AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 4, pp. 651-663
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

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

UDC 636.592:575.174:577.21

doi: 10.15389/agrobiology.2021.4.651eng
doi: 10.15389/agrobiology.2021.4.651rus

GENETIC DIFFERENTIATION OF TURKEY BREEDS WITH MICROSATELLITE MARKERS

V.I. FISININ[1], M.I. SELIONOVA[2] ✉, D.A. KOVALEV[3], L.A. SHINKARENKO[4]

[1]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail fisinin@land.ru;
[2]*Russian State Agrarian University — Timiryazev Moscow Agricultural Academy,* 49, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail m_selin@mail.ru (✉ corresponding author);
[3]*Stavropol Anti-Plague Institute of Rospotrebnadzor,* 13-15, ul. Sovetskaya, Stavropol, 355005 Russia, e-mail kovalev_da.stv@list.ru;
[4]*North Caucasian Zonal Experimental Station for Poultry Breeding — Branch of the Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS*, s. Obil'noe, Georgievskii District, Stavropol Krai, 357812 Russia, e-mail skzospzooteh@yandex.ru
ORCID:
Fisinin V.I. orcid.org/0000-0003-0081-6336
Selionova M.I. orcid.org/0000-0002-9501-8080
Kovalev D.A. orcid.org/0000-0002-9366-5647
Shinkarenko L.A. orcid.org/0000-0003-4959-5415
The authors declare no conflict of interests
Received April 26, 2021

Abstract

One of the trends of modern industrial agriculture is the reduction of breed genetic recourses in farm animals and poultry. Current programs on maintenance of farm animals breeds are giving great attention to the genetic studies, including the use of microsatellite loci. The microsatellite analysis is one of the informative and accessible methods. During the implementation of the Global Project for the Measurement of Domestic Animal Genetic Diversity (MoDAD), 50 populations of different poultry species were studied using microsatellite markers. The works on biodiversity in turkeys initially involved chicken microsatellite loci (*Gallus gallus*), then informative loci were established for the genome of turkeys (*Meleagris gallopavo*). Data on genetic profiles, similarities, differences, and interbreed differentiation of turkeys breeds bred in the USA, Italy, Hungary and other countries have been accumulated. In the present work, the genetic relationship between the Russian turkey breeds and the turkey gene pool population of the University of Minnesota based on microsatellite markers was established for the first time. The obtained data indicate that the genetic distances between breeds is largely determined by their origin, breeding range, and the contribution of the gene pool of some breeds in creating and improving the productive qualities of other breeds. Our purpose was to study genetic diversity and interbreeding differentiation of turkeys of Russian and foreign breeding using microsatellite loci. The research was performed at the North Caucasus zonal experimental station for poultry farming in 2019. Blood samples were taken from 30 individuals of each of seven turkey breeds (*Meleagris gallopavo*) of the Russian selection (Belaya shirokogrudaya, BSH; Bronzovaya Severokavkazskaya, BrSK; Belaya Severokavkazskaya, BeSK; Serebristaya Severokavkazskaya, SSK; Moscowskaya Belaya, MB; Chernaya Tikhoretskaya, CHT; Uzbekskaya palevaya, UP). DNA was isolated according to the protocol for the commercial AmpliPrime DNA-sorb-B kit (InterLabService, Russia). The amount and quality of isolated DNA were assayed using a standard spectrophotometric method (a NanoDrop 2000 spectrophotometer, Thermo Scientific, USA). Genotyping was performed for 12 microsatellite loci (MNT9-MNT20). The described genotypes of turkeys gene pool farm (AM) (Nicholas Turkey Breeding Farms) of the University of Minnesota were used for comparison with the genotypes of turkeys of Russian breeds. The average number and number of effective alleles per locus (Na, Ne), the degree of observed and expected heterozygosity (N_o, N_e), and Shannon index (I) were determined. The genetic structure of populations was assessed based on the F_{ST} values and genetic distances according to M. Nei. The Neighbor Joining Method was used to construct the phylogenetic tree. It was shown that low genetic diversity is characteristic of both Russian breeds of turkeys and the AM population. The number of identified alleles in the microsatellite loci as a whole in the breed sample varied from 1 to 4, the average number of alleles per locus ranged from 1.0 to 1.83. The least genetic difference occurred between the MB and BSH breeds. The BeSK, SSK, and BrSK breeds formed a separate node, with BrSK exhibiting the greatest genetic distance, forming the largest branch by genetic distance. Separate branches at relatively equal distances formed the breeds CHT, UP, and AM population. Thus, our findings confirm an insignificant genetic diversity of the gene pool of the

studied Russian turkeys' breeds and populations as compared to the gene pool of other species of farm animals.

Keywords: turkey breeds, microsatellites, phylogenetic analysis, genetic diversity

One of the problems of modern industrial agriculture is the reduction of the national genetic resources of breeds of farm animals and poultry, a decrease in their genetic diversity. Sometimes there is a threat not only of reduction but also of complete loss of unique, especially valuable gene pools. The priority of reducing the loss of genetic resources, preserving the diversity of existing local breeds, regional groups, types of animals and poultry capable of producing products in different breeding conditions, and ensuring sustainable development of animal husbandry is confirmed by the international convention on biological diversity [1].

An important aspect in the development of programs for the conservation of breeds of farm animals is the study of their genetic characteristics. Multi- and monolocal DNA markers, or microsatellites and single nucleotide polymorphisms (SNPs), are widely used to assess such features, as well as to certify breeds [2].

