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MINI-EXPERIMENT IN BREEDING: GENOTYPING OF APIS MELLIFERA L. COLONIES BY TWO TRAITS

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Between 2020 and 2023, a planned study was conducted on a single apiary (Ukraine, Lviv region) consisting of 50 honey bee subspecies A.m.carnica colonies. Due to insufficient isolation of the apiary from the influence of foreign drones, the work in 2020–2021 focused on achieving a more stable and homogeneous drone background. As a result, by early 2023, two groups of analogous colonies were established, whose queens were sisters. Identifying the subspecies and population affiliation of the chromosomal sets of these queens was performed using morphometric templates for drone wings. Based on comparing wing phenotypes with the templates, the probable affiliation of the queens' genomes to the local "Carnica" population and the Peschetz line was established. During the 2023 season, 17 colonies were tested for two economically valuable traits: flight activity and gentleness. Variance analysis revealed relationships between queen genome composition, environmental factors (weather, nectar availability), and key traits (gentleness, flight activity). The statistical reliability of the results is limited due to the small sample size. However, the observed trends allow for specific recommendations regarding the further use of colonies whose genomic composition and probable chromosomal set affiliations have been established. This approach is recommended as an additional tool to enhance the effectiveness of selective breeding.

Keywords: honey bee, drones, worker bees, genome, chromosomal sets, classical wing morphometry, morphometric templates, economically valuable traits, analysis of variance, discriminant analysis, A.m.carnica.

Introduction. It is well known that achieving successful outcomes in beekeeping is significantly more challenging than in animal husbandry or poultry farming. This is mainly due to the unique biology of the honey bee (*Apis mellifera*) (Sauvager, 2019). One of the primary complications is that offspring within a colony originate from multiple fathers, which results in substantial genetic diversity among honey bee populations (Knoll et al., 2025; Lukic et al., 2024). This phenomenon is not unique to honey bees but is common among Hymenoptera (Bruns et al., 2025). Therefore, to fix desirable traits in offspring, beekeepers must ensure sufficient numbers of drones of the appropriate type are present. (Schaumann et al., 2024; Du et al., 2024). Maintaining complete isolation of apiaries from foreign drones is typically impractical under standard conditions (Rahimi et al., 2023). On the one hand, this contributes positively to biodiversity and enhances population resilience to various diseases due to increased variability both within colonies and across populations. On the other hand, there is a risk of introgression from non-native "breeds," which may displace local populations (Tanasković et al., 2022). Research results (Geslin et al., 2017) have shown that "…, *these Massively Introduced Managed Species (MIMS) integrate local communities and can trigger cascading effects on the structure and functioning of ecosystems*". Moreover, other factors contribute to unpredictable changes in insect diversity in areas that have historically been their natural habitats (Herrera et al., 2024). For instance, land use changes have significantly reduced the taxonomic diversity of honey bees (Toby et al., 2025; Barroso et al., 2025). The influence of additional anthropogenic factors has been explored in the work of Patenković et al. (2022). Both traditional (Park et al., 2020) and emerging diseases continue to impact honey bee populations (Albarrak et al., 2023; Sgroi et al., 2025). Annual colony losses are attributed to various causes depending on the region or country (Aurell et al., 2023; Requier et al., 2018), mainly due to the use of pesticides in general and insecticides in particular (Zhang et al., 2025). Studying such losses requires significant effort from researchers and scientists to mitigate the consequences of invasions (Ferrufino et al., 2024). Annual studies conducted under the auspices of the Canadian Association of Professional Apiculturists (CAPA) for ten provinces revealed a concerning picture of colony losses from 2007 to 2022. The statistical data recorded losses across all provinces ranging from 15.3% to 45.5%, with an average of 27%. The experimental data obtained enabled Canadian researchers to broadly assess the genetic parameters of colonies based on five traits, providing the foundation for planned and proactive measures to ensure colony viability (Maucourt et al., 2021). Currently, researchers have moved beyond merely observing and documenting detrimental factors. They now focus on forecasting the likely negative consequences for pollinating insects. These studies offer practical recommendations to minimize harmful impacts caused by human activity (Kazenel et al., 2024). In light of this, beekeeper associations, scientific institutions, and government authorities—both at regional and national levels—are developing structured bee breeding programs (Uzunov et al., 2022).

Today's primary threat to European honey bee populations is the mite *Varroa destructor*. Given the critical importance of combating Varroa (*V. destructor*) infestation, several countries have developed dedicated breeding programs to address this issue. The conceptual foundations of one of the proposed selective breeding approaches are outlined in the works of Locke (2016) and Blacquière et al. (2019): "*…The scheme is based on the principles of evolution by natural selection: colonies able to survive and repro- duce successfully pass on their genes to the next generation …. Like Darwin's observations, it is possi- ble to observe adaptations without understanding the underlying mechanisms. Here, we use the analogy of a black box from which the content remains hidden while the obvious effects of this content are nonethe- less clear and visible. ….. Inside the black box, alleles associated with a successful phenotype are conserved and will persist in the next generation. Natural selection is therefore 'inclusive' as it maintains genetic diversity by keeping all surviving phenotypes in the black box, including possibly rare alleles beneficial for resistance to parasites and pathogens. Targeted selective breeding programs, on the contrary, are by definition reducing genetic diversity by selecting from the surviving phenotypes only those of their preference of chosen traits, thereby potentially excluding many of the phenotypes despite their shown capability of survival (Uzunov et al. 2017). DBBB selection follows the natural seasonal reproduction cycle of honey bee colonies, mimicking swarming by splitting colonies*", and also in the (DBBB) program (Panziera et al., 2017; Kruitwagen et al., 2017). In the United States, a long-standing program titled "A sustainable approach to managing honey bee varroa mites" operates under the auspices of the National Institute of Food and Agriculture (NIFA) of the U.S. Department of Agriculture (Spivak & Reuter, 2021). The works of Blacquière et al. (2019) and Alphen et al. (2020) express cautious optimism about the practical potential for breeding honey bee colonies resistant to *Varroa destructor*. Similar findings are reported by Luis et al. (2022). At the same time, scientific efforts aimed at developing predictive genome models for Apis mellifera populations (Bernstein et al., 2023) have yielded promising results for most traits (Andonov et al., 2019; Guichard et al., 2020), with the notable exception of V. destructor resistance. Various treatment methods have been employed to control mites, most involving miticides (Jack et al., 2021; Aurell et al., 2024). Occasionally, alternative and non-traditional approaches are proposed (Mukogawa et al., 2024). However, none have entirely eradicated or eliminated the mite from the colony biocenosis. Unable to fundamentally resolve the issue, it is proposed to implement new integrated management and control strategies for *Varroa destructor* (Noël et al., 2020; Sprau et al., 2024).

