusters based on the Disc.sh. feature. In contrast, the Ci.2.1 feature is not as conclusive since the confidence intervals of clusters 2 and 3 overlap and it cannot independently distinguish between them. Despite CV of 7.4% for this feature, its significance is lower, compared to Disc.sh. This information allows for a quick qualitative assessment of the overall situation and the making of an appropriate decision regarding the impact of individual features on the reliability of wing classification.

In both cases shown in Figure 3, the areas where the three wing clusters are located have a consolidated nature. Although there is some mutual overlap of the areas at the boundaries between individual clusters, it is of minor degree. Such data distribution in both cases indicates sufficient reliability of the classification overall. The arrangement of the situation of the three clusters in the space of canonical variables is also significant. For the case (a), each area shares common boundaries with the other two, indicating the similarity of wings near the boundary of the two clusters. For the case (b), there is no common boundary between clusters 1 and 3. This implies a fundamental difference between the wings of clusters 1 and 3. Meanwhile, a portion of the wings of cluster 2 shares common boundaries with clusters 1 and 3, indicating the similarity of some wings to cluster 1 and the rest to cluster 3.

Among the quantitative criteria, we can include the ratio of features that divide and do not divide the data arrays, as well as the values of Mahalanobis distances between them. However, both of these criteria cannot be regarded as an "absolute truth" because the "shares/does not share" ratio and the similarity scale for MD values, obtained empirical-

ly, depend on the number of objects being compared. Thus, both criteria contain a subjective component of the researcher's experience and largely have a qualitative evaluative character of the similarity measure. In general, all the criteria mentioned above are sufficient to reliably assess the classification results and establish numerical values for the characteristics inherent in a particular wing phenotype. Scientists need to pay attention, first and foremost, to the key criteria important for each stage of the research.

If the issue of accuracy of identifying the established wing phenotype of the studied bees using standards is further addressed, the duality of this problem should be noted. Firstly, it is necessary to evaluate the reliability of the subspecific affiliation of the standards. In short, it can be formulated as follows: if the standards are formed based on data from bee colonies with queens of multi-year selection belonging to certain subspecies, ecotypes, populations, lines, confirmed by documentary evidence, the reliability of classification simultaneously ensures the establishment of taxonomy with a high degree of reliability (Yarovets et al., 2022b); or if there is genetic research data (Nikolova et al., 2015; Šťastný et al., 2017; Cherevatov et al., 2019). Otherwise, the standards will lack reliability until their affiliation to certain subspecies is confirmed by genetic research data. However, they can be used as standards for local populations without identifying their breed. Such standards include standards for local populations of Ukrainian steppe bees. Unfortunately, for individual bee colonies, there are no reliable documented data, and genetic studies are extremely outdated or quantitatively insignificant (Metlytska et al., 2010; Hryhorchuk et al., 2020). Secondly, there is a necessity to establish the subspecies affiliation of clusters of wings from newly investigated bee colonies by comparing them with the existing samples. In this case, empirical values of the ranges of similarity distances Mahalanobis obtained in previous studies (Babenko et al., 2023), and other criteria are used to confirm the reliability.

A more complex problem arises when establishing the reliability of the wing phenotype belonging to a specific subspecies, population, ecotype, or line when studying colonies in apiaries consisting predominantly of hybrids. Hybrid honey bees are created through crossbreeding multiple subspecies, resulting in queens or worker bees with a diploid chromosome set containing genetic material from at least two distinct subspecies. These varied chromosome sets differ in structure, leading to genetic heterozygosity and unique characteristics in the hybrid bees. If chromosome sets belong to the same subspecies, they are homozygous. However, in honey bees, as well as in some other Hymenoptera, fully homozygous individuals in the classical sense are only diploid drones, which are destroyed by bees at an early stage of development. Therefore, there is always at least a minimal difference between the haploid genomes received by the zygote from parents of the same subspecies or population.

In case of presence of a hybrid queen, it produces hemizygous drones of two types, which, by majority of characteristics, will correspond to different subspecies. Moreover, due to recombination processes, the formed haploid chromosome sets of these two types of drones will differ structurally from the analogous sets in the queen. Thus, even the haploid genome of a drone relatively quickly undergoes recombination processes (Liu et al., 2015). Morphometrically, such hybridization can be observed if the genome changes are significant, and the classification is carried out accurately. To solve this, it is necessary to use reference samples, which, in turn, requires meticulous routine work. We propose the methodology for identifying the type of hybridization using values of the Mahalanobis distance (MD) between individual wings of the studied hybrid and centroids of standards belonging to certain subspecies. In this variant, the number of wings assigned to specific subspecies standards can also serve as a measure of the degree of hybridization (see in the following section).

