AGRICULTURAL BIOLOGY,
2022, V. 57, Iss. 2, pp. 283-303
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 638.123:577.2

doi: 10.15389/agrobiology.2022.2.283eng
doi: 10.15389/agrobiology.2022.2.283rus

SOME PROBLEMS OF IDENTIFICATION OF HONEYBEE SUBSPECIES AND THEIR SOLUTION ON THE EXAMPLE OF STUDYING THE *Apis mellifera* IN SIBERIA

N.V. OSTROVERKHOVA[1, 2] ✉, O.L. KONUSOVA[1]

[1]*Institute of Biology, National Research Tomsk State University,* 36, prospect Lenina, Tomsk, 634050 Russia, e-mail nvostrov@mail.ru (✉ corresponding author), olga.konusova@mail.ru;
[2]*Siberian State Medical University*, 2, Moskovsky trakt, Tomsk, 634055 Russia
ORCID:
Ostroverkhova N.V. orcid.org/0000-0001-9837-4905 Konusova O.L. orcid.org/0000-0002-2140-9222
The authors declare no conflict of interests
Acknowledgements:
Supported by the Tomsk State University competitiveness improvement program
Received July 7, 2021

Abstract

Studies of the honeybee *Apis mellifera* L. are carried out by both classical morphometric and molecular methods, including whole genome sequencing. Morphometric analysis has revealed thirty subspecies of *A. mellifera* for four evolutionary lineages that corresponded to their geographical origin. mtDNA analysis, e.g., for the variability of COI-COII locus (the sequence between the cytochrome oxidase I and cytochrome oxidase II genes), has identified three major evolutionary lineages, A, M, and C. However, this method has a limitation associated with maternal inheritance of the mitochondrial genome. The honeybee does not have sex chromosomes, so information about inheritance in the paternal line (as well as in the maternal line) can only be obtained from the analysis of autosomal loci, such as SNP (single nucleotide polymorphism) and SSR (simple sequence repeats) markers. The present work, for the first time, evaluates the informativity of morphometric and molecular methods for the identification of *A. mellifera* subspecies inhabiting Siberian apiaries. We have shown that the analysis of the variability of the main parameters of the wing (cubital index, hantel index, and discoidal shift) and the mtDNA COI-COII locus accurately detect the origin of the bee colony. We also studied for the first time the genetic diversity of the *A. mellifera mellifera* Siberian populations for microsatellite loci. Diagnostic alleles specific for subspecies and ecotypes of the honeybees have been identified to differentiate the *A. m. mellifera* subspecies and its ecotypes, as well as subspecies of southern origin (Carpathian bee, Carnica). This work aimed to evaluate prospects for morphometric and molecular analysis methods in differentiation of *A. mellifera* subspecies reared in Siberia. Honeybees from 92 apiaries in 69 settlements located in five regions of Siberia (Tomsk and Kemerovo regions, Krasnoyarsk Territory, Altai Territory, and the Altai Republic) were studied. The first stage was the investigation of worker bees from 414 bee colonies using the morphometric method and analysis of the mtDNA COI-COII locus variability. The second stage was the identification of the *A. m. mellifera* colonies based on a complex of SSR markers. We examined the variability of 31 microsatellite loci. To search for unique or specific SSR markers for different honeybee subspecies, we also examined the genetic diversity of two southern subspecies, *A. m. carpathica* and *A. m. carnica* (a comparison group). Population genetic parameters (allele frequency, observed and expected heterozygosity H_o and H_e) were calculated using GenAlEx 6.5 software (https://biology-assets.anu.edu.au/GenAlEx/). Introgression of the genes of the evolutionary lineage C into the lineage M was assessed based on the microsatellite loci polymorphism data using the STRUCTURE 2.3.4 program (https://web.stanford.edu/group/pritchardlab/home.html). It is shown that three wing parameters, i.e., cubital index, hantel index, and discoidal shift, together with mtDNA polymorphism analysis data, are necessary and sufficient for differentiation of *A. mellifera* subspecies. The discoidal shift parameter is one of the first morphometric trait to deviate from the breed standard values in the honeybee hybridization. Microsatellite analysis revealed loci that differentiate both subspecies of different evolutionary lineages (M and C) and different *A. m. mellifera* ecotypes. Loci A043, Ap081, Ap049, AT139, A113, *mrjp3*, etc. can be considered as diagnostic (subspecies-specific) loci the composition and frequency of the prevailing alleles of which differ in *A. m. mellifera* subspecies (lineage M) and two subspecies of southern origin (*A. m. carpathica* and *A. m. carnica*, lineage C). In *A. m. mellifera* honeybees, alleles 128 bp at the A043 locus, 124 bp at the Ap081 locus, 127 bp at the Ap049 locus, 190 bp at the AT139 locus, 218 bp at the A113 locus, and 529 bp at the *mrjp3* occur in high frequency (0.54-0.99). In honeybees of southern origin (*A. m. carpathica* and *A. m. carnica*), these alleles are rarer (0.01-0.27). The microsatellite locus A008 is the most promising molecular

marker to differentiate *A. m. mellifera* ecotypes from Siberia, the Urals and Europe (eco-specific locus). Based on the genetic diversity of Siberian honeybees for microsatellite loci, a diagnostic panel of molecular markers has been developed to differentiate subspecies and ecotypes of honeybees belonging to the evolutionary M and C lineages (*A. m. mellifera*, *A. m. carpathica*, and *A. m. carnica*).

Keywords: honeybee, *Apis mellifera*, morphometric signs, molecular genetic methods, mtDNA, microsatellite loci, COI-COII, DNA markers, Siberia

Currently, in the study of the honey bee *Apis mellifera* L., both classical morphometric [1, 2] and molecular genetic methods [3-5], including whole genome sequencing [6], are used. Morphometry and mitochondrial DNA (mtDNA) polymorphism analysis are most commonly used to identify honey bee subspecies [1, 7, 8].

The morphometric method involves the analysis of about 40 qualitative traits (coloration of tergites, the shape of the posterior border of the wax mirror, characteristics of the hair border on the abdomen of worker bees, etc.) and quantitative (tarsal index, cubital index, area of the wax mirror, etc.) traits of honeybee [9, 10]. Based on morphometric parameters, 30 subspecies of *A. mellifera* were assigned along four evolutionary lines that corresponded to their geographical origin. These are subspecies of the African continent — the line A (African); subspecies of the western Mediterranean and northwestern Europe — the line M (Mellifera); subspecies of southeastern Europe and the eastern Mediterranean— the line C (Carnica); subspecies of the Middle East and western Asia — the line O (Oriental) [10].

The study of mtDNA polymorphism, for example, the COI-COII locus (the sequence between the genes for cytochrome oxidase I and cytochrome oxidase II), allows three main evolutionary lines, the A, M, and C to be identified [7, 11, 12]. However, mtDNA variants do not always agree with the systematics based on morphology. For example, subspecies of C and O morphological lines are characterized by the same mtDNA variant [12]. According to mtDNA analysis, the C subspecies had the shortest sequence of the COI-COII locus, or Q variant. Conversely, the M and A subspecies had a longer sequence that included at least one Q element, as well as a P element (variants PQ, PQQ, PQQQ, PQQQQ or PQQQQ) [13, 14].

However, the method has a limitation caused by the maternal inheritance of the mitochondrial genome. Since the honey bee does not have sex chromosomes, information about inheritance in the drone line (as well as in the line of the uterus) can only be obtained based on the analysis of autosomal loci, such as SNP (single nucleotide polymorphism, single nucleotide polymorphism) and SSR (simple sequence repeats, microsatellite loci) markers. Thus, microsatellite loci are widely used in honey bee studies, for example, to characterize the population genetic structure [15-17], identify hybridization between different subspecies [5, 18, 19], differentiate subspecies [20, 21] and assess the adaptive potential of bees of different origin and evolutionary lines [22-24]. However, the genetic diversity of different subspecies of the honey bee for microsatellite loci and other nuclear markers has not been studied enough, reference materials have not been developed. Based on the data of polymorphism of SNP markers in bees of 14 subspecies, groups were identified that largely correspond to morphological evolutionary lines. Additional lines of African origin have also been established (e.g., the line Y — Yemenitica, Ethiopia) [7, 25].

In Russia, due to the diversity of natural and climatic conditions, three subspecies of bees are recommended for breeding [23]: the dark forest bee, or the Central Russian breed (*Apis mellifera mellifera* L.), the Caucasian breed (*A. m. caucasica* Gorb.) and the Carpathian breed (*A. m. carpathica*, derived from *A. m. carnica* Poll. *A. m. carnica*, the Italian bee *A. m. ligustica* Spin. and the Far Eastern

bee (the product of unsystematic crossing of Ukrainian steppe *A. m. sossimai* Engel and Central Russian bees with the participation of Caucasian and Italian bees) are also bred. In Siberia, the Central Russian bee (*A. m. mellifera*) brought 230 years ago to the Tomsk province was initially reared. The Central Russian bees have adapted well to the local harsh climatic conditions and plants but the wintering of bee colonies is under human' control. Since the end of the 20th century, active importation of bees of southern origin has been observed in Siberia, which led to mass hybridization [26].

This research, for the first time, assesses the evaluability of morphometric and molecular genetic methods for the identification of subspecies of *A. mellifera* inhabiting the apiaries of Siberia. It was shown that the analysis of the variability of the main parameters of the wing (cubital index, dumbbell index, discoidal displacement) and the mtDNA COI-COII locus provides identification of the bee colony origin. This is the first study of the genetic diversity of the Central Russian bees from Siberian populations using microsatellite loci. Breed- and eco-specific diagnostic alleles have been identified, which make it possible to differentiate the Central Russian breed and its ecotypes, as well as subspecies of southern origin (Carpathian breed, carnica).