In Ukraine, to maintain balance among local bee populations, an official document has been adopted outlining a detailed regional zoning plan for the breeding of *A.m.carnica*, *A.m.mellifera*, and *A.m.macedonica* subspecies (On Beekeeping: Law of Ukraine). Successful implementation of this and other beekeeping and breeding programs requires more than the efforts of individual beekeepers; coordinated action across regions is essential. Honey bee breeding efforts primarily focus on the following traits: honey productivity, varroa tolerance, hygienic behavior, and gentleness (Facchini et al., 2021). Researchers, scientists, agricultural institutions, and beekeeper associations work together to improve these traits to desirable levels (Kistler et al., 2021). To support this, recommendations are provided regarding required measures, methods of work, and procedures (Du et al., 2021; Büchler et al., 2024). These recommendations are based on the concepts of genetic evaluation of individuals, selection index, breeding value assessment, heritability estimation, genetic gain, and others (Bienefeld et al., 2007; Brascamp et al., 2019). A widely used approach among researchers is the "mechanistic approach," which investigates the influence of external factors and colony traits to improve productivity and other characteristics (Quinlan et al., 2023; Barahona et al., 2024). This allows researchers to track how: "*….inter-dependent colony metrics offer insights into environmental-plant-pollinator dynamics*".

Breeding value assessment for bees—commonly used in livestock breeding—can be applied in two primary ways: a) by considering the breeding value of a colony as a function of the queen's identity; b) by assessing it as a function of the traits of the worker bees that comprise the colony (Kistler et al., 2024; Basso et al., 2024).

The feasibility of using genetic information (genotyping) for predictive purposes to identify potentially critical phenotypic traits in the offspring of *A.m.mellifera* and *A.m.carnica* subspecies has been demonstrated in the works of Brascamp & Bijma (2014) and later by Guichard et al. (2023): "…*In this study, heritability estimates for the main traits of interest were calculated for two Swiss bee populations evaluated in the period 2010–2018. The popula- tions were Apis mellifera carnica (Société Romande d'Apiculture, SAR, with a total of 1,009 tested colonies) and Apis mellifera mellifera (mellifera.ch), MEL, with a total of 1,071 tested colonies). The following traits were recorded in the two populations, namely honey production, gentleness, steadiness on comb, swarming tendency, hygienic behavior (pin test) and varroa infestation (infestation in spring and summer and growth rate between the two periods). In addition, the parental information of the queens/colonies was recorded in a pedigree file, tracing back up to 49 generations*". The cited passage shows how long a period was required to obtain the necessary, pervasive experimental data. The research results showed that predictions often indicate a low level of heritability. This outcome is due to: "… *mainly too low measurement accuracies and unidentified environmental effects that superimpose the genetic effects. Therefore, the objective recording of traits is of central importance to achieve better heritability …*".

An important factor in breeding is the generation turnover time. Reducing it makes it possible to accelerate the "genetic gain" of desired traits significantly. Therefore, in beekeeping, instrumental insemination is recognized as a necessity (Du et al., 2023).

In Ukraine, it is currently impossible to carry out work on a similar scale due to several circumstances. The authors of this study are forced to limit themselves to a "targeted" investigation and apply an empirical approach to analyzing the obtained data. The cause-and-effect relationship between genotype and phenotype is understandable, but establishing it in quantitative terms is problematic. This is because large sample sizes of colonies must be studied. Given the typically diverse—often linear, population-specific, or even subspecies-level—origin of the bees, and consequently the different chromosomal sets in the genomes of the queens in the studied colonies, it is impossible to obtain statistically reliable results.

Traditional breeding methods involved a lengthy selection process for specific economically advantageous traits (EUT). For a long time, such approaches allowed the consolidation of desirable characteristics, but they were often based on the strict elimination of deviations from the chosen standard. This, in turn, led to a reduction in genetic diversity in research apiaries and among wild populations in the surrounding environment.

A key stage in the targeted influence on the phenotype of bees was the study of inheritance patterns and gene interaction mechanisms determining valuable traits. However, under current conditions, such studies are conducted to a limited extent, requiring significant resources and time. In this context, modern breeding increasingly focuses not on traditional analysis of trait heritability but on identifying molecular markers associated with them. Identifying such markers allows for effectively classifying bees by phenotypic categories, significantly accelerating the breeding process and ensuring the more accurate selection of desirable genotypes. One example is using the MRJP3 gene (*Major Royal Jelly Protein 3*) as a molecular marker for breeding bees with increased royal jelly production. Studies have shown that specific alleles of this gene (specifically C, D, and E) are associated with higher productivity, which enables their use in the selection of high-producing queens (Ruvolo-Takasusuki et al., 2016).

Another direction is the study of resistance to the mite *Varroa destructor*. In particular, more than 60 significant associations have been found between SNP markers and varroa resistance traits, confirming the polygenic nature of this trait. This indicates the relevance of implementing genomic selection, focused not on individual genes but on the overall genetic profile (Eynard et al., 2025).

Moreover, recent studies demonstrate the high effectiveness of genomic prediction of economically essential traits in honey bees, particularly about productivity, behavioral characteristics, and, to some extent, disease resistance (Bernstein et al., 2023).

One of the significant obstacles for Ukrainian researchers working in breeding programs is the lack of capacity to conduct genetic studies due to a shortage of qualified personnel and unfavorable economic conditions. Considering this, the authors set the following goals: a) to obtain, within the apiary, a controlled micro-population of bees of an interline hybrid for the subspecies *A.m. carnica*; b) to study the possibility of using classical wing morphometry of bees to determine the probable subspecies affiliation of the genome of queens and worker bees; c) to explore the possibility of establishing correlations between the genome composition of queens and worker bees and several economically advantageous traits (EUT).

Unfortunately, due to the limited experimental data, we could not establish the dependence of the studied traits on the composition of the queen's genome with acceptable statistical reliability. However, even though the set goal was not fully achieved, there are grounds to consider it appropriate to continue and scale up the testing and analysis method proposed in this work for evaluating several different traits in honey bee colonies.

Materials and Methods. The study was conducted at an apiary in the Lviv region, which specializes in selling bee packages and consists of 50 colonies. According to previous morphometric studies of worker bee wings, most colonies were classified as belonging to the subspecies *Apis mellifera carnica*. Within a 5 km radius of this apiary, there are six other small apiaries, each consisting of 5 to 10 colonies. The two closest ones are located 1 km away and have 4 and 5 colonies, respectively. In 2020, based on an evaluation of economically valuable and breed-specific traits, one queen was selected as the foundation of the experiment. On June 15 of the same year, unmated F1 queens were obtained from her and introduced into all the colonies. A total of 46 queens successfully mated and began laying eggs. During the 2021 season, the old queens were replaced twice—first with unmated F2 and then with unmated F3. These queens were selected based on previously determined individual genomes, ensuring chromosomal compositions met our subspecies and line affiliation criteria. In 2022, the focus shifted to determining probable genotypes of worker bees and making a preliminary evaluation of several economically valuable traits to form groups of analogous colonies. In the spring of 2023, two groups of analogous colonies (comprising 9 and 8 colonies, respectively) were formed and subjected to testing. One group remained at the primary apiary, while the other was relocated 10 km away to a site with similar nectar conditions. Colony testing in two groups, where the colonies were considered analogs, was conducted ten times during the 2023 season. On each occasion, two beekeepers recorded observations on the same day, assessing two traits—flight activity and gentleness—and two environmental factors—weather conditions and availability of nectar sources. These assessments were made thrice during daytime periods (Tables 1.–4.). Flight activity and weather condition data, assessed on a point-based scale, were recalculated using weighting coefficients (Table 5.) to derive a cumulative daytime score.

