VETERINARY RESEARCH

UDC 638.12+519.24
DOI: 10.15587/2519-8025.2023.275588

II. MORPHOMETRY OF WINGS OF WORKER BEES OF THE SUBSPECIES *APIS MELLIFERA MELLIFERA* L. (POLISSYA POPULATION OF ZHYTOMYR REGION)

Oleksandr Galatiuk, Volodymyr Yarovets, Volodymyr Babenko, Volodymyr Cherevatov, Bohdan Gutiy, Andrii Hryhorenko, Mykhailo Strilchuk, Ihor Stolyar

The uncontrolled spread of the subspecies A. m. carnica, A. m. ligustica, and A. m. caucasica has led to a reduction in the areas of pure "dark forest bees" populations belonging to the subspecies A. m. mellifera in their natural habitats within Ukraine. Due to the need to use dark forest bees in breeding, it became necessary to identify the locations of individual populations of bees belonging to the A. m. mellifera subspecies.

***The purpose of the work** is to create an accessible and at the same time complete methodology for classifying bee wing phenotypes, which would make it possible to determine the probable breed of worker bees, the type and degree of hybridization of the main breed with impurities, and to identify "purebred" bee families by the wing phenotype suitable for further breeding.*

***Material and methods of research:** Using discriminant analysis of data, at the first stage of the study, 1500 wings of bee families were classified using 8 features: Ci, Dbi, Disc.sh, Pci, Ri, Ci.3, Ci.2, Ci.2.1, for which there was preliminary information about the possible belonging of the wing phenotype to the subspecies A. m. mellifera or its hybrids. At the second stage, additional 1212 wings of bee colonies were studied, about which there were doubts about their breed.*

***Results of research and discussion:** The wings are reliably divided into four clusters, indicating the presence of four sufficiently distinct groups among the studied wings in terms of phenotype.*

***Conclusions and prospects for further research:** A classification model has been created that allows for effective discrimination of the wings of working bees of bee colonies in Ukraine, the subspecies A. m. mellifera. Phenotypic values of indices of four Polissia micro-populations of bees, used as reference standards for possible hybridization detection, have been established, which can serve as standards in future research. Four colonies have been found, whose queens produce bees of the A. m. mellifera type of the Polissia population, and three colonies whose queens produce bees of the A. m. macedonica hybrid and can be used for further selection work*

***Keywords:** Morphometry of wings, classification of worker bees, discriminant analysis*

How to cite:
Galatiuk, O., Yarovets, V., Babenko, V., Cherevatov, V., Gutiy, B., Hryhorenko, A., Strilchuk, M., Stolyar, I. (2023). II. Morphometry of wings of worker bees of the subspecies *Apis mellifera mellifera* L. (Polissya population of Zhytomyr region). ScienceRise: Biological Science, 1 (34), 00-00. doi: http://doi.org/10.15587/2519-8025.2023.275588

1. Introduction

Currently, there is significant interest in the subspecies Apis mellifera mellifera L. among beekeepers due to several practical challenges. The observed ability to survive in isolation from human interference motivates the search for representatives of local populations of "dark forest bees" with the potential for resistance to viral and bacterial diseases, as well as tolerance to the Varroa mite [1]. Historically, the Polissya region was the habitat of the subspecies A. m. mellifera. However, as in Western Europe, uncontrolled spread of the bee subspecies A. m. carnica, A. m. ligustica, and possibly to a lesser extent, A. m. caucasica in Central and Eastern Europe has led to a reduction in the areas, inhabited by pure populations of "dark forest bees" belonging to the A. m. mellifera subspecies. This is confirmed by numerous studies. For example, a study on the degree of hybridization of bees in the North Wales region of the UK using wing morphometrics showed that most bee families were hybrids of A. m. mellifera and A. m. ligustica, with a slight predominance of A. m. mellifera [2].

Morphometric and genetic studies of bees in Norway, Sweden, and Finland where the influence of the C-line on local bees was investigated showed that 38 bee families can be considered purebred A. m. mellifera, while 23 are hybrids [3]. The genetic variability of local honeybees in the northeast of Poland, including a special isolated breeding zone in the Augustów Forest, was studied using mitochondrial DNA and nuclear microsatellites [4]. It was found, that approximately 10 to 30 % of the nuclear gene pool and 3 to 50 % of the mitochondria in the studied populations, considered to belong to the "dark forest bee", were obtained from non-local bees.

Regarding the bee populations in Ukraine, based on morphometric studies using 38 morphological traits according to F. Ruttner [5] and reference specimens of subspecies from the morphometric database (Bundesland Hessen, Oberursel), one-third of the samples from the Polissya region of Ukraine were classified as subspecies A. m. mellifera, one-third as A. m. macedonica, and one-third were identified as hybrids [6]. In case of using only wing morphology traits for classification purposes, all studied bee samples were assigned to three clusters: 1) subspecies of evolutionary lineage C (A. m. carnica, A. m. macedonica, A. m. ligustica); 2) subspecies of lineage O (A. m. caucasica and A. m. anatoliaca) together with samples from Ukraine; 3) subspecies A. m. mellifera. Hybrid samples unexpectedly showed a strong influence of the morphological lineage O. It is impossible to establish from the article which exact wing morphometric characteristics were used for classification. It should also be noted, that the sample of bee families, studied in Ukraine, was limited: only 17 families, obtained approximately from Kyiv, Vinnytsia regions, and Western Ukraine (it is impossible to establish the origin of bee samples from the text of the article). This is a reason to believe that the conclusions, drawn from a small number of families, and a selective regional location require further research to identify bee families, supplement with morphometric data, and reveal the real locations of the dark forest bee population or their hybrids. The authors made the first attempt to study the worker bees of several apiaries in the Zhytomyr region using classical wing morphometrics with the use of five traits (indices) in the work [7]. The results showed that the prevailing phenotypic breeds in this area are the Ukrainian steppe bee, A. m. mellifera (Polissya population), and their hybrids. Of the total of 1423 wings examined, only 116 could be reliably attributed to the A. m. mellifera subspecies, while 272 wings belonged to A. m. mellifera hybrids. It was also found, that the use of five indices and the fact that most of the studied families belonged to one beekeeper did not allow for a reliable determination of hybridization types based on wing phenotype and for making general conclusions about bees in the entire Zhytomyr region. Therefore, researchers faced the task of expanding the area of research and creating a classifier of wings that is accessible and accurate enough to determine the subspecies structure of bees. There was also an urgent need to develop a methodology for determining the type and degree of hybridization based on wing phenotype, without which it would be impossible to interpret the results of morphometric studies of wings.