Microsatellites are usually highly polymorphic and include many alleles per locus. The FAO (Food and Agriculture Organization) recommendations for the selection of microsatellite loci in the study of various types of farm animals are based on a list (DAD-IS library, http://www.fao.org/dad-is/), developed by the ISAG-FAO group on genetic diversity. Microsatellites are recognized as informative for the analysis of the origin and mapping of quantitative trait loci [3, 4]. At the same time, with the development of molecular testing technologies, the analysis of SNP using microarrays, or chips, is gaining increasing recognition and advantage over them [5].

For the conservation and rational use of genetic resources of agricultural animals and poultry, FAO carried out a large-scale project for the analysis of their genetic diversity (Global Project for the Measurement of Domestic Animal Genetic Diversity, MoDAD) [6]. More than 50 populations of different bird species have been studied for microsatellite loci [7–9].

The study of microsatellite loci in turkeys (*Meleagris gallopavo*) began in the 2000s using microsatellite panels developed for chickens (*Gallus gallus*). Reed *et al.* [10] used 520 chicken microsatellite markers to work on turkeys. In 280 cases (54%), amplification products were obtained, most of which were either close in size to the fragments amplified with chicken DNA, or completely coincided. When assessing the informative value for genetic mapping of turkey, allelic polymorphism was determined in 57 out of 280 amplified regions. In total, 20 out of 57 markers (35%) were found to be polymorphic (on average, 1.4 alleles per locus). It was concluded that about 20% of chicken microsatellite loci could be used to map the turkey genome [10].

Chicken microsatellites were used to study the gene pool of turkeys of the Brianzolo, Colli Euganei, and Italian Black (Brianzolo, Colli Euganei, Nero d'Italia) breeds. Of the 31 loci, 22 were informative. At that, 12 loci (ADL0112, LEI0192, LEI0234, MCW0014, MCW0016, MCW0037, MCW0067, MCW0098, MCW0103, MCW0111, MCW0165, MCW0183) were studied in single PCR, 10 loci were studied using multiplex panels (Multiplex Master Mix 1 — ADL0268, ADL0278, LEI0094, MCW0216, MCW0248; Master Mix 2 — MCW0034, MCW0069, MCW0081, MCW0222, MCW0295). In nine markers (LEI0166, MCW0020, MCW0078, MCW0080, MCW0104, MCW0123, MCW0248, MCW0284, MCW0330), there were no amplified DNA regions [11]. Eight microsatellite markers of chickens *Gallus gallus* (MCW0111, MCW0067, LEI0104, MCW0123, MCW0081, MCW0069, MCW0104, MCW0183), of which seven were polymorphic, were used to study the gene pool of BIG6 and BIG10 BUT turkeys

(British United Turkeys) [12, 13].

Later, the research team used 772 microsatellite markers developed for three species of birds – chicken, quail, and turkey. As a result of screening for the study of allelic polymorphism and construction of a genetic map of turkeys, 410 microsatellite loci (53.1%) were selected. On a specially created genetic model (family), including direct relatives of three generations (224 individuals), genotyping was carried out at the selected loci. Of the 410 markers, 109 (26.6%) were polymorphic (2.3 alleles per marker). Higher polymorphism (61.1%) was found when using turkey-specific markers. When using markers specific for quail and chicken, polymorphism was 33.3 and 22.7%, respectively. The authors concluded that quail and chicken microsatellite loci could be used to construct a comparative genetic map of turkeys [14].

The next stage was the integration of the data obtained at two research centers – the Roslin Institute (Edinburgh, Scotland) and the University of Minnesota (Minneapolis, USA) [15]. Out of 279 microsatellite markers identified and tested at the Rosslyn Institute, 240 were used for screening on turkeys at the University of Minnesota experimental farm. Of these, 89 turned out to be genetically informative and were used for genotyping F_2 offspring. Analysis using the BLAST (Basic Local Alignment Search Tool) software package made it possible to unify 483 nucleotide sequences of microsatellites.

The researchers also performed BLAST alignment of the marker sequences of the turkey and chicken genomes. There were 263 matches and 1700 sequences with high homology [15].

In the joint work of scientists from the United States and Turkey, the search for informative microsatellite markers was carried out to study the biodiversity of turkeys and create a unified panel. Based on the nucleotide data library, primers were designed for 164 fragments of the turkey genome containing microsatellites. One hundred fifty-four informative genetic markers were identified; however, according to the authors, this is not enough for the general panel [16].

Interest in the study of the genetic diversity of turkeys, including for the genetic certification of commercial breeds, is primarily dictated by the fact that the production of turkey meat in the world is constantly growing. It is 6.1 million tons in the global volume of poultry meat, ranking second. Positive dynamics are also observed in Russia: according to Agrifood Strategies, the growth of turkey meat production in 2019 compared to 2007 amounted to 185 thousand tons (from 37 to 289 thousand tons, or 7.8 times), which characterizes this segment market as the most promising and rapidly growing.

Russia has its own genetic resources in turkey breeding: seven breeds, three crosses, and seven lines of domestic breeding turkeys are registered in the register of breeding achievements. The North Caucasian Zonal Experimental Station for poultry farming is not only the owner of the domestic gene pool of turkeys but also the only enterprise in Russia where systematic work is underway to create new breeding forms.