Table 1.

Evaluation of the trait: frequency of bee departures for pollen and nectar (flight activity)

Nature of flight activity	Evaluation, points
low	0–1
moderate	2–3
high	4–5

Table 2.

Assessment of the factor: weather conditions (meteorological conditions)

Weather conditions	Evaluation*, points
Hot	4–5
Good	3–4
Comfortable	2–3
Rainy	1–2
Cold	0–1

*Note: * – also rated on a qualitative scale: «Hot weathe», «Quality», «Comfortable», «Rain» and «Coldly» respectively.*

Table 3.

Assessment of behavioral trait: gentleness (or the opposite trait in meaning aggressiveness)

Character of behavior	Characteristic of the trait evaluation	Evaluation, points
High gentleness	Calmness during inspection	5 –
Gentleness	Bees fly out of the honeycomb in small numbers	4 –
Moderate gentleness	Bees fly out of the honeycomb and attack the beekeeper	3 –
Gentleness is insignificant; aggressiveness is significant	Bees sting moderately	2 –
Gentleness is absent, aggressiveness is high	Bees overreact to beekeepers' actions and sting a lot.	1–2 –

Table 4.

Taking into account the factor of the nature of the sources of nectar

Main sources of nectar	Type of nectar source	Evaluation*, points
Sunflower	monofloral	5
Rapeseed	monofloral	5
Acacia	monofloral	3–4
Linden	monofloral	3–4
Buckwheat	monofloral	3–4
Phacelia	monofloral	4–5
Miscellaneous grasses, gardens	polyfloral	3–4
Other	polyfloral	1–2
None	none	0

*Note: * – the applied scale system corresponds to the conditions where the studied apiary is located.*

Table 5.

Auxiliary "weighting" coefficients specify the flight activity feature and the weather conditions factor

Period of the day during which the observation is carried out	Time period, hours	Weighting factors
Morning	6.00–10.00	0.286
Noon	10.00–16.00	0.429
Evening	16.00–20.00	0.286

The images of the wings were processed using the TpsDig2 software. The experimental data array was prepared using the previously described method (Yarovets et al., 2023). Drone wing classification was conducted using eight traits: Ci, Dbi, Disc.sh, Pci, Ri, Ci.3, Ci.2.1, and Ci.2.2, through discriminant analysis and the STATISTICA software suite. These traits are accepted as the main identifiers of phenotype clusters for wings. The affiliation of drone wing phenotypes to the subspecies *A.m.carnica*, *A.m.caucasica*, or Ukrainian bee populations ("UkrBee" and "UkrStep") was determined by comparing them with available morphometric templates (Galatiuk et al., 2024; Babenko et al., 2024). The similarity of wing phenotypes to the templates was established using Mahalanobis distance (MD) values and an empirical similarity scale, as proposed by Galatiuk et al. (2024): if MD values range from 0 to 2, the similarity is considered high; 2–2.6 – moderate; 2.6–3.5 – low; >3.5 – absent.

Results. Season 1. 2020. At the beginning of the study, in spring 2020, morphometric analysis of drone wings was performed for 20 colonies. The affiliation of the phenotypes from each of the 40 wing clusters to the corresponding subspecies or populations was established. Based on the obtained information, assumptions were made regarding the subspecies and population origin of the queens' genome chromosome sets. Among them, 14 clusters (35%) belonged to the *A.m. carnica* subspecies, "Carnica" population; 6 (15%) – Peschetz line; 4 (10%) – Sklenar; 2 (5%) – Troiseck; 7 (17.5%) – Ukrainian bees of the "UkrBee" population; 7 (17.5%) – unknown. Considering that the predominant "breed" (65%) in the apiary was *A.m.carnica*, it was decided to concentrate efforts on obtaining such a composition of the apiary, where two types of bees would prevail: "Carnica" and the Peschetz line. The drone wing phenotypes of "Carnica" were identified based on the study of colonies with queens purchased from breeders in the Zakarpattia region of Ukraine. For this purpose, Queen No. 14 was selected as the initial "founder." The morphological characteristics of the drone wings produced by this queen are presented in Table 6 and Figure 1.

Identification of subspecies and population affiliation of drone wing clusters was carried out based on MD values (Table 7) and the results of discriminant data analysis (Fig. 1).

Table 6.

Morphological characteristics of the phenotypes of two drone wing clusters for colonies No. 14 and No. 22 (Cluster 14.1 belongs to the "Carnica" population; clusters 14.2, 22.1, and 22.2 belong to the A.m. carnica subspecies, Peschetz line; Means – average, SE – standard error)

Indices	**Cluster 14.1. Means±SE**	**Cluster 14.2. Means±SE**	**Cluster 22.1. Means±SE**	**Cluster 22.2. Means±SE**
Ci	1.869±0.04	1.809±0.03	2.028±0.05	1.894±0.042
Dbi	1.026±0.007	0.903±0.007	0.865±0.008	0.892±0.008
Pci	2.868±0.015	3.051±0.016	3.079±0.016	3.035±0.017
Disc.sh.	3.381±0.136	0.951±0.157	2.774±0.229	1.179±0.213
Ri	1.565±0.006	1.514±0.008	1.509±0.009	1.49±0.009
Ci.3	1.563±0.014	1.698±0.016	1.615±0.019	1.657±0.016
Ci.2.1	1.98±0.016	1.654±0.009	1.65±0.013	1.659±0.012
C.2.2	3.638±0.045	4.708±0.071	4.064±0.117	4.523±0.094
Number of wings	83	96	50	61