The purpose of the work – Create an accessible and at the same time complete methodology for classifying the phenotypes of bee wings, by means of which it would be possible to determine the probable breed belonging of worker bees; type and degree of hybridization of the main breed with impurities; identify the "purebred" by the phenotype of the wings of bee colonies suitable for further breeding.

2. Materials and methods

A total of 2712 wings of worker bees were used from the apiaries of O. Halatyuk (locations – Zhytomyr city, Buki village, and Horodyshche village in the Zhytomyr district), H. Kevlyuk (Zhytomyr region, Ovruch town); wing samples, provided by A. Hryhorenko (Kyiv region, Kagarlyk village), M. Strilchuk (Mykolaiv region, Veseliniv town) and I. Mozharovskyi (Zhytomyr region, Korosten town), originated from queens from the apiary of I. Stolyar (Hlybochok village, Zhytomyr district) (Fig. 1, Table 1).

The wings of worker bees were collected from naturally obtained winter cluster, in accordance with the recommendations of ARRIVE guidelines for animal experiments and the UK Animals (Scientific Procedures) Act 1986, and relevant guiding principles, or the EU Directive 2010/63/EU on the protection of animals, used for scientific purposes.

Fig. 1. Map of the approximate locations of beekeeping sites in the Zhytomyr region

Table 1

Information on the origin of the queens of the studied bee colonies (n=27)

No	Names of bees colonies	Origin and type of insemination
1	Str.141.(2021)	IO*.(108. Stolyar×F1.110. Stolyar **)
2	Gal.15-1.(2022)	local bees **
3	Gal.15-1.2.(2022)	local bees **
4	Gal.4.(2022)	IO.(6. Galatiuk×110. Stolyar)
5	Gal.4.2.(2022)	IO.(6. Galatiuk×110. Stolyar)
6	Gal.1.(2022)	IO.(107. Stolyar×110. Stolyar)
7	Gal.1.2.(2022)	IO.(107. Stolyar×110. Stolyar)
8	Gryg.2.(2022)	F1., Stolyar **
9	Gryg.2.2.(2022)	F1., Stolyar **
10	Stol.110(4).(2022)	IO.(118. Stolyar×110(1). Stolyar)
11	Stol.111.(2022)	IO.(107. Stolyar x110(1). Stolyar)
12	Stol.126.(2022)	IO.(117. Stolyar×110(1). Stolyar)
13	Str.42.(2022)	F1.110. Stolyar **
14	Str.581.(2022)	F1.141. Strilchuk **
15	Moz.6.(2021)	F1.110. Stolyar **
16	Str.42.(2021)	F1.110. Stolyar **
17	Gal.15.(2021)	local bees **
18	Gal.15.2.(2021)	local bees **
19	Gal.11.(2021)	F1.15. Galatiuk **
20	Gal.11.2.(2021)	F1.15. Galatiuk **
21	Gal.7.(2022)	local bees **
22	Gal.8(6).(2022)	local bees **
23	Gal.8(6).2.(2022)	local bees**
24	Kevl.2.(2022)	local bees **
25	Kevl.3.(2022)	local bees **
26	Kevl.5.(2022)	local bees **
27	Kevl.6(2022)	local bees **

*Note: * – instrumental insemination; ** – natural insemination*

The wing images were processed using the TpsDig software [8]. Index values were calculated using a custom program. In the first stage of the study, 1500 wings from different colonies were classified using eight features (Ci, Dbi, Disc.sh, Pci, Ri, C.2, C.3, C.2.1), as proposed in [9], and based on approximate information about the possible affiliation of the wing phenotype to the subspecies A. m. mellifera or its hybrids. Using discriminant analysis and the StatSoft software package [10], the most reliable result was the classification of the wings into four clusters (Tables 2–5, Fig. 2), indicating the presence of four distinct phenotypic groups among the investigated wings. The phenotype of the wings in this study is defined as the set of values for the eight indices mentioned above.

Table 2

Summary results of discriminant analysis for wings (n=1500) of bee colonies (n=14) in the Zhytomyr region.

Indices	Wilks' Lambda	Partial Lambda	F-remove 3,1489	p-level	Toler.	1-Toler. (R-Sqr.)
Ci	0.08	0.916	45.81	<0.001	0.802	0.198
Dbi	0.084	0.874	71.845	<0.001	0.797	0.203
Pci	0.081	0.911	48.364	<0.001	0.74	0.26
Disc.sh.	0.08	0.913	47.589	<0.001	0.496	0.504
Ri	0.078	0.945	29.12	<0.001	0.448	0.552
Ci.2	0.08	0.914	46.429	<0.001	0.662	0.338
Ci.3	0.08	0.915	45.955	<0.001	0.698	0.302
Ci.2.1	0.086	0.857	83.096	<0.001	0.603	0.397

The sequence of indices according to the increase of their weights in the classification is as follows: Ri, C, C.3, Ci.2, Disc.sh, Pci, Dbi, Ci.2.1 (Table 2). Therefore, the radial Ri and C indices have the smallest influence on the classification of this set of wings from worker bees, while the Dbi and Ci.2.1 indices have the greatest influence.