For the first time, the genetic profile of Russian turkey breeds by microsatellite loci was studied in 2017. The number in the samples of the studied breeds ranged from 9 to 15 individuals. The Hunter-Gaston index was used to assess the representativeness of the samples, the cluster analysis was performed using the UPGMA method, and the dendrogram was built using the START 2 computer program. It was found that the Bronzovaya Severokavkazskaya, Belaya Severokavkazskaya, and Belaya shirokogrudaya breeds had the greatest genetic affinity, followed by Chernaya Tikhoretskaya, Serebristaya Severokavkazskaya, and Mos-

cowskaya Belaya. The most distant was the Uzbekskaya palevaya breed [17]. However, no comparison was made between the gene pool of domestic turkey breeds and the gene pool of imported breeding.

In this work, on the basis of microsatellite markers, for the first time, the genetic relationships between the breeds of turkeys of Russian selection and the gene pool of the University of Minnesota were established. It is shown that the value of genetic distances between breeds is largely determined by their origin, breeding area, as well as the contribution of the gene pool of some breeds to the creation and improvement of the productive qualities of others.

The aim of this work is to study the genetic diversity and inter-breed differentiation of turkeys of Russian and foreign selection using microsatellite loci.

Materials and methods. The work was carried out at the North Caucasian Zonal Experimental Station for poultry farming in 2019. Blood samples were taken from the axillary vein from 30 individuals of each of seven breeds of turkeys (*Meleagris gallopavo*) of Russian selection (Belaya shirokogrudaya, BSH; Bronzovaya Severokavkazskaya, BrSK; Belaya Severokavkazskaya, BeSK; Serebristaya Severokavkazskaya, SSK; Moscowskaya Belaya, MB; Chernaya Tikhoretskaya, CHT; Uzbekskaya palevaya, UP).

DNA was isolated in accordance with the protocol for the commercial kit AmpliPrime DNA-Sorb-B (InterLabService, Russia). The amount and quality of isolated DNA were controlled using a NanoDrop 2000 c spectrophotometer (Thermo Scientific, United States) by a standard spectrophotometric method; the calculation and visualization of the result were performed using the NanoDrop 2000 software, version 1.4.2. Reference solution – TE-buffer pH 7.8-8.2 (FBSI Central Research Institute of Epidemiology, Russia).

Genotyping was performed at 12 microsatellite loci MNT9-MNT20 [18, 19]. PCR was carried out on a T 100 amplifier (Bio-Rad Laboratories, Inc., USA) in a mixture of a final volume of 20 rL containing the following reagents per reaction: 1 rL of forward and reverse primers (Federal Government Health Institution Stavropol Plague Control Research Institute of the Federal Service for the Oversight of Consumer Protection and Welfare, Russia), 2 rl of dNTP solution, 4 rl of RNA-eluent, 10 rl of PCR-mixture-2red (InterLabService, Russia) and 2 rl of DNA samples. The amplification mode was as follows: 15 min at 95 °C; 30 s at 95 °C, 30 s at 58 °C (for loci MNT10, MNT11, MNT20 – 56 °C), 30 s at 72 °C (35 cycles); 5 min at 72 °C.

Capillary electrophoresis was performed using an Experion System station (Bio-Rad Laboratories, Inc., USA) and a kit of reagents for visualization of DNA fragments Ex-perion DNA 1K Analysis Kit (Bio-Rad Laboratories, Inc., USA).

For comparison with the genotypes of turkeys of Russian breeds, the described genotypes of turkeys (AM) of the gene pool of the University of Minnesota (Nicholas Turkey Breeding Farms) were used [18, 19].

The average number of alleles and the number of effective alleles per locus (Na, Ne), the degree of observed and expected heterozygosity (H_o, H_e), and Shannon's index (I) were determined using the Microsoft Excel 2007 and GenAIEx v 6.5 software packages [20]. Means (*M*) and standard deviations (± SD) were calculated. The genetic structure of populations was assessed based on the F_{st} values [21] and genetic distances according to Nei [22]. The phylogenetic tree was constructed using the neighbor-joining method using the Structure 2.3.4 software [23].

Results. For the work, the researchers selected microsatellite loci, which were used in the study of turkeys of the gene pool of the University of Minnesota (NTBF) (Table 1).

1. Primers used for microsatellite genotyping of turkeys (*Meleagris gallopavo*)

Locus	Accession number in GenBank	Nucleotide sequence	Primer sequence (5′→3′)		DNA fragment size, bp
			forward	reverse	
MNT9	AF482368	$(CA)_{18}$	TGGGAGTGGAAAGGTGAAAG	TTCTCCTCAGCTCAGCAACC	164, 168
MNT10	AF482369	$(TG)_{10}+(TTTTG)_5$	TTCCCAGTGCACTACCTGAAC	TGAACAGTGATTCCACTGAAGC	67, 78
MNT11	AF482370	$(TG)_{12}$	TTTCTGACACAGGTACAAGGAAAC	GCCCTCGAGTATTAGCCACTC	90
MNT12	AF482371	$(TG)_{14imp}$	AGGTGTTTTTGGGCAGTCTC	TGCAAGCACCATCTGCTAAG	121, 145
MNT13	AF482372	$(TG)_{20}$	TTAGGGGATGCTGAACTGTG	GCGTAATTGGTGCTTTCTCC	183, 185, 187, 235
MNT14	AF482373	$(CA)_{10}$	AAACAGAACAACCTCAAGGACAG	GAATTGGGTTTGCATTTGAG	177, 181
MNT15	AF482374	$(CA)_{12}$	TTGTTGCTGTTGTTTTTGTGG	TTTCTGTGCCTAAGCTTAATGTG	188
MNT16	AF482375	$(TG)_{13}+(TG)_{11}+(TG)_8+(TG)_5$	TGTTTGCCTGCAATAAGCTG	GCACCCTCCCACTGACTG	219, 226, 234
MNT17	AF482376	$(TA)_5+(CA)_{29}$	AGGAGCACCCAGCTCAAAG	GAGTAATACCAAGGAAAAGTGTGC	181
MNT18	AF482377	$(TG)_{13}$	GCAGGCACAGAGAGCTACG	CCAATGTTGAAGCAGGTGAG	158, 159, 161, 162
MNT19	AF482378	$(TG)_{22}$	GCAGGAGGCTCTGAGCTATG	TTATACGGAAGGCGGTTGAG	224, 250
MNT20	AF482379	$(CA)_{15}$	TAACTGTCTGCCAGGTGGTG	GATCTCGGGTGGTGATTGC	192, 195