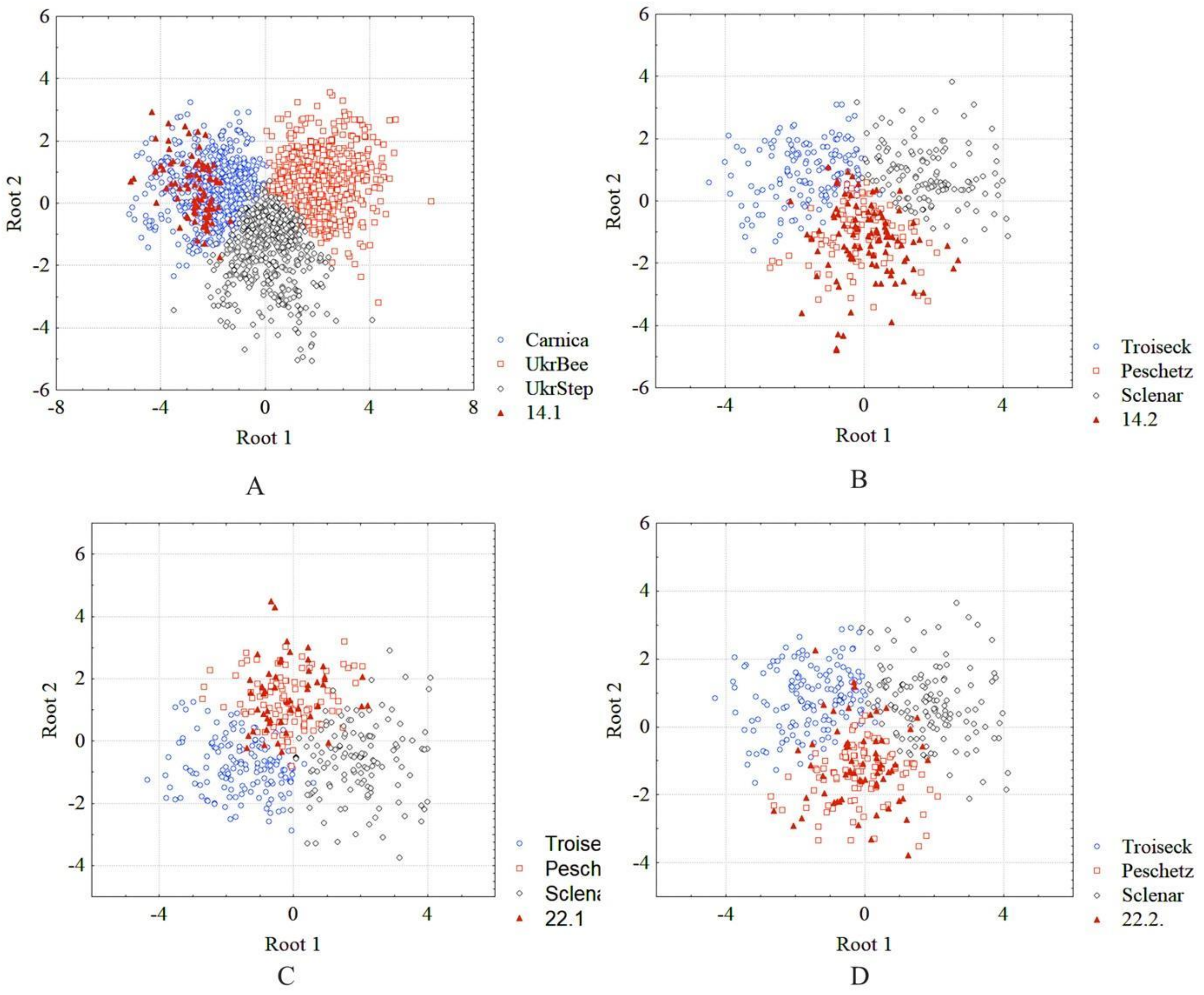

Fig.1** **Location of centroids of templates: "Carnica," "UkrBee," "UkrStep," "Troiseck," "Peschetz," "Sklenar" and centroids of clusters of drone wings of colonies No. 14 (A, B) and No. 22 (C, D) in the space of canonical variables; designations 14.1, 14.2 and 22.1, 22.2 correspond to clusters of drone wings 1 and 2, respectively; experimental data for templates are taken from the works: (Galatiuk et al., 2024; Babenko et al., 2024).

Table 7.

Mahalanobis distances (MD) between the centroids of two wing phenotype patterns of the subspecies A.m.carnica: "Carnica," "Peschetz," and four clusters of drone wings of colonies No. 14, No. 22

Templates**	"Peschetz"	22.2*	14.2*	22.1*	14.1*	"Carnica"
"Peschetz"	0	0.84	1.44	1.75	>2.60	>2.60
22.2	_	0	0.74	1.92	>2.60	>2.60
14.2	_	_	0	2.5	>2.60	>2.60
22.1	_	_	_	0	>2.60	>2.60
14.1	_	_	_	_	0	2.08
"Carnica"	_	_	_	_	–	0

*Note: * – designations 14.1, 14.2, and 22.1, 22.2 correspond to drone wing clusters 1 and 2, respectively; ** – experimental data for the templates are taken from the works (Galatiuk et al., 2024; Babenko et al., 2024).*

June 15, 2020, infertile F1 queens (daughters) were obtained from Queen No. 14 and used to replace all previous queens in the apiary. On July 14, the fertile F1 queens began laying eggs. At that time, each of the 39 colonies received one drone comb to collect drone wing samples later. Typically, young queens were isolated for 4 days in a single-frame isolator made of a Hahnemann grid on a drone comb. Drone wing morphometry was performed in January 2021. The results showed that for 23 queens, one of the two chromosomal sets belonged to the Peschetz line (type – (b)), for 24 –"Carnica" (type – (c)), for five queens – both sets corresponded to type (c/c), for 8 – (b/c), for 15 – (b/x), and 11 – (c/x) (where (x) indicates chromosomal sets off another type). Given a total of 78 chromosomal sets examined, the statistical distribution was: type (c) – 37.2%, type (b) – 33.3%, and type (x) – 29.5%. F1 queens are expected to produce these proportions of drones in the following season.

Season 2. 2021. In order to replace all old queens with new ones, the apiary used fertile queen (F1) No. 22 with its own genome type (b/b) (Table 6). The operation was carried out in May 2021 by replacing old queens with queen cells. After the F2 queens began laying eggs, drones were obtained, and it was established that in 38 colonies, drone wing phenotypes corresponded to types (b) or (c). It was also determined that in these 38 colonies, the genome composition of 22 queens matched type (b/b) and 16 matched (b/c). Colonies in which drones with phenotype (x) were found were excluded from further work and research. Thus, the predicted proportion of drones corresponding to the "Carnica" and Peschetz types of the *A.m.carnica* subspecies on the apiary was 38/50 = >0.76 (76%). On July 5, all queens on the apiary were again replaced with infertile F3 queens obtained from a newly selected queen with a (b/c) genome.

Season 3. 2022. Drone wing morphometry was performed for 42 colonies with fertile F3 queens, resulting in 35 colonies being selected for further study. Morphometric studies of worker bee wings were selectively conducted. However, it was impossible to use the data obtained for worker bees due to the lack of the necessary morphometric templates. As a result, out of 35 colonies, based on a preliminary approximate evaluation of economically useful traits and the queens' own genome composition according to the morphology of drone wings, 24 colonies were selected to form two groups, each consisting of 12 colonies considered to be analogs.

Season 4. 2023. During the season, testing was performed on two economic and functional characteristics (EUT): gentleness and flight activity for 10 and 9 colonies, in groups of colony analogues No. 1 and No. 2, respectively (from the initially selected 24 colonies, five did not survive the winter). Based on a re-analysis of worker bee wing phenotypes, two colonies did not meet the "purebred" condition. Therefore, the final test data analysis was conducted using statistical methods for 9 and 8 colonies in analog groups No. 1 and No. 2, respectively.

Since the distribution pattern of the weighted flight activity data does not statistically differ from a normal distribution ($\chi^2 = 3.83$, $df = 3$, $p = 0.28$), colony test data were analyzed using analysis of variance (ANOVA), which allows the study of flight activity's dependence on one or more qualitative factors. Gentleness was measured on an ordinal scale, so its relationships with other qualitative traits were analyzed using nonparametric statistical methods.

Figure 2 shows the dependence of flight activity on weather conditions and the presence of nectar flows, Figure 3 shows the dependence of gentleness on weather conditions, and the presence of nectar flows, and Figure 4 shows the dependence of flight activity and gentleness on queen genome types.

Table 8.