Table 3

Classification matrix of wings from worker bees in bee colonies (n=14) in the Zhytomyr region

Cluster	Percent Correct	G_1:1 p=0.20333	G_2:2 p=0.32267	G_3:3 p=0.24200	G_4:4 p=0.23200	Total wings
G_1:1	87.9	268	23	9	5	
G_2:2	99.8	1	483	0	0	
G_3:3	97.5	4	0	354	5	
G_4:4	92	11	8	9	320	
Total	95	284	514	372	330	1500

The accuracy (correctness) of classification is satisfactory (95.0 %, Table 3). The arrangement of data (wings) in the space of canonical variables also indicates a qualitative discrimination using the obtained classification model (Fig. 2).

Table 4

Coefficients of linear classification functions for the four clusters of the classification model

Indices	Clusters			
	G_1:1 p=0.20333	G_2:2 p=0.32267	G_3:3 p=0.24200	G_4:4 p=0.23200
Ci	74.34	71.75	79.59	77.38
Dbi	576.47	562.20	608.31	585.70
Pci	510.92	515.69	499.19	500.55
Disc.sh.	–24.84	–25.95	–24.87	–25.20
Ri	659.57	638.94	671.78	655.88
Ci.2	–5.46	–6.18	–8.21	–7.72
Ci.3	249.50	240.93	254.26	242.30
Ci.2.2	158.53	157.60	162.76	143.20
Constant	–1839.54	–1793.75	–1866.21	–1773.17

3. Research results

The nature of the distribution of points in canonical variables (Fig.2) indicates that the phenotypes of wings in clusters 2 and 3 are significantly different from each other and do not have common boundaries. Wings belonging to clusters 1 and 4, upon further analysis, for example using Mahalanobis distances, may consist of two subgroups that differ in phenotype. The phenotype of a larger portion of wings will correspond to clusters 1 and 4, while the phenotype of another portion of wings may be similar to cluster 2 or cluster 3.

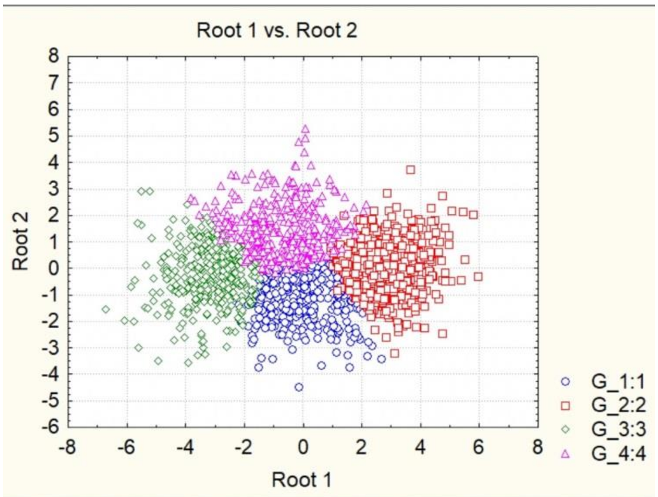

Fig. 2. Data distribution in the canonical variable spaces of the classification model for 8 features (Ci, Dbi, Disc.sh., Pci, Ri, Ci.2, Ci.3, Ci.2.1): G_1:1, G_2:2, G_3:3, G_4:4 – labels for clusters 1–4, respectively

When establishing the tentative affiliation of the wing phenotypes to the four clusters of the classification model (Table 5), literature data for two indices were used: cubital (Ci) and discoidal shift (Disc.sh.) [5, 11]. Based on these indices, the wing phenotype of cluster 2 clearly belongs to the subspecies A. m. mellifera. It can be assumed, that cluster 1 also belongs to the subspecies A. m. mellifera. However, it should be noted, that for cluster 1, the value of Disc.sh. is greater than 0, indicating a significant probability of hybridization. Clusters 3 and 4 correspond to local populations of certain hybrids. Taking into account the value of Disc.sh. for cluster 3 (2.5 > 0) and the relatively high value of Ci (2.04), (according to Avatesyan, the value of Ci for the Ukrainian steppe bee is within the range of 2.0–2.3 [11]; according to Polishchuk, it is 2.16–2.62, Disc.sh. (+) for 72 %–94 % of wings) [12], it can be assumed, that the main component of the bees in cluster 3 is a somewhat hybridized population type of the A. m. macedonica subspecies of the Ukrainian steppe bee.

Table 5

Mean feature (index) values for clusters of the classification model.

Cluster	Indexes								No wings
	Ci		Dbi		Pci		Disc.sh.		
	M*	±m**	M	±m	M	±m	M	±m	
1	1.679	0.013	0.844	0.003	2.959	0.006	0.9	0.082	305
2	1.434	0.008	0.771	0.002	3.051	0.004	-3.259	0.082	484
3	2.04	0.015	0.978	0.003	2.749	0.005	2.501	0.076	363
4	1.967	0.015	0.891	0.003	2.818	0.006	-0.39	0.091	348
								Total	1500
Cluster	Indexes								No wings
	Ri		Ci.2		Ci.3		Ci.2.1		
	M*	±m**	M	±m	M	±m	M	±m	
1	1.415	0.004	4.322	0.037	1.595	0.007	1.686	0.005	305
2	1.261	0.003	4.253	0.024	1.484	0.004	1.611	0.004	484
3	1.495	0.003	3.676	0.027	1.632	0.007	1.677	0.005	363
4	1.36	0.004	3.577	0.024	1.518	0.007	1.511	0.004	348
								Total	1500

*Note: * – mean value of the feature; ** – standard error of the mean*

Table 6

Summary results of classification of wings of worker bees from bee colonies (n=14)