As an example of studying "direct" and "reverse" hybrids of bees, one can refer to the research by Węgrzynowicz et al. (2019), where the methodology of geometric morphometrics was utilized, involving exceptionally labor-intensive work. Hybrids of three subspecies - *A. m. mellifera*, *A. m. caucasica*, and *A. m. carnica* - were obtained through instrumental insemination from three inbred queens. Later, based on the results of morphometric wing phenotypic characteristics of worker bees, an attempt was made to assess the influence of specific subspecies on the wing phenotype of hybrids. In our opinion, there was one drawback that affected the final conclusions, namely, the lack of control over the homozygosity of the inbred queens used to obtain hybrids.

The affiliation of queens to a specific subspecies does not necessarily mean that the degree of homozygosity of their genomes is high. Therefore, these queens could produce worker bees of two types with morphometrically noticeable differences, leading to potential distortion of research results. In our opinion, the starting point of the study should have been the establishment of the required level of homozygosity of the inbred queens using morphometrics of drone wings, and the exclusion of significantly heterozygous individuals from the study. According to the text, this step was not taken. This omission prevented the authors from fully addressing the problem of assessing the impact of individual subspecies on the wing phenotype of worker bees.

Ability to use standards. The obtained combined arrays of sample data allow for the differentiation of wing phenotypes among bee subspecies or differentiation between conditional "breeds" for those standards that do not have precise subspecies identification with a high degree of reliability. However, this is a relatively straightforward task for morphometric methods in their classical form. As an example, we will demonstrate the ability of bee wing morphometrics to solve more complex problems, using the study of the wing phenotype of drones from the *A. m. caucasica* subspecies.

Based on the results of a previous study of one bee colony originating from Georgia (Kutaisi), two small-sized local morphometric samples of drone wings, "Cc307.1" and "Cc307.2," were obtained (Halatiuk et al., 2023a). The reliability of classifying 73 drone wings into two clusters was low because only four out of eight traits reliably differentiated them. The ratio of classification attributes as "distinguishing/not distinguishing" = 4/4 can be interpreted as a negative result. This fact was also confirmed in this study by the data in Table 10, where the value of MD = 2 indicates significant similarity between the specified standards. To improve the differentiation of drone wings in dataset "Cc307", the number of classification traits was increased to 12, namely: six indices Ci, Dbi, Disc.sh, Ri, Ci.3, Ci.2.1, and six angles A1, A4, B3, B4, E9, J10, according to the Dawino method, and a reclassification was performed (Table 13). The accuracy of the new classification variant was 94.6%, which is practically equivalent to the initial variant. The wing ratio between clusters also remained unchanged. However, there was a slight redistribution of wings between clusters, improving the differentiation of wings into two clusters. In this case, out of the 12 applied traits, eight, namely: Dbi, Disc.sh, Ri, Ci.2.1, A4, B3, B4, E9, reliably divided wings into two clusters, compared to the other four – Ci, Ci.3, A1, and J10, which did not differentiate wings among themselves. As a result, two refined samples were obtained: "Cc307.1nov" and "Cc307.2nov," with MD value between them increasing to 2.23, confirming their uniqueness and individuality. Therefore, they could be used as more reliable samples compared to the original data. The new classification variant indicated that by increasing the number of traits, it is possible to achieve the proper level of differentiation of drone wing phenotypes, even in cases of significant similarity between them. It is important to remember that the similarity between two drone wing phenotypes is not a random occurrence but is determined by the high homozygosity of the queen in case of her "purebred" status. Based on the research results, it is reasonable to create small-sized standards for a subspecies, lineage, or even a single bee colony, which can be used to establish a detailed intra-subspecific structure of wing phenotypes. In this case, it is necessary to sacrifice the principle of standardization in favor of the accuracy of benchmarks.