Example of data for a single colony obtained during Season 4

Analogue groups	Types of queens' own genomes *	Flight activity **	Gentleness	Weather conditions **	Availability of nectar sources	Date of inspection
No.2	"4/4"	1.859	2	2.145	1	15.04.2024
No.2	"4/4"	2.002	3	3.146	3	25.04.2024
No.2	"4/4"	2.288	4	3.003	4	17.05.2024
No.2	"4/4"	2.574	4	2.86	4	28.05.2024
No.2	"4/4"	2.288	4	1.859	4	11.05.2024
No.2	"4/4"	1.859	5	1.573	4	10.06.2024
No.2	"4/4"	3.289	4	3.575	2	15.07.2024
No.2	"4/4"	0.858	3	0.858	3	26.07.2024
No.2	"4/4"	1.716	2	2.288	2	13.08.2024
No.2	"4/4"	0.858	2	1.43	1	10.09.2024

*Note: * – the "4/4" designation corresponds to the probable composition of diploid sets in worker bees: (b)/(b), (c)/(c), and (b)/(c); ** – values recalculated with consideration of weighting coefficients (Table 5).*

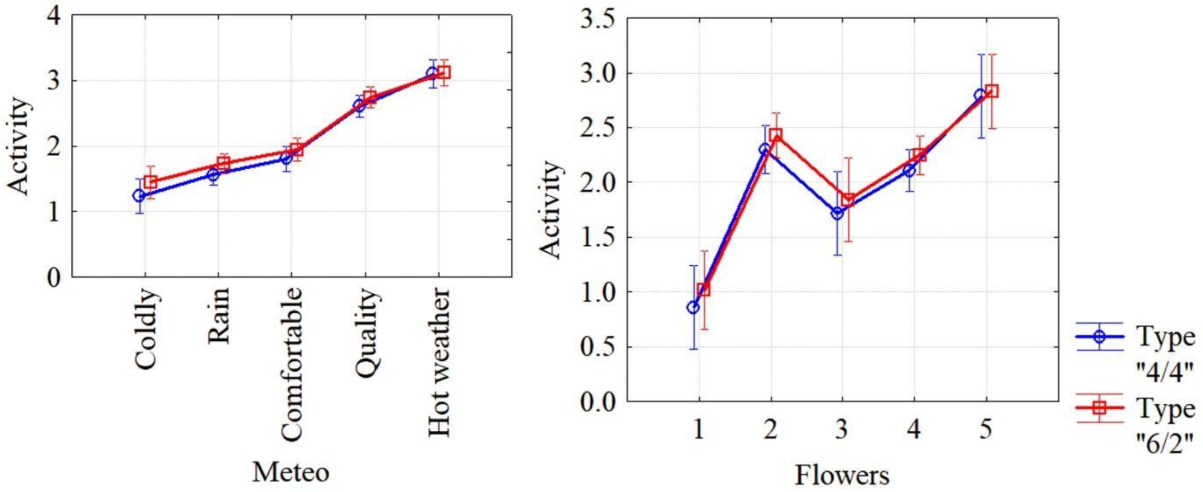

Fig.2 Relationships between two external factors—weather conditions (denoted as "Meteo"), the presence of nectar flows (denoted as "Flowers")—and flight activity (denoted as "Activity"); weather conditions were evaluated using qualitative indicators: "Hot weather", "Quality", "Comfortable", "Rain", and "Cold", respectively.

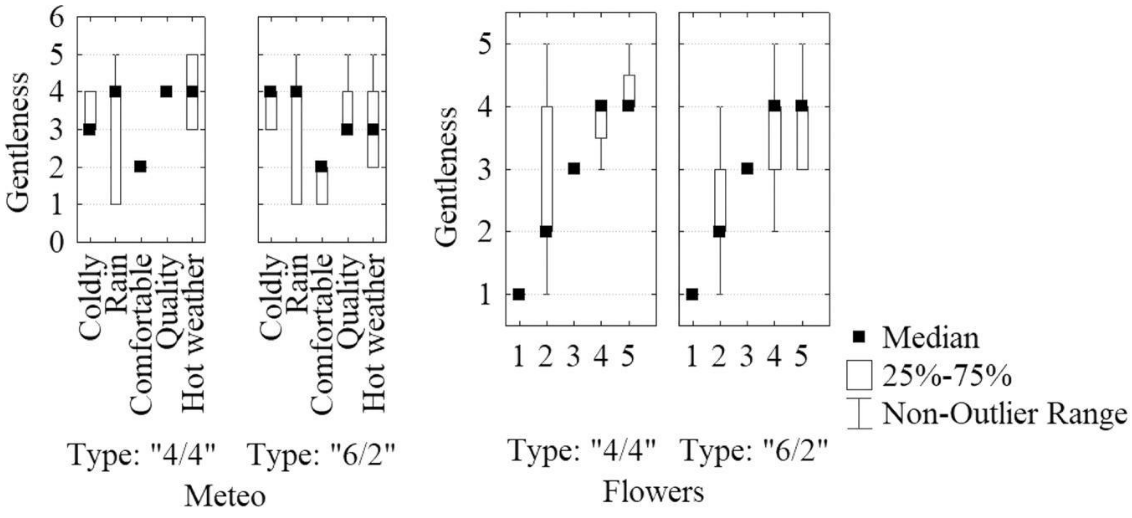

Fig.3 Relationships between three determining factors—weather conditions (denoted as "Meteo"), queen genome types, the presence of nectar flows (denoted as "Flowers")—and gentleness ("Gentleness"); weather conditions were evaluated using qualitative indicators: "Quality", "Comfortable", "Rain", and "Cold", respectively. Queen genome types are labeled as "6/2" and "4/4".

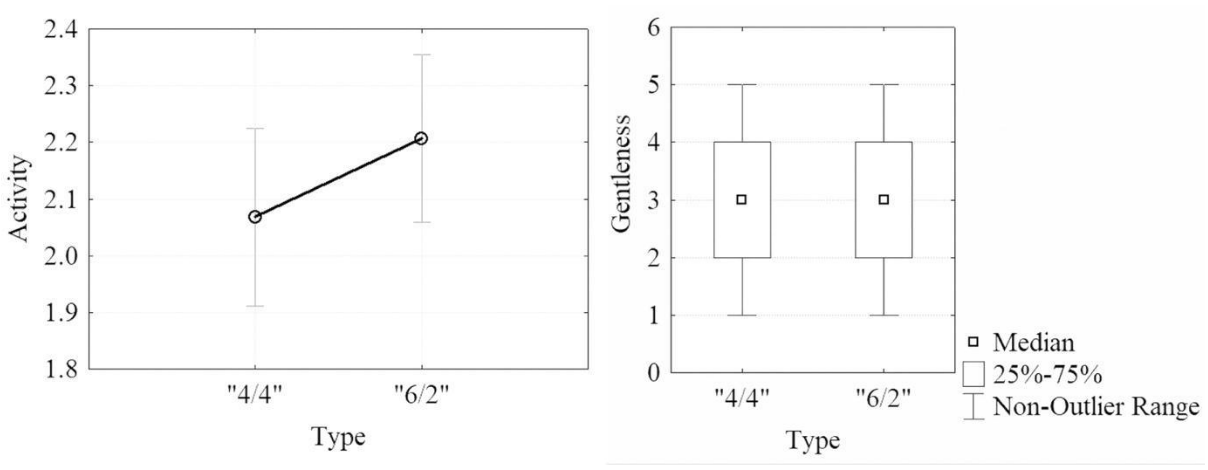

Fig.4. Dependencies between queen genome types, on the one hand, and flight activity (Activity) and gentleness (Gentleness) on the other

Discussion. The objective of the work in Season 1 (2020) was to determine the probable "breed origin" of the chromosomal sets in the queens for a portion of the colonies present in the apiary and to select one founder queen. Here and further in the text, when we talk about own genomes, we mean the probable subspecies affiliation of haploid and diploid genomes partly responsible for the wing phenotype.