No	Names of bees colonies	Number of wings in cluster				No wings
		Cluster 1	Cluster 2	Cluster 3	Cluster 4	
1	Str.141.(2021)	0 0.00 %	52 98.11 %	0 0.00 %	1 1.89 %	53
2	Gal.15-1.(2022)	1 1.00 %	0 0.00 %	77 77.00 %	22 22.00 %	100
3	Gal.15-1.2.(2022)	3 3.00 %	0 0.00 %	64 64.00 %	33 33.00 %	100
4	Gal.4.(2022)	8 8.00 %	4 4.00 %	45 45.00 %	43 43.00 %	100
5	Gal.4.2.(2022)	3 6.38 %	2 4.26 %	19 40.43 %	23 48.94 %	47
6	Gal.1.(2022)	61 61.00 %	2 2.00 %	15 15.00 %	22 22.00 %	100
7	Gal.1.2.(2022)	57 57.00 %	5 5.00 %	22 22.00 %	16 16.00 %	100
8	Gryg.2.(2022)	42 42.00 %	40 40.00 %	12 12.00 %	6 6.00 %	100
9	Gryg.2.2.(2022)	9 20.45 %	25 56.82 %	7 15.91 %	3 6.82 %	44
10	Stol.110(4).(2022)	32 17.68 %	133 73.48 %	6 3.31 %	10 5.52 %	181
11	Stol.111.(2022)	33 23.91 %	98 71.01 %	0 0.00 %	7 5.07 %	138
12	Stol.126.(2022)	11 7.33 %	103 68.67 %	30 20.00 %	6 4.00 %	150
13	Str.42.(2022)	33 16.50 %	1 0.50 %	59 29.50 %	107 53.50 %	200
14	Str.581.(2022)	12 13.79 %	19 21.84 %	7 8.05 %	49 56.32 %	87
Total		305	484	363	348	1500

The same interpretation is also valid for cluster 4, taking into account that the value of Disc.sh.<0 (-0.39) indicates a preference in the phenotype for the subspecies A. m. mellifera, or belonging to the subspecies A. m. caucasica, which is mentioned in the study [6]. This paragraph discusses a study on the morphometrics of the wings of the subspecies A. m. caucasica. The study found that the variation limits and mean values of the indices for seven bee colonies with queens from Georgia were as follows: Si (2.07–2.265), Si (mean) =2.173; Disc.sh. takes values within the range of -0.285 to -1.96, Disc.sh. (mean) =–1.43 [13]. These data are in good agreement with the index values of cluster 4 (Table 4) and with the data of F. Rutner, who reported Si=2.16 [14]. In conclusion, the possibility of the presence of a certain component of the A. m. caucasica subspecies in the genomes of queens from the Polissya region cannot be ruled out. However, a final verdict on this matter can only be made after appropriate genetic research.

The assignment of wing phenotypes from cluster 2 to the subspecies A. m. mellifera, and wing phenotypes from cluster 3 to the hybrid A. m. macedonica, which are phenotypically quite distinct, is consistent with the analysis of the distribution of wings in the canonical variables space, which illustrates a significant difference between clusters 2 and 3 (Fig. 2).

On the second stage of the study, using the obtained classifier, additional 1212 wings from 13 other bee colonies were analyzed, for which there were no previous records or doubts about their belonging to certain breeds, as well as those obtained for processing in the last place (Table 7).

Table 7

Summary of classification results for wings of worker honeybees from other bee colonies (n=13)

No	Names of bees colonies	Number of wings in cluster				N wings
		Cluster 1	Cluster 2	Cluster 3	Cluster 4	
15	Moz.6.(2021)	1 1.85 %	51 94.44 %	0 0.00 %	2 3.70 %	54
16	Str.42.(2021)	2 3.17 %	1 1.59 %	27 42.86 %	33 52.38 %	63
17	Gal.15.(2021)	60 61.86 %	4 4.12 %	24 24.74 %	9 9.28 %	97
18	Gal.15.2.(2021)	49 50.00 %	4 4.08 %	28 28.57 %	17 17.35 %	98
19	Gal.11.(2021)	2 2.00 %	0 0.00 %	48 48.00 %	50 50.00 %	100
20	Gal.11.2.(2021)	1 1.00 %	1 1.00 %	75 75.00 %	23 23.00 %	100
21	Gal.7.(2022)	3 3.00 %	0 0.00 %	73 73.00 %	24 24.00 %	100
22	Gal.8(6).(2022)	14 14.00 %	1 1.00 %	42 42.00 %	43 43.00 %	100
23	Gal.8(6).2.(2022)	10 10.00 %	0 0.00 %	39 39.00 %	51 51.00 %	100
24	Kevl.2.(2022)	2 2.00 %	0 0.00 %	24 24.00 %	74 74.00 %	100
25	Kevl.3.(2022)	1 1.00 %	0 0.00 %	68 68.00 %	31 31.00 %	100
26	Kevl.5.(2022)	7 7.00 %	4 4.00 %	19 19.00 %	70 70.00 %	100
27	Kevl.6.(2022)	25 25.00 %	2 2.00 %	56 56.00 %	17 17.00 %	100
Total		177	68	523	444	1212

The reliability of the classification of bee families No. 15–27 was within 94 %–95 %.

The results of the classification of the wings of 27 bee colonies (Tables 6, 7) were used to obtain morphometric standards of comparison: local micropopulations of Polish bees are designated as Polish, hybrids of the Polish population are designated as Hybrid.Pol, and hybrids of Ukrainian steppe bees are designated as Hybrid.Maced (Tables 8–11).