The purpose of this work is to evaluate the information content of morphometric and molecular genetic methods for the identification of subspecies of *Apis mellifera* in Siberia.

Materials and methods. In the research carried out in 2008-2018, honey bees were obtained from 92 apiaries in 69 settlements located in five regions of Siberia, including Tomsk region (52 points/71 apiaries/340 bee colonies), Kemerovo region (4/5/16), Krasnoyarsk region (6/6/25), Altai Territory (6/9/31) and the Altai Republic (1/1/2). To study the breed composition of honey bees in Siberian apiaries, worker bees from 414 bee colonies were initially studied using the morphometric method and mtDNA analysis.

At the second stage, the identified bee colonies of the Central Russian breed (*A. m. mellifera*) were studied using a complex of SSR markers. The variability of 31 microsatellite loci was studied. In order to search for unique or specific SSR markers for different subspecies of the honey bee, the genetic diversity of bees of two subspecies, *A. m. carpathica* and *A. m. carnica* of southern origin, was investigated (a comparison group).

For morphometric analysis, we studied the main breed-determining parameters of the wing (discoidal displacement, cubital and dumbbell indices) and the color of tergites in 25-30 workers from each family [27]. The obtained morphometric data was compared to the breed standards [28].

DNA was isolated from the pectoral muscles of worker bees using the DNA-Extran-2 kit (ZAO Sintol, Russia) and analyzed by polymerase chain reaction (PCR) using the BioMaster HS-Taq PCR-Color reagent kit (OOO Biolabmix, Russia) on a BioRad T100 cycler (Bio-Rad Laboratories, Inc., USA).

Analysis of mtDNA COI-COII locus variability was performed using the primers F-5'-CACATTTAGAAATTCSSATTA-3', R-5'-ATAAATATGAATCATGTGGA-3' [29] and PCR protocol as follows: 5 min at 95 °C (initial denaturation); 1 min at 95 °C (denaturation), 2 min at 57 °C (primer annealing), 2 min at 72 °C (chain elongation) (35 cycles); 7 min at 72 °C (final elongation). PCR products were separated in 1.5% agarose gel and analyzed using Image LabTM Software (Bio-Rad Laboratories, Inc., USA). In the Central Russian honey bees, the COI-COII locus contains the PQQ and PQQQ alleles, having sizes of 600 and 800 bp, respectively, while the breeds of southern origin (Carpathian breed, carnica, etc.) had the Q allele 350 bp in size. Five workers from each bee colony were

tested.

For SSR analysis, the previously described primer sequences and amplification conditions were used [30-32]. The variability of microsatellite loci A008, Ap049, A043, AC117, Ap243, H110, A024, A113, SV185, Ap066, Ap081, A088, A007, A028, 6339, SV220, K0457B, K1168, Ap033, K0820, Ap06, K1615, K0711, SV167, Ap249, Ap226, A056, AT139 and *mrjp3* was studied. One primer from each pair had a fluorescent label. Genotyping was performed on an ABI Prism 3730 genetic analyzer (Applied Biosystems, Inc., USA; Medical Genomics Center for Collective Use, the Research Institute of Medical Genetics of the Tomsk Scientific Research Center RAS). The GeneScan500-ROX DNA length standard was used under the conditions recommended by the manufacturer. Fragment size was analyzed using GeneMapper Software (Applied Biosystems, Inc., USA). In each sample of bees, a minimum of 27 and a maximum of 534 workers were studied for definite microsatellite loci. Microsatellite analysis was not performed for loci A028, A088, A056, K0711, Ap068, K1615, and Ap249 in bees from the Altai population and for loci Ap033 and SV167 in bees from the Tomsk population.

Population genetic parameters (allele frequency, observed and expected heterozygosity, H_o and H_e) were calculated using GenAlEx 6.5 (https://biology-assets.anu.edu.au/GenAlEx/) [33]. Statistically significant differences between observed and expected heterozygosity were identified using Student's *t*-test. The degree of introgression of genes of the evolutionary line C (Carpathian breed *A. m. carpathica* and Carnica *A. m. carnica*) in the nuclear genome of Central Russian bees (evolutionary line M) from different populations of Siberia was assessed according to the polymorphism of microsatellite loci using STRUCTURE 2.3.4 (https://web.stanford.edu/group/pritchardlab/home.html) based on Bayesian analysis. The Monte Carlo Markov Chain (MCMC) clusterisation was used for a given number of clusters K = 2, K = 3 and K = 5 using the admixture model and 500,000 MCMC iterations [34]. Preliminary data processing was carried out in Microsoft Excel 2010; statistical calculations were performed in StatSoft STATISTICA 8.0 for Windows (StatSoft, Inc., USA). The tables show mean values (*M*) and standard errors of means (±SEM).

Results. According to the data obtained in the study of morphometric parameters and the mitochondrial genome of honey bees, the majority of bee colonies living in Siberia were represented by hybrids of Central Russian and Carpathian breeds (Fig. 1). Moreover, more than 60% of families on the maternal side descended from the Central Russian bee, according to mtDNA analysis data [35].

A pronounced heterogeneous pattern was observed in regions with well-developed beekeeping (Kemerovo region, southern regions of the Tomsk region, Altai Territory), where bees of southern origin (mainly Carpathian breed, carnica) and hybrids are actively imported. For example, in the Kemerovo region, in most apiaries, hybrids of the Central Russian bee and breeds of southern origin were found, but there were also commercial apiaries where breeds of southern origin were reared (*A. m. carpathica*, *A. m. carnica*). We did not find apiaries where purebred Central Russian bees were bred.

In more remote regions of Siberia (the northern regions of the Tomsk region, the Krasnoyarsk Territory, the Republic of Altai), territories were found with a homogeneous array of bees descending from the Central Russian bee *A. m. mellifera*. Of considerable interest were the populations of the Central Russian breed in the Krasnoyarsk Territory (Yenisei population), in the north of the Tomsk region (Ob population) and in the mountainous regions of the

Altai Republic (Altai population), as well as some commercial apiaries in the Altai Territory (Zmeinogorsky and Charyshsky districts) where *A. m. mellifera* was reared (see Fig. 1).

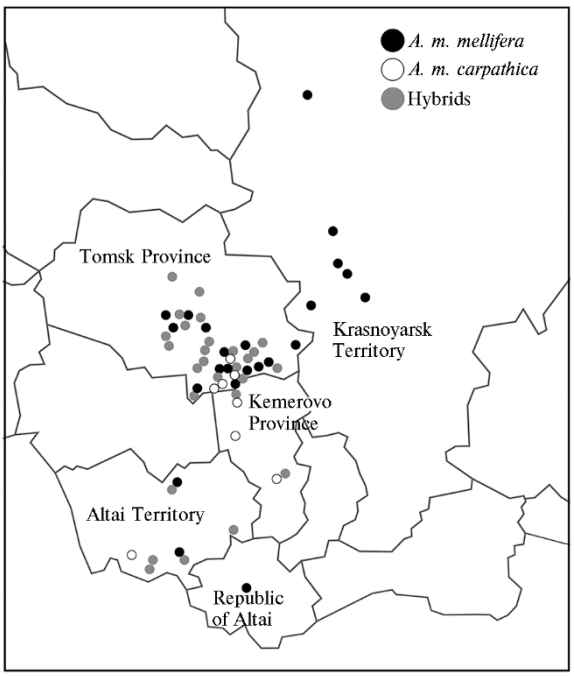

Fig. 1. Distribution map of subspecies *Apis mellifera mellifera* and *A. m. carpathica*, as well as hybrids between these subspecies in the apiaries of the Siberian region of the Russian Federation according to the morphometric study and mtDNA analysis (2008-2018).

Table 1 shows the main parameters of the wing and the variant of the mtDNA COI-COII locus in bees from 34 purebred and hybrid families from Siberia. For some families of the Central Russian breed from isolated apiaries (e.g., family No.3 from the apiary in the village of Ostyatskoe and family No. 1 from the apiary in the vicinity of the village of Turukhansk, Krasnoyarsk Territory; family No. 1 from the apiary in the vicinity of the village of Ongudai of the Altai Republic), a deviation is shown from the breed standard for the cubital index. This may indicate in favor of genetic drift as a result of long-term isolation of families. At the same time, the deviation of some morphometric parameters from the breed standard, for example, in colonies from the apiary of the village of Zarechny (the presence in the family of 27% of individuals with a neutral discoidal displacement), could indicate the influence of subspecies of southern origin (see Table 1). The apiary in the village of Zarechny is located in the southern region of the Tomsk region, where bees of various origins are bred in closely spaced apiaries.

Thus, Siberian honey bee populations are characterized by a complex and mosaic genetic structure. We have identified both purebred bee colonies of various origins and hybrids. Bee colonies *A. m. mellifera* can be considered as a unique material for the conservation and restoration of the Central Russian bee gene pool in Russia.

The genetic diversity of honey bees of the Central Russian breed was studied using 31 microsatellite loci. As a comparison group, we used honey bees from the families of the southern subspecies *A. m. carpathica* and *A. m. carnica*. Among the studied loci, 22 polymorphic SSR markers were selected, which are the most informative for the differentiation of subspecies and ecotypes of the honey bee (Table 2). For the remaining 9 microsatellite loci, a similar composition and frequency of alleles were noted, including those prevailing in bees of different origin (in Table 2, AC117, SV185, and H110 are given as an example of such loci).