Analysis of the data in Table 7 shows that wing clusters 14.1 and 14.2 phenotypes differ significantly according to the MD values. This is understandable, as they are identified as different types: "Carnica" and Peschetz (Table 6, Fig. 1); however, both reliably belong to the subspecies *A.m.carnica*. The chromosomal sets of the own genome of the queen from colony No. 14 are designated as follows: type (c) corresponds to the drone wing phenotype of "Carnica" (cluster 14.1), and type (b) corresponds to the Peschetz line (cluster 14.2). Thus, the chromosomal sets are labeled using the same symbols as the phenotypes they correspond to: (b), (c), and (x) for others. Possible combinations of chromosomal sets for the queens' genomes are designated as (b/b), (c/c), and (b/c). Considering that the composition of haploid chromosomal sets in the spermatozoa stored in the spermatheca of this queen may approximately correspond to the ratio (b+c)/(x)=70%/30%, it was assumed that the vast majority of genomes in worker bees and infertile queens produced by this queen would correspond to the chromosomal combinations: (b/b), (c/c), and (b/c) (Fig.5), and to a lesser extent — (b/x) or (c/x). Combinations with other chromosomal types are not shown in the figure.

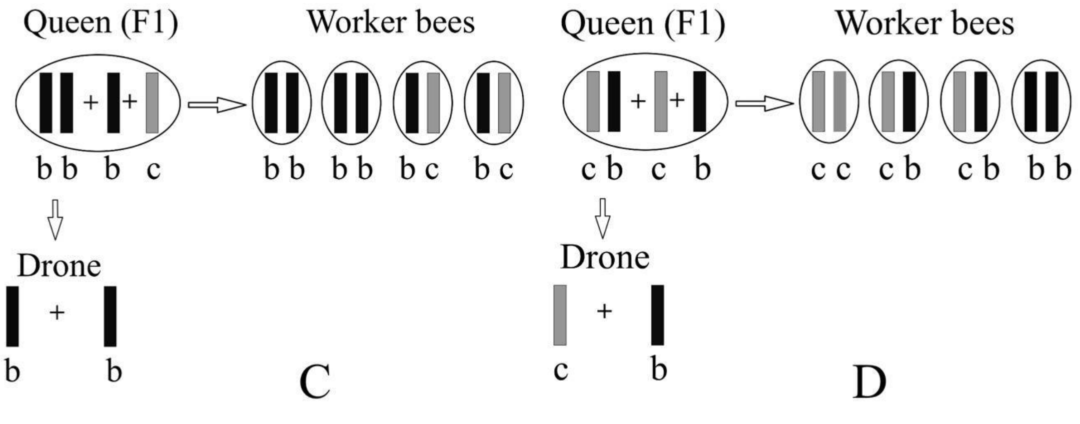

Fig.5 Schemes of two possible variants (C and D) of combinations of chromosome sets of genome compositions of worker bees, infertile queens (F2) (Worker bees), and drones (Drone) producing fertile queens (Queen (F1)) with own genomes of type (b/b) and (b/c). The effect of crossover is ignored

The objective of the work in Season 2 (2021) was to obtain a sufficient number of colonies headed by queens whose own genomes consist exclusively of chromosomal sets belonging to the "Carnica" (c) and Peschetz (b) types of the subspecies *A.m. carnica*. For this purpose, a fertile queen (F1) No. 22 with its genome of the (b/b) type (Table 6) was used to replace all old queens in the apiary. This operation was carried out in May 2021 by replacing the old queens with queen cells.

The goal of the work in Season 3 (2022) was: a) to obtain morphometric reference samples of worker bee wings for all possible combinations of genomic chromosomal sets: (b/b), (c/c), (b/c), (b/x), (c/x), (x/x); b) to form two groups of analogs for testing on two economically advantageous traits, namely: flight activity and gentleness. Given that all phenotypic traits of bee colonies largely depend on the composition of the worker bees, and since the work to obtain colonies of the desired breed composition in the apiary was nearly complete, it became necessary to account for the composition of the diploid genomes of the worker bees. To achieve this, morphometric studies of worker bee wings were conducted. Unfortunately, it was not possible to reliably establish a correspondence between the identified wing phenotypes and the worker bee genomes of the types (b/b), (c/c), and (b/c). This is the subject of a separate study that requires considerable additional effort to create morphometric standards for the wings of inter-population and inter-lineage hybrid worker bees. Therefore, to analyze the results of worker bee wing classification for each colony (from the group of 35 colonies), we applied a "reverse inference principle". If the classification of worker bee wings for a particular colony reveals the presence of two distinct phenotypes in a 50%/50% ratio (an example is shown in Figure 6. Colony: 17.(23)), then, according to Figure 5.C, we assume that the queen's own genome belongs to the (b/b) type. If the classification reveals the presence of three worker bee wing phenotypes in a 2/1/1 ratio. In that case, this corresponds to the classification shown in Fig. 6. Colony: 21.zd.(23). In this case, we assume that the queen's own genome belongs to type (b/c) (Fig.5.D).

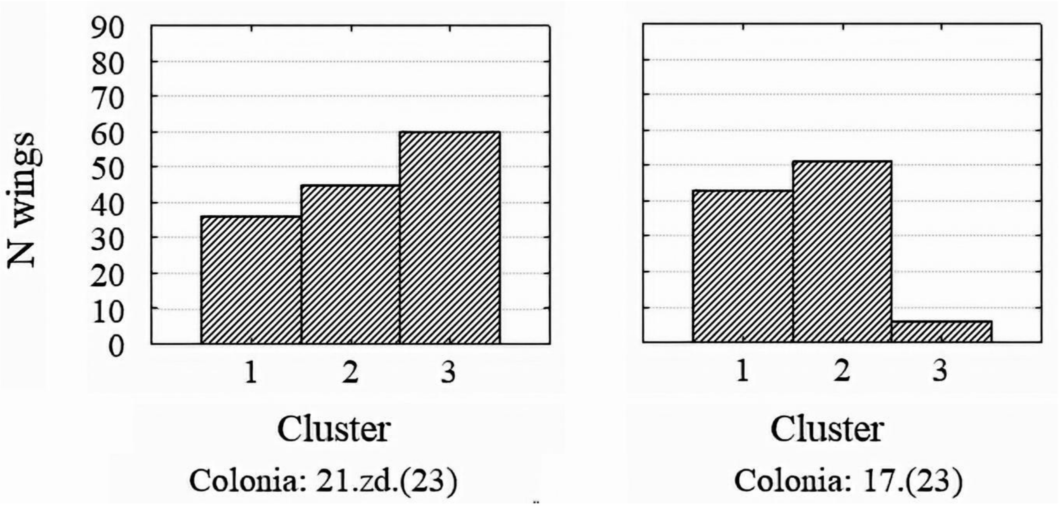

Fig.6. An example of wing classification of worker bees into three clusters for two colonies