Table 8

Wing index values of worker bees of colonies of the morphometric standard of the local Polish micropopulation (Hybrid.Pol.1)

No	Names of bees colonies	Cluster	Indexes				N wings
			Ci	Dbi	Pci	Disc.sh	
6	Gal.1.(2022)	1	1.779	0.845	2.936	1.091	61
7	Gal.1.2.2022)	1	1.779	0.838	2.926	1.415	57
8	Gryg.2.(2022)	1	1.662	0.839	2.985	1.712	42
13	Str.42.(2022)	1	1.824	0.881	2.962	0.617	33
Average:			1.761	0.848	2.948	1.241	193
Standard deviations:			0.069	0.02	0.027	0.469	
Coefficients of variation (%):			3.9	2.4	0.9	37.8	
No	Names of bees colonies	Clus-ter	Indexes				N wings
			Ri	Ci.2	C.3	Ci.2.1	
6	Gal.1.(2022)	1	1.399	4.561	1,682	1,64	61
7	Gal.1.2.2022)	1	1.411	4.531	1.651	1,647	57
8	Gryg.2.(2022)	1	1.47	3.865	1.529	1,738	42
13	Str.42.(2022)	1	1.424	4.338	1.595	1,637	33
Average:			1.422	4.362	1.625	1.663	193
Standard deviations:			0.031	0.321	0.067	0.049	
Coefficients of variation (%):			2.2	7.4	4.1	2.9	

Table 9

Values of wing indices of worker bees of colonies of the morphometric standard of the local Polish micropopulation of the population (Polish)

No	Names of bees colonies	Cluster	Indexes				N wings
			Ci	Dbi	Pci	Disc.sh	
1	Str.141.(2021)	2	1.515	0.789	3.047	–5.673	52
8	Gryg.2.(2022)	2	1.581	0.757	3.072	–3.133	40
9	Gryg.2.2.(2022)	2	1.485	0.73	3.098	–4.125	25
10	Stol.110(4).(22)	2	1.318	0.764	3.026	–2.231	133
11	Stol.111.(22)	2	1.419	0.758	3.045	–2.772	98
12	Stol.126.(22)	2	1.404	0.8	3.054	–4.218	103
15	Moz.6.(21)	2	1.454	0.786	2.935	–4.015	51
Average:			1.42	0.774	3.039	–3,453	502
Standard deviations:			0.085	0.024	0.051	1,138	
Coefficients of variation (%):			6	3.1	1.7	33	
No	Names of bees colonies	Cluster	Indexes				N wings
			Ri	Ci.2	C.3	Ci.2.1	
1	Str.141.(2021)	2	1.18	4.691	1,549	1.527	52
8	Gryg.2.(2022)	2	1.276	4.191	1,405	1.584	40
9	Gryg.2.2.(2022)	2	1.25	4.634	1,434	1.589	25
10	Stol.110(4).(22)	2	1.304	4.137	1,473	1.656	133
11	Stol.111.(22)	2	1.304	4.26	1,49	1.67	98
12	Stol.126.(22)	2	1.209	4.142	1,482	1.582	103
15	Moz.6.(21)	2	1.241	3.912	1,481	1.605	51
Average:			1.262	4.23	1.481	1,617	502
Standard deviations:			0.047	0.282	0.045	0.048	
Coefficients of variation (%):			3.7	6.7	3.1	3	

It is necessary to draw attention to the fact that all 502 wings of the Polish standard come directly from the apiary of Polish bee breeder I. Stolyar (the village of Hlybochok, Zhytomyr district), from which queens were purchased by beekeepers at different times.

Table 10

Values of wing indices of worker bees of colonies of the morphometric reference hybrid of the local micropopulation of the Ukrainian steppe bee (Hybrid.Maced)

No	Names of bees colonies	Cluster	Indexes				N wings
			Ci	Dbi	Pci	Disc.sh	
2	Gal.15-1.(22)	3	2.146	0.982	2.71	2.512	77
3	Gal.15-1.2.(22)	3	1.997	0.993	2.707	2.046	64
4	Gal.4.(22)	3	2.011	0.992	2.725	2.139	45
5	Gal.4.2.(22)	3	1.993	0.996	2.726	1.913	19
6	Gal.1.(22)	3	2.019	0.934	2.764	2.855	15
7	Gal.1.2.(22)	3	2.187	0.949	2.707	2.702	22
12	Stol.126.(22)	3	1.874	0.98	2.83	2.63	30
13	Stol.42.(22)	3	2.076	0.982	2.805	2.82	59
16	Stol.42.(21)	3	2.21	1.017	2.784	2.976	27
20	Gal.11.2.(21)	3	2.042	1.015	2.736	1.776	75
21	Gal.7.(22)	3	2.147	0.997	2.753	2.729	73
24	Kevl.2.(22)	3	2.243	0.969	2.744	2.475	24
27	Kevl.6.(22)	3	2.23	0.979	2.864	2.572	56
Average:			2.096	0.99	2.759	2.428	586
Standard deviations:			0.112	0.023	0.05	0.384	
Coefficients of variation (%):			5.4	2.4	1.8	15.8	
No	Names of bees colonies	Cluster	Indexes				N wings
			Ri	Ci.2	C.3	Ci.2.1	
2	Gal.15-1.(22)	3	1.495	3.597	1.662	1.679	77
3	Gal.15-1.2.(22)	3	1.485	3.697	1.663	1.693	64
4	Gal.4.(22)	3	1.475	3.532	1.633	1.678	45
5	Gal.4.2.(22)	3	1.44	3.656	1.675	1.676	19
6	Gal.1.(22)	3	1.487	3.972	1.694	1.633	15
7	Gal.1.2.(22)	3	1.502	4.391	1.778	1.679	22
12	Stol.126.(22)	3	1.489	3.611	1.613	1.71	30
13	Stol.42.(22)	3	1.529	3.558	1.529	1.645	59
16	Stol.42.(21)	3	1.485	3.637	1.603	1.619	27
20	Gal.11.2.(21)	3	1.484	3.834	1.672	1.661	75
21	Gal.7.(22)	3	1.437	3.766	1.661	1.659	73
24	Kevl.2.(22)	3	1.522	3.453	1.523	1.641	24
27	Kevl.6.(22)	3	1.484	3.75	1.598	1.747	56
Average:			1.486	3.707	1.638	1.676	586
Standard deviations:			0.026	0.242	0.068	0.034	
Coefficients of variation (%):			1.8	6.5	4.2	2	

The origin of the bees of the Hybrid.Pol.1, Hybrid.Maced and Hybrid.Pol.2 standards is more diverse, and covers the points of the city Ovruch and district of Zhytomyr.