Classical morphometrics of bee wings also allows studying "fine effects", for example, determining the degree of similarity not only between individual clusters but also small differences between parts of wings within clusters. The data presented in Table 11 suggest that practically all wings of samples "Cc307.1" and "Cc307.2" are assigned to one cluster 4. Therefore, wing phenotypes belonging to cluster 4 correspond to the phenotype of the local standard of the subspecies *A. m. caucasica*. Total of 30.7% of the wings of group No. 2.nov were also assigned to cluster 4, which correlates with the data analysis in Table 10 and may be the basis for considering that the phenotype of a part of the drone wings of group No. 2.nov is formed as a result of hybridization of queens from local populations of Ukrainian bees with the subspecies *A. m. caucasica*. To a lesser extent, this assumption also applies to the phenotypes of a part of the wings of groups No. 1.nov and No. 3.nov. The values of MD provided in Table 12 indicate that the highest similarity to the wings of drones of the subspecies *A. m. caucasica* is observed in the wing portion of standard No. 2.nov, and a moderate similarity was found in the wing portions of standards

No. 1.nov and No. 3.nov, which also correlates with the data analysis in Table 10. Thus, it can be argued that the greatest influence of the *A. m. caucasica* subspecies is observed in the phenotypes of Ukrainian bees, while it is lower for the Polissya population of the *A. m. mellifera* subspecies. In this case, DS value can be a measure of this influence.

Ranking of reference data. The morphometric characteristics obtained based on the study of individual colonies can be interpreted as primary reference standards (Keller et al., 1997; Kandemir et al., 2011; Yarovets et al., 2022b). If reference data are formed during the study of many colonies from one apiary or several apiaries of a small geographical area, they are interpreted as local standards (Yarovets et al., 2023b). The analysis of the data in Table 9 indicated a high similarity between wing group No. 2.nov and the sample "LocalCarpathic". This fact provides grounds to merge them into a single dataset and elevate it to the rank of a regional morphometric wing sample of a drone for a significant part of the territory of Ukraine. Earlier studies of the wings of worker bees also noted significant similarity in wing phenotypes between the populations of central, northern (standard "U.Step.1") and western (standard "LocalCarpathic") parts of Ukraine (Halatiuk et al., 2023b). At that time, the authors did not find a satisfactory explanation for this phenomenon. As we can see, the study of wing phenotypes of drones confirmed the morphometric studies of the wings of worker bees. Therefore, it is proposed to consider the described wing phenotype of drones as the standard for the distinct population of Ukrainian bees and designate it as "UkrBee". This also allows asserting that the haploid chromosomal sets of drones in this population are similar in the part responsible for the wing phenotype.

Unfortunately, as of today, there are no sources of morphometric data for drones that can be used for comparison, except for the data provided by Ruttner (1988): the values of the Ci index ranged 1.38 to 1.93 for the honey bee (*Apis mellifera* L.), as well as an experimental value of Ci = 1.48 for the *A. m. mellifera* subspecies found by Groeneveld et al. (2020). Thus, considering the low values of cubital indices Ci for standards No. 1.nov and No. 3.nov <1.27, and Disc.sh. <5.05, the wing phenotypes of both standards should be attributed to the local populations of the *A. m. mellifera* subspecies, prevalent in the Polissya region, to which the northern part of Ukraine belongs, and designate them as "Polisca". The reliability of this assumption is currently low and requires confirmation through genetic studies. We note that in case of studying local bee populations, local standards often cannot be unambiguously identified as specific subspecies or hybrids. However, this does not prevent their use for analyzing bee colonies in the region to which the obtained standards belong, without the need to establish the exact taxonomy of the standards themselves and the researched bee colonies. If there is an opportunity to use standard data for the wings of subspecies such as *A. m. carnica*, *A. m. caucasica*, *A. m. mellifera*, *A. m. ligustica*, *A. m. macedonica*, etc., the obtained wing phenotype standards can be identified with specific subspecies. This allows for expanding the scope of their use for other regions where bee populations exist, with the aim of assessing the type and degree of hybridization of queen bees.

Also, we can state that when forming arrays of standard data, two opposite tendencies manifest: a) consolidation of arrays of primary local standard data, leading to increase in the length of confidence interval values, turning them into the status of regional or interregional standards, allowing the use of combined standards to differentiate wing phenotypes within the subspecies taxonomy or establish the type of subspecies hybrids; b) fragmentation of local arrays of standard data to differentiate wing phenotypes within subspecies, ecotype, etc. Such small-sized standards are characterized by a small length of confidence intervals of mean feature values, high precision, and suitability for differentiating wing phenotypes within subspecies, as well as for establishing the probable degree of hybridization of genome of bees of the main subspecies with that of other subspecies.