The comparative analysis of the variability of microsatellite loci in Central Russian, Carpathian and Carnica bees, differences in the composition and/or frequency of alleles between subspecies were found for some SSR markers.

1. Morphometric parameters and variant of the mtDNA COI-COII locus in bees (*Apis mellifera* L.) of some purebred and hybrid families living in Siberia (2008-2018)

Locality	Family No.	mtDNA COI-COII locus	Cubital index		Dumb-bell index		Discoidal shift, %		
			min-max	*M*±SEM	min-max	*M*±SEM	–	0	+
Central Russian bees (Tomsk population)									
Settlement Chalkovo	1	PQQ	1.30-2.00	1.75±0.04	0.720-0.871	0.811±0.010	100.0	0.0	0.0
	2	PQQ	1.32-2.00	1.57±0.04	0.712-0.851	0.789±0.010	100.0	0.0	0.0
Village Teguldet	1	PQQQ	1.44-2.10	1.75±0.03	0.692-1.000	0.854±0.011	100.0	0.0	0.0
	2	PQQQ	1.26-2.22	1.74±0.04	0.701-0.914	0.825±0.010	100.0	0.0	0.0
Village Mogochino	1	PQQ	1.36-2.00	1.73±0.02	0.693-0.923	0.821±0.006	100.0	0.0	0.0
	2	PQQ	1.57-2.82	1.88±0.04	0.770-1.000	0.855±0.015	92.0	8.0	0.0
Settlement Zarechny	1	PQQQ	1.39-2.33	1.65±0.04	0.712-0.932	0.825±0.009	73.5	26.5	0.0
	2	PQQQ	1.19-2.05	1.64±0.06	0.679-1.000	0.865±0.012	73.1	26.9	0.0
Central Russian bees (Keasnoyarsk population)									
Village Ostyatskoe	1	PQQ	1.24-2.00	1.61±0.04	0.675-0.892	0.795±0.011	100.0	0.0	0.0
	2	PQQ	1.25-1.76	1.60±0.04	0.722-0.849	0.812±0.011	100.0	0.0	0.0
	3	PQQ	1.26-1.79	1.51±0.04	0.743-0.910	0.849±0.012	97.0	3.0	0.0
Village Kolmogorovo	1	PQQ	1.32-2.10	1.60±0.05	0.724-0.900	0.820±0.009	97.0	3.0	0.0
	2	PQQ	1.28-1.76	1.58±0.05	0.716-0.919	0.820±0.012	93.0	7.0	0.0
	3	PQQ	1.28-1.86	1.56±0.04	0.746-0.985	0.810±0.011	97.0	3.0	0.0
Village Ozernoe	1	PQQ	1.02-2.00	1.62±0.04	0.746-1.000	0.845±0.011	100.0	0.0	0.0
	2	PQQ	1.45-1.95	1.65±0.04	0.768-1.000	0.867±0.010	93.3	6.7	0.0
	3	PQQ	1.35-2.05	1.65±0.04	0.716-0.951	0.806±0.010	100.0	0.0	0.0
Settlement Yaksha	1	PQQ	1.43-1.82	1.65±0.04	0.803-0.915	0.859±0.011	100.0	0.0	0.0
Village Yartsevo	1	PQQ	1.31-1.85	1.59±0.02	0.711-0.846	0.775±0.008	100.0	0.0	0.0
Village Турухансk	1	PQQ	1.15-1.89	1.52±0.04	0.779-0.919	0.849±0.011	100.0	0.0	0.0
Central Russian bees (Altai population)									
Village Baranovka	1	PQQ	1.42-2.10	1.75±0.04	0.732-0.883	0.833±0.012	100.0	0.0	0.0
The vicinity of the village Ongudai	1	PQQ	1.00-1.67	1.44±0.04	0.740-0.919	0.840±0.012	100.0	0.0	0.0

Continued Table 1

Carpathian bee breed									
Siveria, various regions	1	Q	2.20-3.40	2.80±0.10	0.919-1.208	0.972±0.009	0.0	23.3	76.7
	2	Q	2.00-3.50	2.93±0.07	0.967-1.230	1.118±0.015	0.0	0.0	100.0
	3	Q	2.11-4.10	3.12±0.10	0.905-1.205	1.048±0.015	3.4	10.4	86.2
	4	Q	1.68-3.64	2.51±0.06	0.867-1.210	1.050±0.010	4.0	20.0	76.0
	5	Q	2.00-5.56	3.41±0.02	0.905-1.310	1.125±0.016	0.0	3.0	97.0
Hybrid faminies (apiaries from Tomsk Province)									
Settlement Kurlek	1	PQQQ	1.74-3.29	2.14±0.07	0.857-1.053	0.937±0.100	32.14	57.14	10.72
	2	Q	1.30-2.29	1.66±0.04	0.735-0.965	0.878±0.011	72.40	27.60	0.00
Village Krivosheinj	1	PQQ	1.00-2.67	1.79±0.07	0.707-1.000	0.884±0.018	27.27	18.18	54.55
Settlement Sinii Utes	1	Q	1.83-2.87	2.37±0.06	0.815-1.053	0.931±0.012	6.70	76.70	16.60
Village Podgornoe	1	Q	1.35-2.87	2.19±0.10	0.797-1.100	0.910±0.010	34.30	45.70	20.00
	2	Q	1.72-3.01	2.53±0.06	0.852-1.178	0.977±0.011	51.40	14.30	34.30
The vicinity of Tomsk	1	Q	1.64-2.69	2.12±0.05	0.722-0.946	0.895±0.011	90.00	6.70	3.30
Breed standards									
A. m. mellifera [a]	PQQ/PQQQ		1.30-2.10	1.70	0.600-0.923	–	–	–	–
A. m. mellifera [b]			1.30-1.90	1.5-1.7	0.600-0.923	–	91-100	5-10	0
A. m. carpathica [a]	Q		2.30-3.00	2.65	–	0.925	0-5	0-20	80-100

Note. [a] — the European standard for bee subspecies, developed on the basis of the values of the cubital and dumbbell indices [28], [6] — the breed standard of bees adopted in Russia. At least 25 individuals from the family were studied morphometrically; mtDNA analysis (variability of the COI-COII locus) was carried out for 5 individuals from the bee colony. Dashes indicate no data.

2. Parameters of genetic diversity of 25 microsatellite loci in honey bees (*Apis mellifera* L.) of different origin living in Siberia (2008-2018)

Parameter	Subspecies of *A. mellifera*				
	mellifera			*carpathica*	*carnica*
	evolutionary line M (PQQ, PQQQ of mtDNA locus COI-COII)			evolutionary line C (Q of mtDNA locus COI-COII)	
	Tomsk Province	Krasnoyars Territory	Altai	femilied introduced to Siveria from bee nurseries	
Locus Ap066					
The numer					
of bees	206	323	36	196	97
of alleles	6	7	5	6	3
Prevailing allele frequency:					
90 bp	0.325±0.023[a]	0.130±0.013	0.306±0.054[a]	0.933±0.018[a]	0.921±0.014a
96 bp	0.187±0.019	0.370±0.019[a]	0.417±0.058[a]	0.046±0.015	0.041±0.010
98 bp	0.347±0.024[a]	0.252±0.017	0.222±0.049	0	0
Ho	0.782±0.029	0.625±0.027***	0.694±0.077	0.148±0.025	0.113±0.032
He	0.726±0.010	0.728±0.007	0.682±0.026	0.150±0.024	0.127±0.032
Locus A024					
The numer					
of bees	307	534	34	183	106
of alleles	6	8	4	6	9
Prevailing allele frequency::					
92 bp	0.660±0.019[a]	0.473±0.015[a]	0.779±0.050[a]	0	0
96 bp	0.007±0.003	0.156±0.011	0	0	0
98 bp	0.011±0.004	0.028±0.005	0.029±0.021	0.219±0.022	0.099±0.021
100 bp	0.186±0.016	0.126±0.010	0.162±0.045	0.301±0.024[a]	0.448±0.034[a]
102 bp	0.007±0.003	0.029±0.005	0	0.156±0.019	0.297±0.031
104 bp	0	0.059±0.007	0	0.169±0.020	0
106 bp	0.130±0.014	0.085±0.009	0	0.101±0.016	0.057±0.016
Ho	0.505±0.029	0.468±0.022***	0.265±0.076	0.563±0.037***	0.566±0.048*
He	0.513±0.020	0.722±0.012	0.365±0.065	0.798±0.009	0.693±0.021
Locus Ap081					
The numer					
of bees	150	372	33	193	137
of alleles	5	4	3	6	4
Prevailing allele frequency:					
124 bp	0.820±0.022[a]	0.973±0.006[a]	0.955±0.026[a]	0.204±0.024	0.042±0.010
130 bp	0.037±0.011	0.009±0.004	0.030±0.021	0.762±0.022[a]	0
132 bp	0	0	0	0.101±0.015	0.708±0.028[a]
Ho	0.120±0.027***	0.032±0.009	0.091±0.050	0.337±0.034	0.489±0.043
He	0.318±0.034	0.053±0.011	0.088±0.047	0.404±0.030	0.452±0.031
Locus A043					
The numer					
of bees	305	418	33	177	106
of alleles	4	4	3	4	3
Prevailing allele frequency:					
128 bp	0.831±0.015[a]	0.974±0.006[a]	0.864±0.042[a]	0.071±0.014	0.222±0.029
132 bp	0.012±0.004	0.006±0.003	0.121±0.040	0.020±0.007	0
138 bp	0	0	0	0.105±0.016	0.009±0.007
140 bp	0.156±0.015	0.014±0.004	0.015±0.015	0.805±0.021[a]	0.769±0.029[a]
Ho	0.279±0.026	0.022±0.007*	0.030±0.030**	0.266±0.033	0.443±0.048
He	0.285±0.021	0.052±0.011	0.239±0.063	0.336±0.030	0.360±0.032
Locus A007					
The numer					
of bees	206	381	36	193	114
of alleles	3	6	3	9	8
Prevailing allele frequency:					
104 bp	0.063±0.012	0.154±0.013	0.444±0.059[a]	0.005±0.004	0.081±0.019
108 bp	0.820±0.019[a]	0.810±0.014[a]	0.542±0.059[a]	0.357±0.025[a]	0.257±0.030
112 bp	0.117±0.016	0.013±0.004	0	0.378±0.025[a]	0.114±0.022
114 bp	0	0.008±0.003	0.014±0.014	0.114±0.016	0.252±0.030
131 bp	0	0	0	0.082±0.014	0.200±0.028
Ho	0.199±0.028**	0.297±0.023	0.806±0.066	0.609±0.036*	0.495±0.049***
He	0.309±0.027	0.320±0.020	0.509±0.020	0.708±0.013	0.808±0.011
Locus A008					
The numer					
of bees	306	451	34	193	135
of alleles	6	6	3	9	7
Prevailing allele frequency:					
162 bp	0.868±0.014[a]	0.938±0.008[a]	0.868±0.041[a]	0.106±0.016	0.315±0.028[a]
170 bp	0	0	0.118±0.039	0.065±0.013	0.067±0.015