Analysis of the data obtained made it possible to establish that the turkeys of Russian breeds and the gene pool of the University of Minnesota were characterized by low genetic diversity. The number of identified alleles in microsatellite loci as a whole for the breed sample varied from 1 to 4 (Table 2).

2. The number of alleles of microsatellite loci in turkeys (*Meleagris gallopavo*) of Russian breeds (North Caucasian Zonal Poultry Experimental Station, Stavropol Territory, 2019) **and Gene Pool Populations of Nicholas Turkey Breeding Farms** (University of Minnesota)

Locus	Breed							
	BSH	BrSK	BeSK	SSK	MB	CHT	UP	AM
MNT9	2	2	2	1	2	2	1	2
MNT10	1	1	1	2	2	1	1	1
MNT11	1	1	1	1	1	1	1	3
MNT12	2	2	2	1	2	1	1	1
MNT13	4	3	3	2	2	1	1	2
MNT14	2	1	1	1	2	1	1	2
MNT15	1	1	1	1	1	1	1	2
MNT16	2	2	2	1	1	2	1	2
MNT17	1	1	1	1	1	1	1	2
MNT18	3	2	2	1	3	1	1	1
MNT19	1	1	1	1	2	2	2	1
MNT20	2	1	1	1	2	2	2	2

Note. BSH — Belaya shirokogrudaya, BrSK — Bronzovaya Severokavkazskaya, BeSK — Belaya Severokavkazskaya, SSK — Serebristaya Severokavkazskaya, MB — Moscowskaya Belaya, CHT — Chernaya Tikhoretskaya, UP — Uzbekskaya palevaya, AM — population of a gene pool of the University of Minnesota [18, 19].

The average number of alleles (Na) per locus ranged from 1.0 to 1.83, with the largest number being characterized by the BSH (1.83), MB (1.75) breeds, and the AM population (1.75). One allele per locus was identified in the SSK and UP breeds. Similar patterns were observed in relation to the number of effective alleles (Ne): the highest value of this indicator was in the BSH breed and the AM population (1.58 and 1.55), the minimum − in the SSK and UP breeds (1.0), the Moscowskaya Belaya and Chernaya Tikhoretskaya occupied an intermediate position (1.30 and 1.16). The revealed low number of alleles per microsatellite locus in turkeys of Russian breeding is consistent with the data of foreign researchers, who indicate a low genetic diversity of commercial turkey breeds in comparison with other species of farm animals and poultry, as well as wild turkey populations [24, 25].

3. Genetic diversity of turkeys (*Meleagris gallopavo*) of Russian breeds ($n = 30$, North Caucasian Zonal Poultry Experimental Station, Stavropol Territory, 2019) **and Gene Pool Populations of Nicholas Turkey Breeding Farms** (University of Minnesota) inferred from microsatellite loci ($M \pm SD$)

Breed	Na	Ne	H_o	H_e	$H_o–H_e$	I
BSH	1.83±0.27	1.58±0.19	0.273±0.07	0.279±0.07	–0.006	0.416±0.12
BrSK	1.50±0.19	1.07±0.03	0.063±0.02	0.065±0.02	–0.002	0.122±0.04
BeSK	1.50±0.19	1.43±0.18	0.207±0.07	0.212±0.07	–0.005	0.304±0.11
SSK	1.00±0.01	1.00±0.00	0.000±0.00	0.000±0.00	0.000	0.000±0.00
MB	1.75±0.17	1.30±0.12	0.189±0.05	0.194±0.05	–0.005	0.312±0.08
CHT	1.33±0.14	1.16±0.08	0.106±0.04	0.111±0.04	0.005	0.162±0.07
UP	1.00±0.01	1.00±0.00	0.000±0.00	0.000±0.00	0.000	0.000±0.00
AM	1.75±0.17	1.55±0.15	0.286±0.07	0.291±0.07	0.005	0.419±0.14

Note. BSH — Belaya shirokogrudaya, BrSK — Bronzovaya Severokavkazskaya, BeSK — Belaya Severokavkazskaya, SSK — Serebristaya Severokavkazskaya, MB — Moscowskaya Belaya, CHT — Chernaya Tikhoretskaya, UP — Uzbekskaya palevaya, AM — population of a gene pool of the University of Minnesota [18, 19]. Na and Ne — average and effective numbers of alleles per locus, H_o and H_e — observed and expected heterozygosity, I — Shannon index.

Comparison of the values of expected and observed heterozygosity ($H_o–H_e$) showed a lack of heterozygotes in all studied breeds and populations from

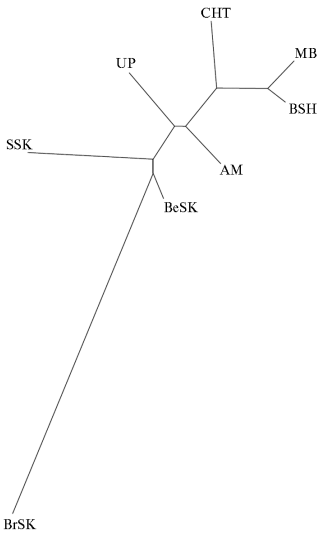
0.2 to 0.6%. The low genetic diversity was also evidenced by the Shannon information index, which did not exceed 0.50, and in the SSK and UP breeds it was equal to zero (Table 3).