There is an essential caveat in using morphometry of worker bee wings. If, during the insemination of virgin queens, the 50%/50% ratio between the two types of sperm components is disrupted, it will lead to changes in the proportions of wing types among the worker bees. This significantly complicates the analysis of morphometric results and increases the risk of errors (not to mention the possibility of the sperm containing other components, which is quite realistic). Such a deviation of experimental data from theoretical expectations is observed in Fig.6 for colony 21.zd.(23). Therefore, we believe that the advantage in establishing the reliable “breed identity” of queens and their genomes belongs to drone wing morphometry. However, because the use of drone wings is complicated by the need to have drones present at the right moment and requires more research time, the use of worker bee wing morphometry to determine queen “breed identity” is feasible at the current research stage and may be more appropriate in the long term.

The objectives of the 2023 Season 4 study were: a) to perform trait testing in groups of colonies of analogs; b) to conduct additional morphometric studies of worker bee wings in the tested colonies; c) to attempt to establish the relationship between queen genome composition and economically advantageous traits (EUTs).

We acknowledge that it is impossible to guarantee the "purity" of sperm in queen spermathecae using worker bee wing morphometry. However, it is highly probable that under the proposed propagation method, most drone sperm chromosomal sets in spermathecae will belong specifically to types (b) and (c). Then, the diploid genomes of most worker bees will match the following combinations: for a queen with genome (b/b): (b/b) and (b/c), in a 50%/50% ratio (similarly for queens with (c/c): (c/c) and (b/c)); for a queen with genome (b/c): (b/b), (c/c), and (b/c), in a 25%/25%/50% ratio. We denote the genome type (b/b) as "6/2" and (b/c) as "4/4" (numbers indicate the total number of chromosomal sets of types (b) and (c) in worker bee genomes, respectively). It was found that the proportion of such colony types in the group of colonies of analogues №1 is: N("6/2")/N("4/4")=5/4=55.6%/44.4%, and N("6/2")/N("4/4")=4/4=50%/50% – for group №2. According to the results of the analysis of the data presented in figure 2, no differences were found between the studied economically useful traits in groups No. 1 and No. 2. (flight activity: $\chi^2 = 7.77$, $df = 6$, $p = 0.28$; gentleness: $\chi^2 = 7.33$, $df = 4$, $p = 0.12$). This result is expected given the small distance (10 km) between apiary locations and comparable environmental conditions.

Using analysis of variance (ANOVA), a dependence of flight activity on weather conditions (Fig.2 Meteo) and nectar source availability (Fig.2 Flower) was established: Fisher statistics: F=80.012, $p<10^{-4}$ and F=31.932, $p<10^{-4}$, respectively. Flight activity for both genome types increases predictably with better weather. The most significant difference between them is observed under the poorest weather conditions. As the weather improves, the difference in flight activity decreases. There is virtually no difference between the two genome types regarding the "flight activity/nectar availability" relationship (Fig.3 Flower). Without nectar, flight activity is the lowest, which is logical. The next section of the graph, corresponding to medium nectar availability ("Flowers" values: 2–4), shows complex flight activity dynamics. From practical experience, flight activity is low even under excellent weather when nectar is absent. Thus, flight activity is influenced by the cumulative effect of weather and nectar availability. However, due to limited data, assessing the combined effect of both factors was impossible. Flight activity sharply increases and exceeds all other levels at high nectar availability. Notably, genome type "6/2" tends to show higher flight activity than "4/4" regarding weather and nectar base quality.

A dependence of gentleness on weather conditions was identified (Kruskal-Wallis statistic: H=19.6, p=0.0002) and on nectar availability (H=82.9, $p<10^{-4}$) (Fig.3 Meteo). Spearman's rank correlation confirms the relationship of EUTs with environmental factors: flight activity vs. weather (ρ=0.76, $p<10^{-4}$), flight activity vs. nectar availability (ρ=0.42, $p<10^{-4}$), gentleness vs. nectar availability (ρ=0.67, $p<10^{-4}$). There is no rank correlation between gentleness and weather. However, for both genome types, a notable reduction in aggression is observed under comfortable weather.

The complex relationship between gentleness and weather (Fig.3 Meteo) can be explained: under cold conditions ("Rain" or "Coldly"), bee activity in the hive and flight is low, so the reaction to external interference is minimal—hence, low aggression—as the weather improves (higher temperature, mild cloudiness—"Comfortable"), hive activity increases. If nectar sources are still scarce, the reaction to interference increases, reflected as a peak in the graph. Further improvement in weather (sunny, 20–25°C—"Quality") with some nectar sources reduces aggression and increases gentleness as bees focus on foraging. The final graph represents ideal weather (sunny, 25–30°C—"Hot weather") aligning with peak nectar flow when bees entirely focus on collecting nectar for winter and show the least reaction to disturbance.

Data in Fig.3 Flowers show gentleness increases for both genome types with improved weather. There is no difference in graph trends between "6/2" and "4/4" (Fig.4). The absence of differences is supported by the Mann-Whitney statistic: $U = 3125.5$, $p = 0.14$. The inability to establish discrepancies between colony types may be due to insufficient sample size. In practice, a minimum of 100 colonies should be studied.

Figure 3 shows that the flight activity of bees with queen genome "6/2" is higher across weather conditions. This is supported by Fisher's statistic (F=3.94, p=0.049). A similar trend is seen for most nectar availability levels, though not statistically significant overall (Student's T=1.27, p=0.21). The observed relationships between two economically advantageous traits(EUTs) and the composition of the queens' genomes can be perceived as a trend that requires further research and setting up an experiment on a larger scale to achieve the necessary reliability. However, even with such a conclusion, it is possible to make suggestions to improve the necessary economic and functional characteristics (EUT): to enhance flight activity, queens with genome type (b/b) — "6/2" — should be used. Such queens produce two types of bees, (b/b) and (b/c), making up 75% of chromosomal sets of type (b) in worker genomes. Given the gentleness-weather dependency (Fig.3 Meteo), preference should go to queens with genome (b/c)—type "4/4"—which produce three bee genome types: (b/b), (c/c), and (b/c), in a 1:1:2 ratio. For such colonies, the total share of (b) and (c) chromosomal sets in worker genomes is 50%.