Continued Table 2

174 bp	0.023±0.006	0.009±0.003	0.015±0.015	0.443±0.025[a]	0.415±0.030[a]
178 bp	0	0	0	0.153±0.018	0.096±0.018
H_o	0.226±0.024	0.093±0.014	0.265±0.076	0.580±0.036***	0.607±0.042*
H_e	0.243±0.023	0.119±0.015	0.233±0.062	0.751±0.018	0.710±0.017
		Locus AC117			
The number					
of bees	301	497	34	185	137
of alleles	4	4	3	4	4
Prevailing allele frequency:					
177 bp	0.098±0.012	0.139±0.011	0.029±0.021	0.014±0.006	0.157±0.022
181 bp	0.292±0.019	0.163±0.012	0.044±0.025	0.203±0.021	0.190±0.024
185 bp	0.517±0.020[a]	0.687±0.015[a]	0.927±0.032[a]	0.778±0.022[a]	0.650±0.029[a]
H_o	0.389±0.028***	0.302±0.021***	0.147±0.061	0.297±0.034	0.219±0.035***
H_e	0.629±0.014	0.482±0.016	0.139±0.055	0.353±0.026	0.517±0.028
		Locus AT139			
The number					
of bees	38	162	34	129	135
of alleles	3	3	2	7	5
Prevailing allele frequency:					
177 bp	0.211±0.047	0.080±0.015	0.015±0.015	0.124±0.021	0.656±0.029[a]
179 bp	0	0	0	0.349±0.030[a]	0
182 bp	0	0	0	0.349±0.030[a]	0.230±0.026
186 bp	0.118±0.037	0	0	0.012±0.007	0
190 bp	0.671±0.054[a]	0.880±0.018[a]	0.985±0.015[a]	0.008±0.006	0.022±0.009
H_o	0.500±0.081	0.154±0.028	0.029±0.029	0.674±0.041	0.430±0.043
H_e	0.491±0.053	0.218±0.029	0.029±0.028	0.718±0.013	0.510±0.028
		Locus Ap243			
The number					
of bees	212	316	32	198	107
of alleles	8	8	8	4	7
Prevailing allele frequency:					
252 bp	0.014±0.006	0.137±0.014	0.141±0.044	0.970±0.009[a]	0.869±0.023[a]
255 bp	0.427±0.024[a]	0.242±0.017	0.031±0.022	0	0.037±0.013
257 bp	0	0.008±0.004	0.563±0.062[a]	0	0
263 bp	0.330±0.023[a]	0.390±0.020[a]	0.125±0.041	0.005±0.004	0.033±0.012
H_o	0.439±0.034***	0.468±0.028***	0.500±0.088	0.020±0.010*	0.140±0.034
H_e	0.694±0.014	0.753±0.010	0.642±0.058	0.059±0.016	0.241±0.039
		Locus SV185			
The number					
of bees	243	278	36	57	30
of alleles	5	5	3	4	5
Prevailing allele frequency:					
263 bp	0.288±0.021	0.158±0.016	0.417±0.058[a]	0.149±0.033	0.100±0.039
266 bp	0.117±0.015	0.385±0.021[a]	0.097±0.035	0.149±0.033	0.300±0.059[a]
269 bp	0.578±0.022[a]	0.421±0.021[a]	0.486±0.059[a]	0.386±0.046[a]	0.233±0.055
272 bp	0.010±0.005	0.002±0.002	0	0.316±0.044[a]	0.350±0.062[a]
H_o	0.527±0.032	0.550±0.030**	0.583±0.082	0.439±0.066***	0.867±0.062*
H_e	0.569±0.017	0.649±0.009	0.581±0.027	0.707±0.019	0.723±0.023
		Locus Ap049			
The number					
of bees	309	442	36	183	85
of alleles	5	7	4	9	5
Prevailing allele frequency:					
120 bp	0.123±0.013	0.002±0.002	0	0.227±0.022	0.194±0.030
127 bp	0.673±0.019[a]	0.761±0.014[a]	0.542±0.059[a]	0.030±0.009	0.035±0.014
130 bp	0.175±0.015	0.154±0.012	0.250±0.051	0.160±0.019	0.235±0.033
139 bp	0.023±0.006	0.042±0.007	0	0.511±0.026[a]	0.453±0.038[a]
152 bp	0	0.008±0.003	0.181±0.045	0.055±0.012	0.082±0.021
H_o	0.447±0.028	0.378±0.023	0.389±0.081*	0.564±0.037*	0.506±0.054**
H_e	0.501±0.020	0.394±0.019	0.611±0.040	0.658±0.019	0.694±0.022
		Locus A113			
The number					
of bees	290	509	33	194	136
of alleles	11	4	2	9	6
Prevailing allele frequency:					
212 bp	0.107±0.013	0.048±0.007	0	0.874±0.017[a]	0.599±0.030[a]
218 bp	0.571±0.021[a]	0.818±0.012[a]	0.879±0.040[a]	0.034±0.009	0.272±0.027
220 bp	0.255±0.018	0.129±0.011	0.121±0.040	0.039±0.010	0.015±0.007
H_o	0.521±0.029*	0.236±0.019**	0.000±0.000***	0.180±0.027	0.456±0.043*
H_e	0.597±0.017	0.312±0.017	0.213±0.060	0.233±0.029	0.562±0.025

Continued Table 2

Locus H110					
The number					
of bees	282	439	36	195	77
of alleles	3	5	5	5	6
Prevailing allele frequency:					
154 bp	0	0	0	0.005±0.004	0.110±0.025
158 bp	0	0.040±0.007	0.014±0.014	0.126±0.017	0.013±0.009
162 bp	0.789±0.017[a]	0.452±0.017[a]	0.111±0.037	0.385±0.025[a]	0.097±0.024
166 bp	0.027±0.007	0.126±0.011	0.722±0.053[a]	0.413±0.025[a]	0.630±0.039[a]
170 bp	0.184±0.016	0.342±0.016[a]	0.028±0.019	0.072±0.013	0.143±0.028
H_o	0.333±0.028	0.421±0.024***	0.333±0.079	0.631±0.035	0.584±0.056
H_e	0.343±0.022	0.660±0.009	0.450±0.065	0.661±0.012	0.561±0.040
Locus A028					
The number					
of bees	126	343	0	170	109
of alleles	3	4	No data	5	2
Prevailing allele frequency:					
126 bp	0.770±0.027[a]	0.848±0.014[a]		0.071±0.014	0.243±0.029
132 bp	0.167±0.024	0.054±0.009		0.829±0.020[a]	0.757±0.029[a]
H_o	0.397±0.044	0.274±0.024		0.341±0.036	0.431±0.047
H_e	0.376±0.034	0.268±0.021		0.301±0.031	0.368±0.030
Locus Ap226					
The number					
of bees	120	345	55	167	100
of alleles	5	3	2	5	5
Prevailing allele frequency:					
227 bp	0.033±0.012	0.197±0.015	0.200±0.127	0.087±0.015	0.065±0.017
233 bp	0.871±0.022[a]	0.801±0.015[a]	0.800±0.127[a]	0.051±0.012	0.260±0.031
235 bp	0.004±0.004	0	0	0.210±0.022	0.090±0.020
237 bp	0.017±0.008	0	0	0.608±0.027[a]	0.400±0.035[a]
247 bp	0	0	0	0.045±0.011	0.185±0.028
H_o	0.225±0.038	0.035±0.010***	0.000±0.000*	0.473±0.039	0.620±0.049
H_e	0.235±0.035	0.319±0.018	0.320±0.143	0.575±0.025	0.726±0.017
Locus A088					
The number					
of bees	82	236	0	82	77
of alleles	2	2	No data	2	3
Prevailing allele frequency:					
141 bp	0.927±0.020[a]	0.998±0.002[a]		0.098±0.023	0.234±0.034
150 bp	0	0		0.902±0.023[a]	0.753±0.035[a]
H_o	0.122±0.036	0.004±0.004		0.024±0.017*	0.260±0.050
H_e	0.136±0.035	0.004±0.004		0.176±0.037	0.378±0.037
Locus A056					
The number					
of bees	39	41	0	146	110
of alleles	3	2	No data	5	4
Prevailing allele frequency:					
280 bp	0.026±0.018	0		0.188±0.023	0.336±0.032[a]
282 bp	0.667±0.053[a]	0.720±0.050[a]		0.089±0.017	0.127±0.023
284 bp	0.256±0.049	0.281±0.050		0.630±0.028[a]	0.473±0.034a
H_o	0.013±0.013	0.463±0.078		0.473±0.041	0.536±0.048*
H_e	0.039±0.022	0.404±0.044		0.555±0.029	0.643±0.019
Locus K0711					
The number					
of bees	82	170	0	127	53
of alleles	3	3	No data	5	3
Prevailing allele frequency:					
212 bp	0.976±0.012[a]	0.918±0.015[a]		0.020±0.009	0
219 bp	0.018±0.011	0.071±0.014		0.815±0.024[a]	0.868±0.033[a]
222 bp	0	0		0.130±0.021	0.113±0.031
H_o	0.049±0.024	0.094±0.022		0.299±0.041	0.264±0.061
H_e	0.048±0.023	0.153±0.025		0.318±0.035	0.234±0.050
Locus Ap068					
The number					
of bees	125	242	0	157	55
of alleles	10	5	No data	7	9
Prevailing allele frequency:					
146 bp	0.020±0.009	0.074±0.012		0.255±0.025	0.118±0.031
150 bp	0.008±0.006	0		0.242±0.024	0.073±0.025
152 bp	0.092±0.018	0		0.303±0.026[a]	0.209±0.039
154 bp	0.036±0.012	0.008±0.004		0.045±0.012	0.227±0.040
158 bp	0.392±0.031[a]	0.552±0.023[a]		0.061±0.014	0.173±0.036
162 bp	0.212±0.026	0.337±0.022[a]		0.083±0.016	0.073±0.025