Using three groups of drone wings as standard morphometric data to establish the "breed" of a new, previously unstudied colony No. Krv.27 allowed for obtaining interesting results. Firstly, despite the fact that six features (Ci, Dbi, Disc.sh., Ri, Ci.3, Ci.2.1) out of eight reliably distinguished drone wings in bee colony No. Krv.27 between two clusters (Table 14), the data presented in Table 15 indicate that the phenotypes of wings in both clusters, Krv.27.1 and Krv.27.2, show moderate similarity to group No. 2.nov. This is a basis for identifying the phenotypes of these clusters with the phenotype of Ukrainian bees. Although, considering the average value of Ci = 1.285 for cluster

Krv.27.1, it could be mistakenly assigned to the phenotype of group No. 3.nov. However, in reality, this was not the case, and a detailed analysis of the distribution of individual wings provided a more accurate picture. Specifically, only a small portion of wings in cluster Krv.27.1 (6 pieces) can be attributed to group No. 3.nov, which is likely associated with the Polissya population of subspecies *A. m. mellifera* (Table 16). For cluster Krv.27.2, such an influence on the phenotype of drone wings from the subspecies *A. m. mellifera* was not observed. Secondly, considering that the wings of drones in the bee colony are produced by a single bee mother, the value of DS can be used to assess the degree of genome hybridization in drones. The identification results of drone wings suggest that the nuclear genome of the queen bee from bee colony No. Krv.27 likely consists of two chromosomal sets, which overall belong to the population of Ukrainian bees "UkrBee". In this case, we can observe a slight hybridization (DS = 13%) of one of the two chromosomal sets with the subspecies *A. m. mellifera* in part responsible for the wing phenotype. Thirdly, this queen bee may be recommended for use as a "parental" for obtaining drones of the desired type.

It is necessary to note that establishing the similarity of wing phenotypes, in cases where they belong to the same subspecies, is interpreted as common similarity. If the wings are assigned to different subspecies, the interpretation of similarity, if proven, is fundamentally different. Namely, the degree of similarity can be equated with the degree of hybridization of the main species by admixture.

Today, morphometric characteristics play a leading role in determining the status of individual taxa at different levels. The variability and applicability of classical morphometrics depend on insect taxa and sets of features used to define specific species (Parhomenko et al., 2002; Aytekin et al., 2007; Favret, 2009; Wilke et al., 2016). In particular, establishing the taxonomic status of representatives of the order Hymenoptera often occurs through the application of wing morphometrics (Favret, 2009; Laciny et al., 2017; Katzke et al., 2018). Answers to questions of phylogeny and subspecies are sought through studying the geometry of wing venation (wing vein patterns) of insects. Such research is conducted systematically worldwide. In most cases, they concern several orders (Hymenoptera, Diptera) of the class Insects and are primarily related to cryptic species (Patterson & Schofield, 2005; Yazdi et al., 2012; Yazdi, 2014; Laciny et al., 2017; Katzke et al., 2018; Sontigun et al., 2019). This paper proposes the method for obtaining morphometric standards of wings of the honeybee (*A. m. mellifera* L.) using classical morphometrics. This allows using data-processing programs that are freely available, unlike geometric morphometrics. However, the accuracy and reliability of the obtained results under both approaches do not significantly differ and are at the acceptable level (Tofilski, 2008).

Conclusion

The main stages of studying the wing phenotype of bees using classical morphometrics have been clearly demonstrated. Important points and peculiarities in processing primary statistical data have been emphasized. We utilized the two-step method to form arrays of reference data for the local bee populations. The use of such standards allows for intersubspecific differentiation of phenotypes of bee wings or differentiation between hypothetical "breeds" for those standards lacking precise subspecies identification with a high degree of reliability. Criteria for the reliability of classification results and identifying the affiliation of bee wing phenotypes to specific subspecies have been discussed.

Classical morphometrics has demonstrated high capability to distinguish between clusters of wings, even in cases of significant similarity based on Mahalanobis distance values. This allows obtaining a greater number of accurate local standards within one subspecies, contributing to the detection of discrete intrasubspecific structure of wing phenotypes. The methodology has been developed with available, precise local standards, which show the degree of wing phenotype similarity and potential degree of hybridization of the main breed with admixture.

The paper highlighted the issue of using drone-wing morphometrics to predict the likely composition of the haploid genome of drones and the diploid genome of queens and worker bees.

The authors express gratitude to beekeepers: O. M. Krivchenko, I. V. Stolyar, and M. S. Strilchuk for providing samples of drone wings, which contributed to the expansion of the study and the significance of the obtained results.

The authors declare no conflict of interest.

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