This study's results highlight the potential of bee wing morphometry, especially of drones, in improving selection quality and predictability. However, there are "weak points" in the study, particularly in reliably identifying subspecies origin based on drone wing phenotypes and the haploid chromosomal sets they reflect. Using MD (Mahalanobis distance) values helps determine phenotypic similarity to reference patterns. However, DNA analysis is needed to confirm established "breed identity." The study's length (2020–2023) is a limiting factor in honeybee breeding. Poor apiary isolation forced extra work to eliminate queens with genomes from other subspecies. Out of 50 colonies, only 17 were usable. It is necessary to pay attention to the absence of such an essential characteristic as honey productivity in the list of studied characteristics. However, considering that the work of the apiary as a whole is aimed at selling packages, and obtaining honey was not a priority, it is possible to understand why this characteristic was not tested. The authors used a relatively simple approach, where phenotyping by EUTs traits is carried out mainly as a function of the composition of the chromosome sets of the queen's own genome and drone sperm in the spermatheca. However, given that the properties of a colony are largely determined by worker bees, it is still necessary to investigate the breeding value of a colony, apiary, or population in a broader sense. To achieve this, it is necessary to determine the ratio of worker bee groups with different types of diploid genomes within the colonies. From this point of view, it becomes clear that this was not done in this study. It was assumed that the proportions of the two types of sperm in queens were equal and, therefore, that there was a proportional ratio of the types of worker bees in colonies. This is unlikely to happen in natural mating. Therefore, to obtain more accurate and reliable results, it is essential to perform wing morphometry of worker bees for each colony and to determine the actual ratios between groups of bees with different genome types. This approach allows us to quantitatively assess the interaction of different combinations of chromosome sets and establish relationships between specific genomes of worker bees in line with economically valuable traits. The queens' chromosome sets indicate the genetic potential, while the genome composition of worker bees reflects how this potential is expressed. By analyzing the work's results, you can find other problematic points that require attention and resolution.

For the experiment, colonies with queens whose genomes consisted of significantly heterozygous chromosome sets of the (b/c) type were specifically used. As expected, this approach made the manifestation of the heterosis effect noticeable, allowing it to be recorded with the necessary accuracy and contrast. At the same time, in 2021–2022, efforts were directed at finding queens with significantly homozygous chromosome sets of their genome of the type (b/b) or (c/c). Queens with their genomes can be considered conditionally inbred. They are undesirable to use but can consolidate properties over generations. For this purpose, infertile queens were bred each subsequent year, replacing all previous queens. The order of replacement with queens with genomes of type (b/b) or (c/c) alternated with type (b/c). The queens' genomes were used to produce infertile queens and were monitored using drone wing morphometry. A by-product of the research was the acquisition of data arrays of morphometric traits (eight indices): Ci, Dbi, Disc.sh, Pci, Ri, Ci.3, Ci.2.1, Ci.2.2 for drones of the types "Carnica" and Peschetz, which served as local morphometric templates for the given apiary.

The study applied a "closed" breeding system, which reduces genetic diversity but enables faster fixation of desired traits at the genetic level. However, due to the insufficient isolation of the apiary, the "closed" breeding system, in this case, is somewhat conditional. Instrumental insemination would significantly improve the reliability and accuracy of achieving the desired composition of queen and worker bee genomes (Plate et al., 2019; Du et al., 2023). We believe it is advisable to implement this practice at dedicated apiaries located within the distribution area of a single population, with the goal of further using such queens in an "open" breeding system within that population. At this stage, natural queen fertilization likely has its advantages. After all, natural selection, despite its lack of controllability, always leads to greater species resilience to both old and new environmental challenges.

Beekeepers are well aware that changes in weather conditions during the day, or the beginning and end of nectar secretion by melliferous plants, significantly affect flight activity and, to a lesser extent, bee aggressiveness. Therefore, if accurate data for these factors—recorded three times during the daytime—are obtained, it will be possible to determine the genetic basis of worker bee behavioral traits depending on environmental changes. This study did not reach such a level of depth due to lack of experience, although observations were made three times during the day. Hence, we emphasize to researchers the critical importance of meticulous attention to recording values of the traits being tested and external factors at the apiary. In addition, our experience shows the need to use many colonies for testing. Two groups of 10 colonies each are insufficient for achieving acceptable statistical reliability of data analysis results. This work is a pilot study to test the possibility of identifying the relationship between bee genotype and EUTs under the condition that the likely "breed identity" of queen and worker bee genomes is known. The use of variance analysis makes it practically feasible to implement such intentions. It was demonstrated that even under conditions of partial apiary isolation, it is possible to obtain the desired composition of inter-lineage hybrids successfully.

Since it was impossible to reliably determine the structure of worker bee genome types in the 17 studied colonies, the predicted characteristics for two EUTs (flight activity and gentleness) are only qualitatively assessed as a function of the queen's native genome composition. This limitation of the adopted model significantly affected the ability to determine the specific form of the traits of all key factors. Therefore, it is necessary to improve the morphometric research methodology to accurately determine the proportions of wing clusters among worker bees produced by each queen and to relate them to genotypes.

Conclusion. The proposed method for determining genotype–phenotype associations can support recommendations on selecting individual queens as donors. In addition to the standard practice of selecting superior queens based on EUTs, the authors recommend prioritizing breeding candidates based on the queen's genome composition and the chromosomal sets in the spermatheca.

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The requirements of the recommendations of the EU Directive 2010/63/EU on the protection of animals used for scientific purposes have been met.

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МІНІ-ЕКСПЕРИМЕНТ У СЕЛЕКЦІЇ: ГЕНОТИПУВАННЯ КОЛОНІЙ APIS MELLIFERA L. ЗА ДВОМА ОЗНАКАМИ

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На протязі 2020–2023 років проведено сплановане дослідження однієї пасіки (Україна, Львівська область), що налічує 50 колоній бджіл медоносних підвиду A.m.carnica. Оскільки ізоляція пасіки від впливу сторонніх трутнів була недостатньою, робота у 2020–2021 роках концентрувалась на досягненні більш стабільного та однорідного трутневого фону. В результаті на початок 2023 року створено дві групи колоній аналогів, королеви яких є сестрами. Ідентифікація підвидової та популяційної належності хромосомних наборів вказаних королев, здійснювалась за допомогою морфометричних шаблонів для крил трутнів. За результатами порівняння фенотипів крил з шаблонами, встановлено імовірну належність власних геномів королев до місцевої популяції «Carnica», та лінії Peschetz. Для 17 колоній протягом сезону 2023 року здійснено тестування за двома господарсько-корисними ознаками: льотної активності та лагідності. За допомогою дисперсійного аналізу даних, встановлено залежності між імовірним складом геномів королев з одного боку, та двома факторами – метеоумовами та наявністю медодаїв), а також вказаними ознаками -лагідністю, льотною активністю, з іншого. Одержані результати не мають статистичної достовірності у повному обсязі, що обумовлено недостатньою кількістю експериментальних даних. Однак, виявлені тенденції дозволяють робити конкретні пропозиції щодо подальшого використання колоній, для яких встановлено склад та імовірна належність хромосомних наборів геномів королев. Такий підхід рекомендується як додатковий інструмент, з метою підвищення ефективності у селекційній роботі.

Ключові слова: бджола медоносна, трутні, робочі бджоли, геном, хромосомні набори, класична морфометрія крил, морфометричні шаблони, господарсько-корисні ознаки, дисперсійний аналіз, дискримінантний аналіз, A.m.carnica.

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