Continued Table 2

164 bp	0.128±0.021	0		0	0
H_o	0.816±0.035	0.488±0.032*		0.605±0.039***	0.782±0.056
H_e	0.769±0.018	0.576±0.015		0.772±0.010	0.841±0.013
		Locus Ap033			
The number					
of bees	0	274	35	90	84
of alleles	Нет данных	6	6	11	9
Prevailing allele frequency:					
221 bp		0.113±0.014	0.300±0.055[a]	0.006±0.006	0
225 bp		0.007±0.004	0.286±0.054	0.017±0.010	0.131±0.026
227 bp		0	0	0.233±0.032	0.262±0.034
229 bp		0	0	0	0.113±0.024
231 bp		0	0	0.356±0.036[a]	0.107±0.024
233 bp		0.016±0.005	0.100±0.036	0.200±0.030	0.030±0.013
235 bp		0.312±0.020[a]	0.229±0.050	0.061±0.018	0.262±0.034
239 bp		0.146±0.015	0.043±0.024	0	0
241 bp		0.405±0.021[a]	0.043±0.024	0	0
H_o		0.631±0.029*	0.857±0.059	0.489±0.053***	0.607±0.053***
H_e		0.704±0.010	0.762±0.021	0.770±0.017	0.816±0.014
		Locus SV167			
The number					
of bees	0	251	36	80	82
of alleles	Нет данных	4	3	8	8
Prevailing allele frequency:					
198 bp		0.492±0.022[a]	0.458±0.059[a]	0	0
201 bp		0.400±0.022[a]	0.444±0.059[a]	0.475±0.040[a]	0.482±0.039[a]
204 bp		0	0	0	0.177±0.030
207 bp		0.104±0.014	0.097±0.035	0.188±0.031	0.037±0.015
H_o		0.558±0.031	0.444±0.083	0.475±0.056***	0.512±0.055**
H_e		0.587±0.011	0.583±0.026	0.716±0.030	0.711±0.030
		Locus SV220			
The number					
of bees	81	362	36	82	81
of alleles	7	7	5	7	6
Prevailing allele frequency:					
176 bp	0.062±0.019	0.003±0.002	0	0.117±0.025	0.277±0.037
179 bp	0.031±0.014	0	0.028±0.019	0.599±0.039[a]	0.453±0.041[a]
182 bp	0	0.354±0.018[a]	0.097±0.035	0.031±0.014	0.014±0.010
185 bp	0.617±0.038[a]	0.403±0.018[a]	0.681±0.055[a]	0.037±0.015	0.047±0.017
188 bp	0.173±0.030	0.079±0.010	0.167±0.044	0.043±0.016	0.101±0.025
191 bp	0.090±0.023	0.006±0.003	0	0.173±0.030	0.095±0.024
H_o	0.432±0.055*	0.503±0.026***	0.528±0.083	0.500±0.058**	0.519±0.056
H_e	0.574±0.039	0.693±0.010	0.498±0.061	0.697±0.026	0.594±0.037
		Locus K1615			
The number					
of bees	42	282	0	49	40
of alleles	5	4	No data	5	5
Prevailing allele frequency:					
208 bp	0.512±0.055[a]	0.910±0.012[a]		0.010±0.010	0.025±0.018
210 bp	0	0		0	0.175±0.043
212 bp	0	0		0.796±0.041[a]	0.700±0.051[a]
214 bp	0.429±0.054[a]	0.016±0.005		0.102±0.031	0
H_o	0.667±0.073	0.181±0.023		0.367±0.069	0.250±0.069*
H_e	0.553±0.026	0.170±0.021		0.351±0.058	0.473±0.059
		Locus Ap249			
The number					
of bees	114	247	0	159	85
of alleles	6	4	No data	9	8
Prevailing allele frequency:					
207 bp	0	0.020±0.006		0.110±0.018	0.038±0.015
213 bp	0.013±0.008	0.010±0.005		0.211±0.023	0.294±0.036
215 bp	0.013±0.008	0		0.289±0.025	0.019±0.011
219 bp	0.254±0.029	0.004±0.003		0.233±0.024	0.469±0.040[a]
221 bp	0.544±0.033[a]	0.966±0.008[a]		0.098±0.017	0.069±0.020
H_o	0.588±0.046	0.061±0.015		0.679±0.037**	0.663±0.053
H_e	0.624±0.026	0.067±0.016		0.795±0.009	0.682±0.027
		Locus *mrjp3*			
The number					
of bees	89	244	27	145	129
of alleles	4	5	3	9	8
Prevailing allele frequency:					
391 bp	0.034±0.014	0.041±0.009	0.370±0.066[a]	0.110±0.018	0.043±0.013
406 bp	0	0	0	0.486±0.029a	0.399±0.031[a]

Continued Table 2

464 bp	0.084±0.021	0.027±0.007	0	0.097±0.017	0.194±0.025
518 bp	0	0	0	0.197±0.023	0.194±0.025
529 bp	0.832±0.028[a]	0.812±0.018[a]	0.574±0.067[a]	0.041±0.018	0.019±0.009
H_o	0.067±0.027***	0.283±0.029	0.333±0.091	0.621±0.040	0.550±0.044***
H_e	0.298±0.043	0.329±0.024	0.530±0.039	0.700±0.022	0.753±0.017

N o t e. The subspecies of the honey bee were established according to the data of morphometric study and mtDNA analysis. H_o — observed heterozygosity, H_e — expected heterozygosity; [a] — predominant alleles the frequency of which is more than 30%.

*, **, *** Differences between observed and expected heterozygosity are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Thus, for loci Ap081, A008, A043, A139, A113, Ap243, Ap049, A024, A088, Ap226, K0711, SV220, K1615, and *mrjp3*, the predominant alleles (the frequency of occurance more than 0.40) were identified, the composition of which differed in bees of the Central Russian breed (line M) and southern subspecies (line C). For the A043 locus, in *A. m. mellifera* (line M), a 128 bp allele prevailed with the frequency over 0.83, while in *A. m. carpathica* and *A. m. carnica* (line C), 140 bp allele predominated with the frequency more than 0.76. For the A113 locus, the predominant allele in *A. m. carpathica* and *A. m. carnica* had a size of 212 bp, in A. m. mellifera — 218 bp (the frequency more than 0.57). For a number of loci (e.g., Ap066 and A007), common predominant alleles were identified, but with different frequencies of occurrence in the subspecies of lines M and C (see Table 2).

The characterization of genetic diversity based on heterozygosity indicators revealed similar results for most of the studied loci in different bee subspecies: lower values of observed heterozygosity (H_o) compared to expected heterozygosity (H_e) were shown. For some SSR markers, e.g., the Ap066 locus in Central Russian bees and the A043 and SV185 loci in Carnica bees, the H_o value was higher than H_e. Among the groups of Central Russian bees, this was most typical for the Altai population which may be due to the small number of individuals in the sample.