Calculation of Nei's genetic distances (Table 4) and cluster analysis using the neighbor-joining tree method made it possible to obtain a graphical display of the phylogenetic relationship (Fig.) between the Russian breeds of turkeys and the population of the gene pool of the University of Minnesota.

4. Genetic differentiation of turkeys (*Meleagris gallopavo*) of Russian breeds (North Caucasian Zonal Poultry Experimental Station, Stavropol Territory, 2019) **and Gene Pool Populations of Nicholas Turkey Breeding Farms** (University of Minnesota) inferred from microsatellite loci

Порода	BSH	BrSK	BeSK	SSK	MB	CHT	UP	AM
BSH	0.000	0.250	0.127	0.594	0.344	0.457	0.162	4.872
BrSK	0.346	0.000	0.046	0.425	0.493	0.430	0.461	–
BeSK	0.180	0.127	0.000	0.579	0.514	0.469	0.212	–
SSK	0.495	0.423	0.514	0.000	0.131	0.363	1.386	–
MB	0.300	0.467	0.443	0.178	0.000	0.228	0.760	–
CHT	0.392	0.431	0.415	0.502	0.267	0.000	0.762	–
UP	0.241	0.612	0.339	0.870	0.672	0.672	0.000	3.684
AM	0.600	0.728	0.643	0.735	0.633	0.705	0.723	0.000

Примечание. BSH — Belaya shirokogrudaya, BrSK — Bronzovaya Severokavkazskaya, BeSK — Belaya Severokavkazskaya, SSK — Serebristaya Severokavkazskaya, MB — Moscowskaya Belaya, CHT — Chernaya Tikhoretskaya, UP — Uzbekskaya palevaya, AM — population of a gene pool of the University of Minnesota [18, 19]. Nei's genetic distance [22] is above the diagonal, Fst values in pairwise comparison is under the diagonal. Dashes indicate no indicators.



Genetic links between turkeys (*Meleagris gallopavo*) of Russian breeds (North Caucasian Zonal Poultry Experimental Station, Stavropol Territory, 2019) **and Gene Pool Populations of Nicholas Turkey Breeding Farms** (University of Minnesota) **inferred from Nei's genetic distance [22]:** BSH — Belaya shirokogrudaya, BrSK — Bronzovaya Severokavkazskaya, BeSK — Belaya Severokavkazskaya, SSK — Serebristaya Severokavkazskaya, MB — Moscowskaya Belaya, CHT — Chernaya Tikhoretskaya, UP — Uzbekskaya palevaya, AM — population of a gene pool of the University of Minnesota [18, 19].

The genetic distance dendrogram showed the smallest genetic difference between the MB and BSH breeds. The BeSK, SSK, and BrSK breeds formed a separate node, while the BrSK breed showed the greatest genetic removal, both in this node and with other breeds, forming the largest branch in terms of genetic distance. CHT, UP, and AM populations formed separate branches at relatively equal distances. The location of turkey breeds on the genetic distance tree, apparently, was due to the history of their creation. The Belaya shirokogrudaya breed is one of the oldest breeds in the world, created in the 1960s in the United States. She is of genetic origin from Dutch white turkeys. The Belaya shirokogrudaya turkey breed, namely four lines of the Hidon cross (A, B, C, D), were brought to the North Caucasian zonal station for poultry in 1980 from Holland. On the basis of lines B and D, the parental forms were selected, which have remained pure to the present day. Their DNA samples were used in the present study. The Moscowskaya Belaya breed was created in the Moscow Region. Initially, local white turkeys and Beltsville turkeys were used, and finally, white Dutch turkeys were used. That is, the gene pool of white turkeys was used for a long time to create breeds of Belaya shirokogrudaya and Moscowskaya Belaya, which led to their close location on the tree of genetic distances.

The first Russian breed of turkeys, the Bronzovaya Severokavkazskaya one,

was bred in the 1950s-1960s by crossing local aboriginal turkeys with producers of the Bronzovaya and Bronzovaya shirokogrudaya breeds. The second domestic breed, the Belaya Severokavkazskaya breed, was created in the 1970s-1980s by crossing native Bronzovaya Severokavkazskaya turkeys with males of the Belaya shirokogrudaya breed of English origin. When breeding the Serebristaya Severokavkazskaya breed released in 2008, the population of Uzbekskaya palevaya turkeys in the first stages was improved by the Belaya shirokogrudaya breed, mainly males of the O4 line of the maternal type, in order to increase reproduction and meat productivity. At the final stage of the creation of the breed, individuals with different shares of the gene pool of the white broad-breasted were bred "in themselves" with strict culling of phenotypes that did not meet the requirements. Consequently, the Belaya Severokavkazskaya and Serebristaya Severokavkazskaya breeds were created using the gene pool of Belaya shirokogrudaya, which, apparently, determined their great genetic proximity to each other and some distance from Bronzovaya Severokavkazskaya, which was created exclusively using the bronze plumage breeds. It is possible that the formation of a common node by the Bronzovaya, Belaya, and Serebristaya Severokavkazskaya breeds was also influenced by the fact that they were all created in the North Caucasian region. Habitat factors of the same type, apparently, contributed to the selection of closely related genotypes.