Table 11

Wing index values of worker bees of colonies of the morphometric standard of the local Polish micropopulation (Hybrid.Pol.2)

No	Names of bees colonies	Cluster	Indexes				N wings
			Ci	Dbi	Pci	Disc.sh	
4	Gal.4.(22)	4	1.861	0.897	2.78	–0.887	43
5	Gal.4.2.(22)	4	1.901	0.913	2.81	–0.606	23
13	Str.42.(22)	4	2.05	0.916	2.818	–1.002	107
16	Str.42.(21)	4	2.031	0.927	2.83	–0.255	33
22	Gal.8(6).(22)	4	2.08	0.887	2.744	–0.228	43
23	Gal.8(6).2.(22)	4	2.001	0.883	2.748	–0.38	51
Average:			2.005	0.905	2.791	–0,656	300
Standard deviations:			0.087	0.018	0.037	0,329	
Coefficients of variation (%):			4.3	1.9	1.3	50.1	
No	Names of bees colonies	Cluster	Indexes				N wings
			Ri	Ci.2	C.3	Ci.2.1	
4	Gal.4.(22)	4	1.349	3.334	1.467	1.508	43
5	Gal.4.2.(22)	4	1.336	3.286	1.423	1.526	23
13	Str.42.(22)	4	1.367	3.582	1.542	1.474	107
16	Str.42.(21)	4	1.381	3.682	1.553	1.507	33
22	Gal.8(6).(22)	4	1.389	3.352	1.437	1.604	43
23	Gal.8(6).2.(22)	4	1.391	3.464	1.446	1.6	51
Average:			1.371	3.482	1.492	1,527	300
Standard deviations:			0.022	0.156	0.056	0,053	
Coefficients of variation (%):			1.6	4.5	3.7	3.5	

4. Discussion

For the arrays of wings, formed by the Hybrid.Pol.1, Polish, and Hybrid.Pol.2 standards, the coefficients of variation of the Disc.sh. index range from 33 % to 50.1 %, which exceeds the "limit of reasonableness," while the coefficients of variation of the other seven indices have entirely acceptable values (<8 %). This fact cannot be explained solely by errors in establishing landmarks on the wing, which determine the value of the discoidal shift index, but indicates that the Disc.sh. index is critically sensitive to minor changes in the genomes of the mothers that determine the wing phenotype within a particular micro-population. This peculiarity can be used advantageously in the future as a criterion for differentiation between ecotypes, populations, and lines.

Clusters 1, bee colonies No: 10, 11, 17, 18, 27; clusters 3, bee colonies No: 17, 18, 19, 22, 23; and clusters 4, bee colonies No: 3, 6, 7, 14, 21, 25, 26, for which the values of Disc.sh.>0, and No: 2, 5, 13, 19, 20, 24, for which Disc.sh. <0, were not included in the formation of the array of values of the Hybrid.Pol.1, Hybrid.Maced, and Hybrid.Pol.2 standards, respectively, due to the significant increase in the coefficients of variation of the indices, although these bee colonies, identified in the first approximation according to the established classification, were indicated by Euclidean distances. To assess the breed affiliation of the wing phenotypes of the indicated groups of bee colonies, some of them were analyzed using Mahalanobis distances and the created standard data (Tables 12, 13).

Table 12

The result of the analysis of the similarity of wing clusters of some bee colonies to the formed standards

No	Names of bees colonies	Cluster	Mahalanobis distance	Standard
10	Stol.110(4).(2022)	1	3.144	Hybrid.Pol.1
27	Kevl.6.(2022)	1	3.433	Hybrid.Pol.1
19	Gal.11.(2022)	3	3.971	Hybrid.Maced
19	Gal.11.(2022)	4	3.535	Hybrid.Pol.2
22	Gal.8(6).(2022)	3	3.354	Hybrid.Maced
14	Str.581.(2022)	4	3.342	Hybrid.Pol.2

Based on the empirical data, accumulated by the authors, and the analysis of the results, presented in Table 12, approximate boundary values of similarity are used, namely: "0–2" – high similarity; "2–3.5" – significant similarity; "> 3.5" – insignificant (or absent) similarity. Four out of five clusters of wings that were tested can be considered significantly similar to the comparison standards we created, except for cluster 3 of bee colony No: 19, for which the Mahalanobis distance is 3.971>3.5.

The data, given in Table 13, allow to obtain information about a more detailed structure of wing phenotypes for each individual cluster.

Table 13

Distribution of wings of worker bees, some bee colonies according to the nearest centroids of comparison standards.

No	No wings	%	Standard
10	14	43.8	Hybrid.Pol.1
Cluster	18	56.3	Polish
1	0	0	Hybrid.Maced
	0	0	Hybrid.Pol.2
Total	32	100.1	
27	18	72	Hybrid.Pol.1
Cluster	0	0	Polish
1	4	16	Hybrid.Maced
	3	12	Hybrid.Pol.2
Total	25	100	
22	0	0	Hybrid.Pol.1
Cluster	0	0	Polish
3	41	97.6	Hybrid.Maced
	1	2.4	Hybrid.Pol.2
Total	42	100	
14	11	22.4	Hybrid.Pol.1
Cluster	6	12.2	Polish
4	1	2	Hybrid.Maced
	31	63.3	Hybrid.Pol.2
Total	49	99.9	
19	0	0	Hybrid.Pol.1
Cluster	0	0	Polish
4	0	0	Hybrid.Maced
	49	100	Hybrid.Pol.2
Total	49	100	