We found statistically significant differences between H_o and H_e values for different samples for most loci, except for AT139, A028, and K0711. Thus, statistically significant differences between H_o and H_e were characteristic: in Central Russian bees (Yenisei population) for loci Ap066, A024, AC117, Ap243, H110, Ap226 and SV220 ($t \geq 3.69$, $p < 0.001$), SV185 and A113 ($t \geq 2.98$, $p < 0.01$), A043, Ap033 and Ap068 ($t \geq 2.30$, $p < 0.05$); in the Tomsk population for Ap081, AC117, Ap243, *mrjp3* ($t \geq 4.55$, $p < 0.001$), A007 ($t = 2.83$, $p < 0.01$), A113 and SV220 ($t \geq 2.26$, $p < 0.05$); in the Altai population for A113 ($t = 3.55$, $p < 0.001$), A043 ($t = 3.00$, $p < 0.01$), Ap049 and Ap226 ($t \geq 2.46$, $p < 0.05$); in *A. m. carpathica* for A024, A008, SV185, SV167, Ap068, Ap033 ($t \geq 3.90$, $p < 0.001$), SV220 and Ap249 ($t = 3.10$, $p < 0.01$), A007, Ap243, Ap049, A088 ($t \geq 2.07$, $p < 0.05$); in *A. m. carnica* for A007, AC117, Ap033, *mrjp3* ($t \geq 4.30$, $p < 0.001$), Ap049 and SV167 ($t \geq 3.18$, $p < 0.01$), A113, A008, A024, SV185, A056 and K1615 ($t \geq 2.13$, $p < 0.05$) (see Table 2).

To assess the introgression of the genes of breeds of southern origin (Carpathian breed, carnica, evolutionary line C) into the M line (Central Russian breed), as well as to identify the boundaries of A. m. mellifera, we conducted a comparative study of the genetic diversity of the Central Russian breed, the Carpathian breed, and the Carnica breed according to the complex of nuclear genome markers (Fig. 2). The histogram constructed on the basis of data on the variability of 24 microsatellite loci, on the one hand, clearly shows the low degree of introgression of the C line genes into the M line, that is, the purebred Central Russian bees of Siberian populations. On the other hand, an important characteristic of the Siberian populations of the Central Russian bees was genetic polymorphism,

with the greatest diversity found for the bees of the Tomsk population (apiaries of the northern and southern regions), and the bees of the Altai population turned out to be more homogeneous (mainly from the reproducer of the Central Russian breed, as well as an isolated apiary of the Altai Republic). There was an overlap of genetic variants in Central Russian bees from different populations, which is probably associated with the movement of bee colonies, which beekeepers carry out in Siberia (for example, from isolated apiaries of the Krasnoyarsk Territory or the Altai Republic to the territory of the Tomsk Region). Finally, high genetic relatedness is shown for the subspecies A. m. carnica and A. m. carpathica (a derivative of carnica), despite the different habitat conditions (the Carpathian breed was cultivated for a long time in the conditions of the Carpathians).

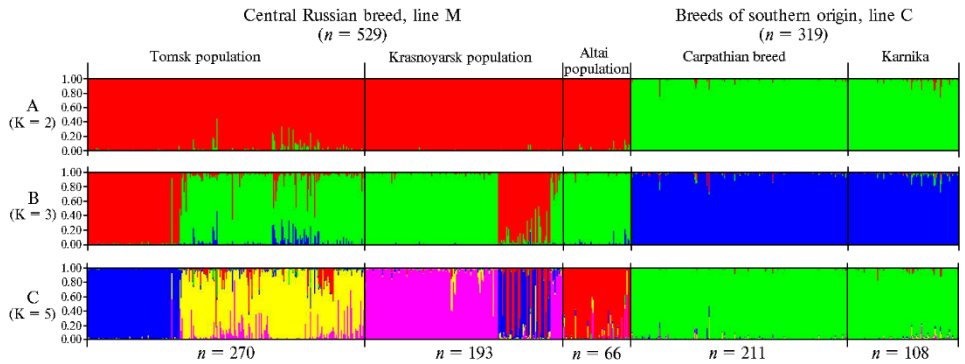

Fig. 2. Histogram constructed in the STRUCTURE 2.3.4 program based on data on the variability of 24 microsatellite loci in honey bees (*Apis mellifera* L.) of the Central Russian breed and breeds of southern origin living in Siberia (2008-2018). The vertical lines represent the proportion of the individual's membership in the color-coded cluster.

A (K = 2): green color reflects the representation of nuclear genes characteristic of breeds of southern origin (Carpathian A. m. carpathica and A. m. carnica), red color - genes of the Central Russian breed A. m. mellifera.

B (K = 3): blue color reflects the representation of nuclear genes characteristic of breeds of southern origin, the other colors - genes of the Central Russian breed.

B (K = 5): green color reflects the representation of nuclear genes characteristic of breeds of southern origin, the other colors - genes of the Central Russian breed.

When studying honey bees from Siberian apiaries, we encountered some problems in identifying bee subspecies and identifying hybrids, which led to the need to develop a research algorithm and select the most informative markers to differentiate different breeds, primarily the Central Russian bee.

The morphometric method, on the one hand, is simple and quite economical, but on the other hand, it is a laborious approach that involves the analysis of a large number of morphological features, which greatly complicates and lengthens the procedure for studying bee colonies [6]. To simplify morphometric analysis, geometric morphometry is used (analysis of the wing shape instead of measuring the angles and distances of cells) [6, 36, 37], special programs for measurements [38, 39], as well as approaches that reduce the number of bee morphometric parameters to be assessed [27] or use a single wing cell [40]. In Russia, the morphometric method is widely used to identify breeds (subspecies) of bees, often with the use of computer programs [41, 42]. However, to eliminate errors in the interpretation of the results, verification of the obtained data is required.

When using the morphometric method, it is also important to take into account the fact that environmental factors can influence the variability of some morphological characters, such as proboscis length [9]. Finally, the morphometric method is not sufficiently informative in assessing hybrid families [43], and additional markers, such as the mtDNA locus, must be studied [27]. Therefore, the

question of both the choice of the most informative morphometric markers and the algorithm for studying bees as a whole is relevant.

In this paper, we have shown that among the morphometric parameters, three indicators of the wing (discoidal displacement, cubital and dumbbell indices) are highly informative and sufficient (minimally necessary) to identify subspecies of *A. mellifera* [26, 27]. It was found that some bee colonies in some respects do not meet the morphometric standards of honey bee subspecies. Moreover, this situation is observed not only in the zones of hybridization of bees, but also in isolated apiaries (see Table 1). In addition, some bee colonies that corresponded to the breed standard in terms of morphological parameters (e.g., the standard of the Central Russian breed) had maternal origin from breeds of southern origin, according to mtDNA analysis (the Q variant of the COI-COII locus was detected), that is, they are "changeling families".

Therefore, the use of the mtDNA COI-COII locus as an additional marker allowed us to refine the data of morphometric analysis, despite the fact that the analysis of the mitochondrial genome evaluates the genetic contribution only along the uterine line. The research algorithm used in the study of the breed composition of honey bees living in Siberia includes i) mtDNA analysis (detection of variability of the COI-COII locus) to establish the origin of the family on the maternal line, ii) evaluation of morphometric parameters of the wing (cubital index, dumbbell index, discoidal displacement), and iii) identification of compliance of morphometric and mtDNA analysis data with the breed standard. Thus, an integrated approach using the morphometric method and mtDNA analysis allowed us to significantly simplify the morphometric analysis of bees and to accurately identify subspecies and hybrids of honey bees living in Siberia.

Using data on the variability of 31 SSR markers, a database was created on microsatellite loci (standard allelic ladder) for Central Russian bees of Siberian populations, and a search was made for molecular markers that are informative for differentiating subspecies of bees of evolutionary lines M and C. The most promising microsatellite loci, which can be included in the diagnostic DNA panel to differentiate the subspecies *A. m. mellifera* (line M) and two subspecies of southern origin *A. m. carpathica* and *A. m. carnica* (line C) turned out to be A043, Ap081, Ap049, AT139, A024, A113, A088, A028, A008 and *mrjp3.* In general, among the studied microsatellite loci, three classes of markers can be distinguished, differing in their information content for diagnosing honey bee breeds and ecotypes.

The first class includes loci for which breed-specific alleles have been found (e.g., A043, Ap081, Ap049, AT139, A024, A113, A088, A028, A008 and *mrjp3*). For these loci, we found predominant allele (alleles) in bees of the Central Russian breed (*A. m. mellifera*) but not in *A. m. carpathica* and *A. m. carnica* where this allele or alleles, if occurred, had a low frequency (see Table 2). For example, for the A043 locus, the 128 bp allele dominated in *A. m. mellifera* from different Siberian populations (the frequency over 0.83), the Burzyanskaya population (Bashkortostan) and most European populations (frequency of occurrence from 0.68 to 0.90) [11, 12, 21], while for the southern subspecies it was a 140 bp allele is characteristic (frequency of occurrence more than 0.76). For *mrjp3*, different predominant alleles were also identified in bees of evolutionary lines M and C. A 529 bp allele was registered with a high frequency (more than 0.57) in the Central Russian bees, while in the Carpathian bees and bees of the *carnica* subspecies it was rare (the frequency less than 0.05). On the contrary, the 406 bp allele dominated in *A. m. carpathica* and *A. m. carnica* (the frequency 0.49 and 0.40, respectively) and was not found in Central Russian bees of Siberian populations. A similar situation was observed for loci Ap081, Ap049, AT139, A024, A113 (see Table

2). In addition, for some loci (Ap081, AT139, etc.), differences in the composition and frequency of dominant alleles in *A. m. carpathica* and *A. m. carnica*, which may be informative for differentiating the two southern subspecies.