The genetic remoteness of the Chernaya Tikhoretskaya and Uzbekskaya palevaya breeds is explained by the fact that they were created to a greater extent using populations of local turkeys. The Uzbekskaya palevaya breed was bred on the basis of the Uzbekskaya Bronzovaya turkeys, which were pointwise improved by the Belaya shirokogrudaya breed. The use of the Belaya shirokogrudaya breed in the breeding of Uzbekskaya palevaya should have influenced their genetic affinity. However, the Chernaya Tikhoretskaya breed turned out to be closer to the Belaya shirokogrudaya and Moscowskaya Belaya breeds. It can be assumed that the significant geographical remoteness of the area of creation of the Uzbekskaya palevaya breed determined a greater genetic difference with the Moscowskaya Belaya and Belaya Severokavkazskaya breeds, which, like the Chernaya Tikhoretskaya, were created in the North Caucasian region.

The population of turkeys of the gene pool of the University of Minnesota showed a certain genetic distance from Russian breeds. However, this distance was not as pronounced as expected. Probably, the gene pool of the AM population includes both the gene pool of breeds with bronze plumage and the gene pool of the most widespread white broad-breasted breed in the world, which determines its equal distance from the studied Russian turkey breeds.

The data obtained in this study largely coincide with the results of Fisinin et al. [17]. Constructing a dendrogram based on genetic distances using the same microsatellite loci, but using a smaller sample and using the START 2 program, distributed the studied breeds into two clusters. The first cluster was formed by a part of the genotypes of Belaya shirokogrudaya and all genotypes of the Uzbekskaya Palevaya breed, the second — by two large subclusters. The first subcluster was formed by the genotypes of the Bronzovaya Severokavkazskaya, Belaya Severokavkazskaya, and Belaya shirokogrudaya breeds, the second — by the genotypes of the Chernaya Tikhoretskaya, Serebristaya Severokavkazskaya and Moscowskaya Belaya breeds [17]. In the above and the present study, the closest were the Belaya shirokogrudaya and Moscowskaya Belaya, Belaya Severokavkazskaya and Serebristaya Severokavkazskaya breeds; the Chernaya Tikhoretskaya and Uzbekskaya palevaya were more genetically remoted. The use in the presented work of a larger number of individuals and the method of the nearest neighbor in the Structure 2.3.4 program revealed a more significant genetic differentiation of the Bronzovaya

Severokavkazskaya breed.

In another study, also carried out at the North Caucasian Zonal Experimental Station for Poultry, using the DNA fingerprinting method, it was shown that the most similar breeds were Bronzovaya Severokavkazskaya and Belaya Severokavkazskaya, followed by Serebristaya Severokavkazskaya and Uzbekskaya palevaya. Chernaya Tikhoretskaya showed a significant genetic distance from the breeds of the Belaya shirokogrudaya, Belaya Severokavkazskaya, Serebristaya Severokavkazskaya, Bronzovaya Severokavkazskaya, and Uzbekskaya palevaya [26].

Many authors point out that the main factors affecting the degree of genetic differentiation of domestic breeds and wild populations of turkeys are the use of the gene pool of some breeds when creating others and the geographical area of their breeding. At the same time, scientists are unanimous in the opinion that the genome of turkeys is much less diverse than the genome of other types of farm animals and poultry.

Latch et al. [24] investigated wild oriental (*M. gallopavo silvestris*) and Russian turkey (*M. gallopavo*) using seven microsatellite markers. The number of alleles per locus varied from 5 to 15, while Russian turkeys compared to eastern wild ones were characterized by significantly fewer alleles per locus and general heterozygosity.

Kamara et al. [27] studied the genetic differentiation between commercial and non-commercial turkey breeds — Narra-gansett, Bourbon Red, Blue Slate, Spanish Black, and Royal Palm from the gene pool of the Virginia College farm 10 microsatellite loci (RHT0009, RHT0011, RHT0024, RHT0095, RHT0131, RHT0216, RHT0294, TUM16, TUM20, ADL0023). Using phylogenetic analysis, it was found that the Narra-ganset, Bourbon Red, and Blue Slate breeds had greater genetic similarity to commercial breeds than Spanish Black and Royal Palm [27]. Similar data for these breeds were obtained with other genetic marker systems (SNPs and DNA fragments of random sequences − random amplification of polymorphic DNA, RAPD) [28]. Kusza et al. [25] carried out a clear genetic differentiation between the Hungarian bronze and Belaya shirokogrudaya turkey breeds based on 15 microsatellite loci. The Hungarian Bronze breed was more polymorphic (average number of alleles per locus 3.20) than Belaya shirokogrudaya (average number of alleles per locus 2.77).

Mock et al. [29] used microsatellite markers and mitochondrial DNA in its most variable part to study genetic relationships between wild turkey populations. They studied 24 populations of six subspecies of wild turkeys: seven — Rio Grande (*M. gallopavo intermedia*), six — eastern turkey (*M. gallopavo silvestris*), three — Florida (Florida, *M. gallopavo osceola*), five — Merriam's (*M. gallopavo merriami*), three — Gould's (*M. gallopavo mexicana*). The authors established the correspondence of the modern division of wild turkey subspecies, based on the morphological description, to their genetic characteristics, except for the eastern turkey and Florida, which showed no genetic differences. The populations of Merriam and Rio Grande showed a positive relationship between genetic and geographic distance, while no such relationship was found in populations of eastern turkey.