The wings of cluster 1, family No: 10, which are very similar to the Hybrid.Pol.1 standard (Table 12), are distributed somewhat unexpectedly between the standards: Hybrid.Pol.1/Polish = 43.8 %/56.3 %, which actually indicates a greater similarity of the entire cluster to the Polish standard (Table 13). This fact correlates with the significant ambiguity in the distribution of wings between clusters 1 and 2 (Table 3) and, as a result, unsatisfactory accuracy of classifying the wings of cluster 1 (87.9 %). For the other considered bee colonies, No: 27, 19, 22, and 14, the majority of wings are assigned to the standards, determined in Table 12. The presence of a certain portion of wings, assigned to other standards, can be considered as a possible degree of hybridization with a certain breed. For example, for bee colony No. 27, the ratio of wings in cluster 1, Hybrid.Pol.1/Hybrid.Maced = 72/16, which may indicate a slight increase in the influence of the subspecies A. m. macedonica to a phenotype that most likely belongs to the subspecies A. m. mellifera.

A comparison of the average values of the cubital index C of cluster 3 of the classification model (2.04) and the standard Hybrid.Maced (2.096) indicates that the initial assumption that the bees of this cluster belong to the partially hybridized subspecies A. m. macedonica is correct. For 6 bee colonies out of 13 that form the Hybrid.Maced standard, the value of C>2.14, which corresponds to the typical values for Ukrainian steppe bee populations (Table 10), for the other seven – C<2.076. That is, in a real attempt to classify the wings of "purebred" Ukrainian steppe bees using this classifier, they will be assigned to cluster 3. It should be understood, that this fact does not at all indicate their hybridization. The type and degree of hybridization determine the specific values of the phenotypes and their comparison with the reference data.

In summary, based on the analysis of data, presented in Tables 8–12, it can be concluded, that purebred bees of the Polish population of the subspecies A. m. Mellifera by phenotype, were found only at one apiary location, Stolyar's in the village of Hlybochok, Zhytomyr district. This result provides optimism for the next stages of work, aimed at the selective consolidation of necessary economically beneficial traits (EBTs) in the established micro-population of Polissya bees, namely: forming groups of analogues, evaluating the values of EBTs, and selecting breeding material.

Hybrids of Polish bees of the subspecies A. m. mellifera are present in all other studied apiaries without exception, which correlates with the data [4]. However, we cannot confirm or deny the assumption of the influence of bees of the evolutionary lineage O on the population of local bees due to the fact that it is extremely problematic to morphometrically establish the difference between the subspecies A.m.mellifera and A. m. Caucasica.

All four arrays of formed standards for 8 wing features are included in the morphometric data bank of bee wings, managed by the authors of this work. Considering the fact that the reference data Hybrid.Pol.1 and Hybrid.Pol.2 refer to local hybrids of bee micropopulations, they will have the advantage of being used within the Zhytomyr region and its adjacent territories, in contrast to the standards Polish and Hybrid.Maced, which, based on the values of the wing phenotypes, probably belong directly to the subspecies A. m. mellifera and A. m. macedonica, and therefore can be used as standards of comparison without territorial restrictions.

Limitations of the study. It is necessary to state the fact that the number of received drone wings was insufficient, which did not allow adding research results to the discussion.

Prospects for further research. However, we believe that the morphometry of drone wings is an extremely effective tool in establishing the species affiliation of bees, which, a priori, significantly increases the credibility of works of the kind as this work. Therefore, in the future, the authors will direct their research precisely in the direction of filling the gap in the study of drone wing morphometry.

It should be noted, that the wings of bee colonies No: 17, 18, 25 and 26 did not receive a reliable interpretation of the breed of the phenotype of the wings in this work, therefore no comments about them are given in the text.

5. Conclusions

1. With the help of statistical methods of data analysis, a classification model was created, which allows for a sufficiently efficient analysis of worker bees in the Polissia region.

2. Discrimination of 1500 bee wings, which according to the preliminary assessment were positioned as the Polish population of A. m. macedonica, showed that 1137 of them can be attributed to the subspecies A. m. mellifera, or to its hybrids; a smaller part of the wings, namely 363 – to the subspecies A. m. macedonica, or to its hybrids. Of the 1212 wings that did not have a prior reliable prediction of breed, 523 were assigned to the subspecies A. m. Macedonian. In general, this distribution shows that despite the significant influence of the subspecies A. m. macedonica on the wing phenotype of worker bees, the Polissia region remains the natural habitat of autochthonous bees of the subspecies A. m. mellifera.

3. Phenotypic values of wing indices of worker bees from four micro-populations of Polissya honeybees have been established, which can serve as comparison standards in future research. One of these micro-populations likely exhibits characteristics of purity and belongs to the local Polissya micro-population in the Zhytomyr region, belonging to the subspecies A. m. mellifera. In addition, the availability of established comparison standards by the authors makes it possible to assess the type and degree of probable hybridization based on the wing phenotype of worker bees.

4. Four bee colonies were found: No: 1 (Str.141(21)) and No: 10 (Stol.110(4)(22)), 11 (Stol.111(22)), 12 (Stol.126(22)), whose queens produce worker bees of the purebred A. m. mellifera phenotype of the local Polissya micro-population; three bee colonies – No: 20 (Gal.11.2(21)), No: 2 (Gal.15-1(22)), 21 (Gal.7(22)), where the majority of worker bees are clustered in group 3 (in a ratio of 4/1), which is identified as a hybrid of Ukrainian steppe bees, and can be used as founders of lines.