The second class included loci for which eco-specific alleles were found, that is, different composition and frequency of alleles were registered in *A. m. mellifera* from different populations of Russia and Europe. The microsatellite locus A008 is of considerable interest. In Central Russian bees of Siberian populations, the162 bp allele occurred at a high frequency (more than 0.86). In bees of the *carniaca* subspecies, this allele was also registered, but with a lower frequency (less than 0.32). In this regard, the A008 locus is of interest for differentiation not so much of subspecies as of ecotypes of *A. m. mellifera*: differences in the composition of alleles between the bees *A. m. mellifera* from different populations of Russia and Europe. So, if for the Central Russian bees of Siberia the 162 bp allele was predominant, then in *A. m. mellifera* of the Ural and European populations, shorter alleles of 154 and 148 bp, respectively, were dominant [11, 12, 21]. In the Central Russian bees of the Siberian populations, the 148 bp allele was not found at all. Since the bees *A. m. mellifera* from different populations of Russia and Europe revealed a geographic gradient in the size of the dominant allele (148 bp−154 bp−162 bp) in the west-east direction, it can be assumed that the A008 locus is associated geographical/ecological conditions of bee habitat. The problem of genetic specificity of different subspecies/ecotypes of bees to local environmental conditions is actively discussed in scientific publications [7, 44-46].

Nonspecific loci (for example, AC117, H110, SV185), belonging to the third class, are markers for which a similar composition and close allele frequencies have been shown in bees of different origin and/or geographical localization.

In general, among microsatellite loci, the variability of which has been studied in Siberian bee populations, a number of markers can be used to establish the origin of subspecies and/or ecotypes of the honey bee. Despite the existing limitations in the use of DNA markers of the nuclear genome (the absence or inaccessibility of a database and reference materials on the variability of SSR markers in bees of different populations/ecotypes/subspecies), in some cases microsatellite loci are highly informative and widely used in assessing the introgression of genes from one evolutionary line into another and identifying traces of hybridization [5, 19-21].

Thus, at present, there is no universal method or diagnostic marker (morphometric, molecular) for identifying subspecies of *A. mellifera*, but with the complex application of different methods, morphometric and DNA markers complement each other.

The importance of an integrated approach is noted in many works on the taxonomy and phylogeny of animals. Thus, when describing new species of wasps (*Hymenoptera*), it was shown that, along with the morphological approach, molecular genetic, cytogenetic, and other methods are important [47]. Comprehensive analysis of nuclear and mitochondrial markers is the most informative for identifying hybrids and assessing introgression or gene flow [3, 21].

The choice of a reliable DNA marker for molecular genetic studies, as well as the search for a morphological trait, are difficult and poorly developed [48, 49]. A good molecular marker implies the presence of a sufficient number of informative sites, a low degree of homoplasia, and a relatively uniform rate of evolution within the analyzed group of organisms [50]. If the analysis of large taxa characterized by significant divergence (tribes, families) requires a DNA marker with a low degree of variability, then when studying groups of a low taxonomic level (species), it is desirable to use a rapidly evolving marker with variability that has not reached the saturation limit [49]. Finally, before using a new DNA locus

as a phylogenetic marker, it is necessary to determine the degree of its variability and informativeness, for example, in a group of organisms with a well-studied taxonomy and evolutionary history [50].

It should be emphasized that various approaches, such as morphological and molecular genetic methods used in the systematics and phylogeny of organisms, complement each other and are not competing or mutually exclusive, especially since molecular genetic methods will not replace morphological species identification system [51]. The optimal and most informative for identifying species and other taxa, as well as establishing the boundaries between species and describing species diversity is an integrated approach involving a wide arsenal of methods and taking into account data from various disciplines (comparative anatomy, ecology, ethology, population genetics, philogeography) [52]. Particularly credible studies are those in which the phylogenetic hypothesis is substantiated by the analysis of several independently evolving molecular markers [52, 53] or the variability of a molecular marker is preliminary studied and its information content and reliability are shown to resolve phylogenetic relationships at a given taxonomic level [48, 50, 54].

So, in the study of honey bees in Siberia, we used an integrated approach, including the analysis of morphometric traits, markers of the mitochondrial and nuclear genomes, and assessed the information content of different methods. The optimal research algorithm, with regards to the differentiating ability, information content and efficiency of the methods used, can be as follows: i) analysis of the origin of the family on the maternal line using markers of the mitochondrial genome; ii) study of morphometric features and assessment of their compliance with mtDNA analysis data; iii) microsatellite analysis to clarify the breed affiliation of families, the origin of hybrids, as well as to identify the genetic diversity of bees of different evolutionary lines. This approach allowed us to identify honey bee subspecies, identify populations of the Central Russian breed Apis mellifera mellifera and characterize their genetic diversity, evaluate the genotypic composition of bee colonies, and determine bee hybridization zones. The results obtained in this work are a scientific basis for the genetic certification of bees and breeding work on the selection of purebred families with the necessary biological and economically significant traits, which is an important condition for the conservation and rational use of native breeds/ecotypes.

REFERENCES

1. Bouga M., Alaux C., Bienkowska M., Büchler R., Carreck N.L., Cauia E., Chlebo R., Dahle B., Dall'Olio R., De la Rúa P., Gregorc A., Ivanova E., Kence A., Kence M., Kezic N., Kiprijanovska H., Kozmus P., Kryger P., Le Conte Y., Lodesani M., Murilhas A.M., Siceanu A., Soland G., Uzunov A., Wilde J. A review of methods for discrimination of honey bee populations as applied to European beekeeping. *Journal of Apicultural Research*, 2011, 50(1): 51-84 (doi: 10.3896/IBRA.1.50.1.06).
2. Nawrocka A., Kandemir İ., Fuchs S., Tofilski A. Computer software for identification of honey bee subspecies and evolutionary lineages. *Apidologie*, 2018, 49: 172-184 (doi: 10.1007/s13592-017-0538-y).
3. Pinto M.A., Henriques D., Chávez-Galarza J., Kryger P., Garnery L., van der Zee R., Dahle B., Soland-Reckeweg G., De la Rúa P., Dall'Olio R., Carreck N.L., Johnston J.S. Genetic integrity of the Dark European honey bee (*Apis mellifera mellifera*) from protected populations: a genome-wide assessment using SNPs and mtDNA sequence data. *Journal of Apicultural Research*, 2014, 53(2): 269-278 (doi: 10.3896/IBRA.1.53.2.08).
4. Muñoz I., Henriques D., Johnston J.S., Chávez-Galarza J., Kryger P., Pinto M.A. Reduced SNP panels for genetic identification and introgression analysis in the dark honey bee (*Apis mellifera mellifera*). *PLoS ONE*, 2015, 10(4): e0124365 (doi: 10.1371/journal.pone.0124365).
5. Parejo M., Henriques D., Pinto M.A., Soland-Reckeweg G., Neuditschko M. Empirical comparison of microsatellite and SNP markers to estimate introgression in *Apis mellifera mellifera*. *Journal of Apicultural Research*, 2018, 57(4): 504-506 (doi: 10.1080/00218839.2018.1494894).