For deeper information on the genetic diversity of turkey breeds and lines, Aslam et al. [30] used a more modern and informative method — whole-genome sequencing. As a result of scanning the genome of 32 turkeys from different populations, 5.49 million SNPs were identified in relation to the described reference turkey genome (UMD 2.01), which is 1.1 billion bases [31, 32]. The heterozygosity of individuals varied from 0.17 to 2.73 SNP, and throughout the entire sample ranged from 0.73 to 1.64 SNP per thousand base pairs. The authors concluded that the studied commercial breeds and lines of turkeys had a common origin, while the genetic basis for their breeding was wild forms of turkeys, which are

characterized by higher heterozygosity. The authors also emphasize that the genome of turkeys, in comparison with the genome of other species of farm animals and poultry, is characterized by a much greater conservatism.

Thus, the genetic peculiarity and differences in the genotypes of turkey breeds of the breeding and genetic center for breeding and preserving the gene pool of domestic turkey breeds in comparison with the turkey population of the gene pool of the University of Minnesota are shown. The smallest genetic differences were found between the breeds of Moscowskaya Belaya and Belaya shirokogrudaya, Belaya Severokavkazskaya and Serebristaya Severokavkazskaya. The Chernaya Tikhoretskaya and Uzbekskaya palevaya breeds, as well as the population of turkeys of the University of Minnesota gene pool, showed great genetic remoteness both from the above breeds and among themselves. The highest genetic differentiation was demonstrated by the Bronzovaya Severokavkazskaya breed. Intra-breed characteristics and inter-breed differentiation of seven Russian turkey breeds by microsatellite DNA markers largely reflect the history of their creation and improvement. Analysis of the number of alleles per locus made it possible to confirm the regularity revealed by other researchers, according to which the gene pool of breeds and populations of domestic turkeys is characterized by insignificant genetic diversity. For further rational use of the gene pool of domestic turkey breeds, as well as obtaining new information about their genetic characteristics and place in genetic differentiation among other turkey breeds bred in the world, it is advisable to use additional methods and modern markers of genetic analysis, such as SNP and MLST sequencing.

REFERENCES

1. Stolpovskii Yu.A., Zakharov-Gezekhus I.A. *Vavilovskii zhurnal genetiki i selektsii*, 2017, 21(4): 477-486 (doi: 10.18699/VJ17.266) (in Russ.).
2. Jarne P., Lagoda P.J.L. Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution*, 1996, 11(10): 424-429 (doi: 10.1016/0169-5347(96)10049-5).
3. Gholizadeh M., Mianji G.R. Use of microsatellite markers in poultry research. *International Journal of Poultry Science*, 2007, 6(2): 145-153 (doi: 10.3923/ijps.2007.145.153).
4. Putman A.I., Carbone I. Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecology Evolution*, 2014, 4(22): 4399-4428 (doi: 10.1002/ece3.1305).
5. Tan C., Bian C., Yang D., Li N., Wu Z.-F., Hu X.-X. Application of genomic selection in farm animal breeding. *Yi Chuan*, 2017, 39(11): 1033-1045 (doi: 10.16288/j.yczz.17-286).
6. Weigend S., Romanov M.N. The world watch list for domestic animal diversity in the context of conservation and utilisation of poultry biodiversity. *World's Poultry Science Journal*, 2002, 58(4): 411-430 (doi: 10.1079/WPS20020031).
7. Groeneveld L.F., Lenstra J.A., Eding H., Toro M. A., Scherf B., Pilling D., Negrini R., Finlay E.K., Jianlin H., Groeneveld E., Weigend S., the GLOBALDIV Consortium. Genetic diversity in farm animals — a review. *Animal Genetics*, 2010, 41(s1): 6-31 (doi: 10.1111/j.1365-2052.2010.02038.x).
8. Soller M., Weigend S., Romanov M.N., Dekkers J.C.M., Lamont S.J. Strategies to assess structural variation in the chicken genome and its associations with biodiversity and biological performance. *Poultry Science*, 2006, 85(12): 2061-2078 (doi: 10.1093/ps/85.12.2061).
9. Wilkinson S., Wiener P., Teverson D., C.S.Haley, Hjcking P.M. Characterization of the genetic diversity, structure and admixture of British chicken breeds. *Animal Genetics*, 2012, 43(5): 552-563 (doi: 10.1111/j.1365-2052.2011.02296.x).
10. Reed K.M., Mendoza K.M., Beattie C.W. Comparative analysis of microsatellite loci in chicken and turkey. *Genome*, 2000, 43(5): 796-802.
11. Colombo E., Strillacci G., Cozzi M.C., Madeddu M., Mangiagalli M.G., Mosca F., Zaniboni L., Bagnato A., Cerolini S. Feasibility study on the FAO chicken microsatellite panel to assess genetic variability in the turkey (*Meleagris gallopavo*). *Italian Journal of Animal Science,* 2014, 13(4): 887-890 (doi: 10.4081/ijas.2014.3334).
12. Novgorodova I.P., Gladyr' E.A., Fisinin V.I., Zinov'eva N.A. *Dostizheniya nauki i tekhniki APK*, 2015, 29(11): 88-90 (in Russ.).
13. Novgorodova I.P., Volkova V.V., Gladyr' E.A., Selionova M.I., Rastovarov E.I., Fisinin V.I.,