Practical Significance. Thus, this work has significant practical implications as it allows beekeepers to independently classify the wings of worker bees from a particular area using classification functions, coefficients of which are provided in Table 4. In the first approximation, it is possible to predict the probable affiliation of bees to certain subspecies, ecotypes, populations, or lines based on Euclidean distances, using the values of indices for the formed comparison standards (Tables 8–12). The validity of such predictions can be confirmed by comparing the phenotype of the studied wings with the standard samples, such as the four, formed in this study. However, rdinary beekeepers will not be able to perform the final stage of the work on their own. In order to solve the problem of reliability, they should contact the custodians of the "bank of reference data", which is currently being created by the authors and is available for public use. In addition, the existence of such a database allows not only for determining the affiliation of a wing phenotype to a particular "breed", but also for estimating the probable degree of hybridization of the main breed with an impurity.

Conflict of Interest

The authors declare that they have no conflict of interest regarding this research, including financial, personal, authorship or any other kind of conflict that could influence the research and its results, presented in this article.

Funding

The research was conducted without any financial support.

Data Availability

The data will be provided upon a reasonable request.

References

1. Halatiuk, O. Ye., Petrenko, S. O. (2020). Bdzhilnytstvo ta profilaktyka khvorob. Odesa: Astroprynt, 324.

2. Keller, E. M., Harris, I., Cross, P. (2014). Identifying suitable queen rearing sites of Apis mellifera mellifera at a regional scale using morphometrics. Journal of Apicultural Research, 53(2), 279–287. doi: https://doi.org/10.3896/ibra.1.53.2.09

3. Groeneveld, L. F., Kirkerud, L. A., Dahle, B., Sunding, M., Flobakk, M., Kjos, M., Henriques, D., Pinto, M. A., Berg, P. (2020). Conservation of the dark bee (Apis mellifera mellifera): Estimating C-lineage introgression in Nordic breeding stocks. Acta Agriculturae Scandinavica, Section A – Animal Science, 69 (3), 157–168. doi: https://doi.org/10.1080/09064702.2020.1770327

4. Oleksa, A., Chybicki, I., Tofilski, A., Burczyk, J. (2011). Nuclear and mitochondrial patterns of introgression into native dark bees (Apis mellifera mellifera) in Poland. Journal of Apicultural Research, 50 (2), 116–129. doi: https://doi.org/10.3896/ibra.1.50.2.03

5. Ruttner, F. (1988). Zuchttechnik und Zuchtauslese bei der Biene. Ehrenwirth-Verlag, 138.

6. Meixner, M. D., Worobik, M., Wilde, J., Fuchs, S., Koeniger, N. (2007). Apismellifera melliferain eastern Europe – morphometric variation and determination of its range limits. Apidologie, 38 (2), 191–197. doi: https://doi.org/10.1051/apido:2006068

7. Yarovets, V., Babenko, V., Halatiuk, O., Strilchuk, M., Mozharovskyi, I. (2022). Morphometry of bees wings of the south-eastrn region of polisya (Zhytomyr region). Scientific and production journal “Beekeeping of Ukraine,” 1 (8), 72–76. doi: https://doi.org/10.46913/beekeepingjournal.2022.8.11

8. TpsDig 2. Available at: https://ru.freedownloadmanager.org/Windows-PC/tpsDig2.html

9. Yarovets, V. I., Babenko, V. V., Galatiuk, O. Ie. (2022). Morphometry of bee wings on eight signs indices: Ci, Dbi, Disc.sh, Pci, Ri, Ci.2, Ci.2.1, Ci.3. Scientific and production journal “Beekeeping of Ukraine,” 1 (8), 65–71. doi: https://doi.org/10.46913/beekeepingjournal.2022.8.10

10. StatSoft (2001). Moscow. Available at: http://www.StatSoft.ru/home/textbook/default.htm

11. Avetisian, G. A. (1983). Razvedenie i soderzhanie pchel. Moscow: Izdanie «Kolos», 271.

12. Polishchuk, V. P. (2001). Bdzhilnytstvo. Kyiv: Vydavnytstvo «Ukrainskyi pasichnyk», 296.

13. Yarovets, V., Babenko, V., Mozharovskyi, I., Sakara, P. (2021). Morfometriia kryl deiakykh linii porody «Sira hirska kavkazska». Ukrainskyi pasichnyk, 6, 4.

14. Ruttner, F., Tassencourt, L., Louveaux, J. (1978). Biometrical-statistical analysis of the geographic variability of apis mellifera L. I. Material and Methods. Apidologie, 9 (4), 363–381. doi: https://doi.org/10.1051/apido:19780408

Received date 02.02.2023
Accepted date 15.03.2023
Published date 31.03.2023

Oleksandr Galatyuk, Doctor of Veterinary Sciences, Professor, Head of Department**,** Department of Microbiology, Polis National University, Staryi blvd., 7, Zhytomyr, Ukraine, 10002

Volodymyr Yarovets*, PhD, Beekeeper**,** Lviv, Ukraine

Volodymyr Babenko, Senior Lecturer, Department of Higher Mathematics**,** Ivan Franko National University of Lviv**,** Universytetska str., 1, Lviv, Ukraine, 79000

Volodymyr Cherevatov, PhD, Associate Professor, Department of Molecular Genetics and Biotechnology, Yuriy Fedkovych Chernivtsi National University**,** Kotsiubynskoho str., 2, Chernivtsi, Ukraine, 58012

Bogdan Gutyj, Doctor of Veterinary Sciences, Professor, Department of Molecular Genetics and Biotechnology, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv**,** Pekarska str., 50, Lviv, Ukraine, 79010

Grigorenko Andrii, Beekeeper, Breeder of Bee Queens, Uterine apiary «AMG», Kyiv, Ukraine

Mykhailo Strilchuk, Safety Engineer, Veselinivska Central District Hospital, Odeska str., 82, Veselynove, Ukraine, 57001, Beekeeper, Breeder of Bee Queens

Ihor Stolyar, Beekeeper, Breeder of bee Queens, Queen apiary of the Polish Population of A.m.mellifera, Hlybochok village, Berdychiv district, Ukraine

Corresponding author: *Volodymyr Yarovets, e-mail: 1951nadija@gmail.com*