6. Henriques D., Chávez-Galarza J.C., Quaresma A., Neves C.J., Lopes A.R., Costa C., Costa F.O., Rufino J., Pinto M.A. From the popular tRNA^{leu}-COX2 intergenic region to the mitogenome: insights from diverse honey bee populations of Europe and North Africa. *Apidologie*, 2019, 50(2): 215-229 (doi: 10.1007/s13592-019-00632-9).
7. Meixner M.D., Pinto M.A., Bouga M., Kryger P., Ivanova E., Fuchs S. Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. *Journal of Apicultural Research*, 2013, 52(4): 1-28 (doi: 10.3896/IBRA.1.52.4.05).
8. Porrini L.P., Quintana S., Brasesco C., Porrini M.P., Garrido P.M., Eguaras M.J., Müller F., Iriarte P.F. Southern limit of Africanized honey bees in Argentina inferred by mtDNA and wing geometric morphometric analysis. *Journal of Apicultural Research*, 2020, 59(4): 648-657 (doi: 10.1080/00218839.2019.1681116).
9. Alpatov V.V. *Porody medonosnoi pchely* [Honeybee breeds]. Moscow, 1948 (in Russ.).
10. Ruttner F. *Biogeography and taxonomy of honey bees*. Berlin, Germany, 1988.
11. Franck P., Garnery L., Solignac M., Cornuet J.-M. Molecular confirmation of a fourth lineage in honeybees from the Near East. *Apidologie*, 2000, 31(2): 167-180 (doi: 10.1051/apido:2000114).
12. Garnery L., Cornuet J.M., Solignac M. Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. *Molecular Ecology,* 1992, 1(3): 145-154 (doi: 10.1111/j.1365-294x.1992.tb00170.x).
13. Cornuet J.M., Garnery L., Solignac M. Putative origin and function of the intergenic region between *COI* and *COII* of *Apis mellifera* L. mitochondrial DNA. *Genetics*, 1991, 128(2): 393-403 (doi: 10.1093/genetics/128.2.393).
14. Rortais A., Arnold G., Alburaki M., Legout H., Garnery L. Review of the Dra*I COI-COII* test for the conservation of the black honeybee (*Apis mellifera mellifera*). *Conservation Genetics Resources*, 2011, 3(2): 383-391 (doi: 10.1007/s12686-010-9351-x).
15. Dall'Olio R., Marino A., Lodesani M., Moritz R.F.A. Genetic characterization of Italian honeybees, *Apis mellifera ligustica*, based on microsatellite DNA polymorphisms. *Apidologie*, 2007, 38(2): 207-217 (doi: 10.1051/apido:2006073).
16. Cánovas F., de la Rúa P., Serrano J., Galián J. Microsatellite variability reveals beekeeping influences on Iberian honeybee populations. *Apidologie*, 2011, 42(3): 235-251 (doi: 10.1007/s13592-011-0020-1).
17. Ostroverkhova N.V., Kucher A.N., Konusova O.L., Kireeva T.N., Sharakhov I.V. Genetic diversity of honeybees in different geographical regions of Siberia. *International Journal of Environmental Studies*, 2017, 74(5): 771-781 (doi: 10.1080/00207233.2017.1283945).
18. Soland-Reckeweg G., Heckel G., Neumann P., Fluri P., Excoffier L. Gene flow in admixed populations and implications for the conservation of the Western honeybee, *Apis mellifera*. *Journal of Insect Conservation*, 2009, 13: 317-328 (doi: 10.1007/s10841-008-9175-0).
19. Oleksa A., Chybicki I., Tofilski A., Burczyk J. Nuclear and mitochondrial patterns of introgression into native dark bees (*Apis mellifera mellifera*) in Poland. *Journal of Apicultural Research*, 2011, 50(2): 116-129 (doi: 10.3896/IBRA.1.50.2.03).
20. Nikolova S. Genetic variability of local Bulgarian honey bees *Apis mellifera macedonica (rodopica)* based on microsatellite DNA analysis. *Journal of Apicultural Science*, 2011, 55(2): 117-129.
21. Il'yasov R.A., Poskryakov A.V., Petukhov A.V., Nikolenko A.G. *Genetika*, 2016, 52(8): 931-942 (doi: 10.7868/S0016675816060059) (in Russ.).
22. Zinov'eva N.A., Krivtsov N.I., Fornara M.S., Gladyr' E.A., Borodachev A.V., Berezin A.S., Lebedev V.I. Microsatellites as a tool for evaluation of allele pool dynamics when creation of prioksky type of middle russian honey bee *Apis mellifera*. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2011, 6: 75-79 (in Russ.).
23. Krivtsov N.I., Zinov'eva N.A., Borodachev A.V., Lebedev V.I., Fornara M.S. *Vestnik Ryazanskogo gosudarstvennogo agrotekhnologicheskogo universiteta imeni P.A. Kostycheva*, 2011, 4(12): 23-27 (in Russ.).
24. Hassett J., Browne K.A., McCormack G.P., Moore E., Native Irish Honey Bee Society, Soland G., Geary M. A significant pure population of the dark European honey bee (*Apis mellifera mellifera*) remains in Ireland. *Journal of Apicultural Research*, 2018, 57(3): 337-350 (doi: 10.1080/00218839.2018.1433949).
25. Whitfield C.W., Behura S.K., Berlocher S.H., Clark A.G., Johnston J.S., Sheppard W.S., Smith D.R., Suarez A.V., Weaver D., Tsutsui N.D. Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science*, 2006, 314(5799): 642-645 (doi: 10.1126/science.1132772).
26. Ostroverkhova N.V., Rosseikina S.A., Konusova O.L., Kucher A.N., Kireeva T.N. *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya*, 2019, 47: 142-173 (doi: 10.17223/19988591/47/8) (in Russ.).
27. Konusova O.L., Ostroverkhova N.V., Kucher A.N., Kurbatskii D.V., Kireeva T.N. *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya*, 2016, 1(33): 62-81 (doi: 10.17223/19988591/33/5) (in Russ.).
28. Căuia E., Usurelu D., Magdalena L.M., Cimponeriu D., Apostol P., Siceanu A., Holban A., Gavrilă L. Preliminary researches regarding the genetic and morphometric characterization of

honeybee (*A. mellifera* L.) from Romania. *Scientific Papers Animal Science and Biotechnologies*, 2008, 41(2): 278-286.
29. Nikonorov Yu.M., Ben'kovskaya G.V., Poskryakov A.V., Nikolenko A.G., Vakhitov V.A. *Genetika*, 1998, 34(11): 1574-1577 (in Russ.).
30. Solignac M., Vautrin D., Loiseau A., Mougel F., Baudry E., Estoup A., Garnery L., Haberl M., Cornuet J.-M. Five hundred and fifty microsatellite markers for the study of the honeybee (*Apis mellifera* L.) genome. *Molecular Ecology Notes*, 2003, 3(2): 307-311 (doi: 10.1046/j.1471-8286.2003.00436.x).
31. Baitala T.V., Faquinello P., de Toledo V.d.A.A., Mangolin C.A., Martins E.N., Ruvolo-Takasusuki M.C.C. Potential use of major royal jelly proteins (MRJPs) as molecular markers for royal jelly production in Africanized honeybee colonies. *Apidologie*, 2010, 41: 160-168 (doi: 10.1051/apido/2009069).
32. Albert S., Klaudiny J., Šimúth J. Molecular characterization of MRJP3, highly polymorphic protein of honeybee (*Apis mellifera*) royal jelly. *Insect Biochemistry and Molecular Biology*, 1999, 29(5): 427-434 (doi: 10.1016/s0965-1748(99)00019-3).
33. Peakall R., Smouse P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. *Bioinformatics*, 2012, 28(19): 2537-2539 (doi: 10.1093/bioinformatics/bts460).
34. Pritchard J.K., Stephens M., Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*, 2000, 155(2): 945-959 (doi: 10.1093/genetics/155.2.945).
35. Ostroverkhova N.V., Konusova O.L., Kucher A.N., Kireeva T.N., Vorotov A.A., Belykh E.A. *Genetika*, 2015, 51(1): 89-100 (doi: 10.7868/S0016675815010105) (in Russ.).
36. Kandemir İ., Özkan A., Fuchs S. Reevaluation of honeybee (*Apis mellifera*) microtaxonomy: a geometric morphometric approach. *Apidologie*, 2011, 42(5): 618-627 (doi: 10.1007/s13592-011-0063-3).
37. Özkan A.K., Kandemir İ. Comparison of two morphometric methods for discriminating honey bee (*Apis mellifera* L.) populations in Turkey. *Turkish Journal of Zoology*, 2013, 37(2): 205-210 (doi: 10.3906/zoo-1104-10).
38. Klingenberg C.P. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 2011, 11(2): 353-357 (doi: 10.1111/j.1755-0998.2010.02924.x).
39. Charistos L., Hatjina F., Bouga M., Mladenovic M., Maistros A.D. Morphological discrimination of Greek honey bee populations based on geometric morphometrics analysis of wing shape. *Journal of Apicultural Science*, 2014, 58(1): 75-84 (doi: 10.2478/JAS-2014-0007).
40. Francoy T.M., Prado P.R.R., Gonɜalves L.S., Costa L.F., De Jong D. Morphometric differences in a single wing cell can discriminate *Apis mellifera* racial types. *Apidologie*, 2006, 37(1): 91-97 (doi: 10.1051/apido:2005062).
41. Lyuto A.A., Ivanova O.V., Tolstopyatov L.P. *Pchelovodstvo*, 2015, 9: 21-22 (in Russ.).
42. Brandorf A.Z., Ivoilova M.M. *Biomika*, 2016, 8(2): 73-75 (in Russ.).
43. Guzmín-Novoa E., Page R.E.Jr., Fondrk M.K. Morphometric techniques do not detect intermediate and low levels of Africanization in honey bee (*Hymenoptera*: *Apidae*) colonies. *Annals of the Entomological Society of America*, 1994, 87(5): 507-515 (doi: 10.1093/aesa/87.5.507).
44. De la Rúa P., Jaffé R., Dall'Olio R., Muñoz I., Serrano J. Biodiversity, conservation and current threats to European honeybees. *Apidologie*, 2009, 40: 263-284 (doi: 10.1051/apido/2009027).
45. Meixner M.D., Büchler R., Costa C., Francis R.M., Hatjina F., Kryger P., Uzunov A., Carreck N.L. Honey bee genotypes and the environment. *Journal of Apicultural Research*, 2014, 53(2): 183-187 (doi: 10.3896/IBRA.1.53.2.01).
46. Hatjina F., Costa C., Büchler R., Uzunov A., Drazic M., Filipi J., Charistos L., Ruottinen L., Andonov S., Meixner M.D., Bienkowska M., Dariusz G., Panasiuk B., Le Conte Y., Wilde J., Berg S., Bouga M., Dyrba W., Kiprijanovska H., Korpela S., Kryger P., Lodesani M., Pechhacker H., Petrov P., Kezic N. Population dynamics of European honey bee genotypes under different environmental conditions. *Journal of Apicultural Research*, 2014, 53(2): 233-247 (doi: 10.3896/IBRA.1.53.2.05).
47. Gokhman V.E. *Zhurnal obshchei biologii*, 2017, 78(5): 37-45 (in Russ.).
48. Bannikova A.A. *Zhurnal obshchei biologii*, 2004, 65(4): 278-305 (in Russ.).
49. Abramson N.I. *Trudy Zoologicheskogo instituta RAN*, 2009, 1: 185-198 (in Russ.).
50. Tarasov O.V., Zhuravleva G.A., Abramson N.I. *Molekulyarnaya biologiya*, 2008, 42(6): 937-946 (in Russ.).
51. Vinarskii M.V. *Zhurnal obshchei biologii*, 2015, 76(2): 99-110 (in Russ.).
52. Sinev S.Yu. *Entomologicheskoe obozrenie*, 2011, XC(4): 821-832 (in Russ.).
53. Lukhtanov V.A, Shapoval N.A. *Doklady Akademii nauk*, 2008, 423(3): 421-426 (in Russ.).
54. Wiens J.J., Kuczynski C.A., Townsend T., Reeder T.W., Mulcahy D.G., Sites J.W.Jr. Combining phylogenomics and fossils in higher-level squamate reptile phylogeny: molecular data change the placement of fossil taxa. *Systematic Biology*, 2010, 59(6): 674-688 (doi: 10.1093/sysbio/syq048).