Zinov'eva N.A. *Dostizheniya nauki i tekhniki APK*, 2011, 10: 66-67 (in Russ.).
14. Reed K.M., Chaves L.D., Garbe J.R., Da Y., Harry D.E. Allelic variation and genetic linkage of avian microsatellites in a new turkey population for genetic mapping. *Cytogenetic and Genome Research*, 2003, 102(1-4): 331-339 (doi: 10.1159/000075771).
15. Reed K.M., Chaves L.D., Knutson T.P., Krueth S.B., Ashwell C.M., Burt D.W. Integration of microsatellite — based genetic maps for the turkey (*Meleagris gallopavo*). *Genome*, 2006, 49(10): 1308-1318 (doi: 10.1139/g06-084).
16. Smith E.J., Geng T., Long E., Pierson F.W., Sponenberg D.P., Larson C., Gogal R. Molecular analysis of the relatedness of five domesticated turkey strains. *Biochemical Genetics*, 2005, 43(1-2): 35-47 (doi: 10.1007/s10528-005-1065-5).
17. Fisinin V.I., Selionova M.I., Shinkarenko L.A., Shcherbatova N.G., Kononova L.V. Study of microsatellites in the Russian breeds of turkey. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2017, 52(4): 739-748 (doi: 10.15389/agrobiology.2017.4.739eng).
18. Reed K.M., Chaves L.D., Rowe J.A. Twelve new turkey microsatellite loci. *Poultry Science*, 2002, 81(12): 1789-1791 (doi: 10.1093/ps/81.12.1789).
19. Reed K.M., Roberts M.C., Murtaugh J., Beattie C.W., Alexander L.J. Eight new dinucleotide loci in turkey (*Meleagris gallopavo*). *Animal Genetics*, 2000, 31(2): 140-157 (doi: 10.1046/j.1365-2052.2000.00571.x).
20. Peakall R., Smouse P.E. GenAIEx 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).
21. Weir B.S., Cockerham C.C. Estimating F-statistics for the analysis of population structure. *Evolution*, 1984, 38(6): 1358-1370 (doi: 10.2307/2408641).
22. Nei M. Genetic distance between populations. *American Naturalist*, 1972, 106: 283-392.
23. Falush D., Stephens M., Pritchard J.K. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 2003, 164(4): 1567-1587.
24. Latch E.K., Smith E.J., Rhodes O.E. Isolation and characterization of microsatellite loci in wild and domestic turkeys (*Meleagris gallopavo*). *Molecular Ecology Note*, 2002, 2(2): 176-178 (doi: 10.1046/j.1471-8286.2002.00183.x).
25. Kusza S., Mihók S., Czeglédi L., Javor A., Arnyasi M. Testing the breeding strategy of Hungarian Bronze turkey strains for maintaining genetic diversity with microsatellites. *Arch. Anim. Breed.*, 2011, 54(4): 419-429 (doi: 10.5194/aab-54-419-2011).
26. Shinkarenko L.A., Terletskii V.P., Tyshchenko V.I. *Ptitsevodstvo*, 2020, 9: 17-21 (doi: 10.33845/0033-3239-2020-69-9-17-21) (in Russ.).
27. Kamara D., Gyenai K.B., Geng T., Hammade H. Microsatellite marker-based genetic analysis of relatedness between commercial and heritage turkeys (*Meleagris gallopavo*). *Poultry Science*, 2007, 86(1): 46-49 (doi: 10.1093/ps/86.1.46).
28. Knutson T.P., Chaves L.D., Hall M.K., Reed K.M. One hundred fifty-four genetic markers for the turkey (*Meleagris gallopavo*). *Genome*, 2004, 47(6): 1015-1028 (doi: 10.1139/g04-076).
29. Mock K.E., Theimer T.C., Rhodes O.E., Greenberg D.L., Keim P. Genetic variation across the historical range of the wild turkey (*Meleagris gallopavo*). *Molecular Ecology*, 2002, 11(4): 643-657 (doi: 10.1046/j.1365-294X.2002.01467.x).
30. Aslam M.L., Bastiaansen J.W.M., Elferink M.G, Megens H.J., Crooijmans R.P.M.A., Blomberg L.A., Fleischer R.C., Tassell C.P., Sonstegard T.S., Schroeder C.G., Groenen M.A.M, Long J.A. Whole genome SNP discovery and analysis of genetic diversity in Turkey (*Meleagris gallopavo*). *BMC Genomics*, 2012, 13: 391-404 (doi: 10.1186/1471-2164-13-391).
31. Flicek P., Amode M.R., Barrell D., Beal K., Brent S., Carvalho-Silva D., Clapham P., Coates G., Fairley S., Fitzgerald S., Gil L., Gordon L., Hendrix M., Hourlier T., Johnson N., Кдндri A. K., Keefe D., Keenan S., Kinsella R., Komorowska M., Koscielny G., Kulesha E., Larsson P., Longden I., McLaren W., Muffato M., Overduin M.M.B., Pignatelli M., Pritchard B., Riat H.S., Ritchie G., Ruffier M., Schuster M.R.B., Sobral D., Tang A., Taylor T., Trevanion S., Vandrovcova J., White S.J., Wilson M., Wilder S.P., Aken B.L., Birney E., Cunningham F., Dunham I., Durbin R., Fernandez-Suarez X., Harrow J., Herrero J., Hubbard T., Parker A., Proctor G., Spudich G., Vogel J., Yates A., Zadissa A., Searle S. Ensembl 2012. *Nucleic Acids Research*. 2012, 40(D1): D84-D90 (doi: 10.1093/nar/gkr991).
32. Dalloul R. A., Long J.A., Zimin A.V., Aslam L., Beal K., Blomberg L. A., Bouffard P., Burt D.W., Crasta O., Crooijmans R.P.M.A., Cooper K., Coulombe R.A., De S., Delany M.E., Dodgson J.B., Dong J.J., Evans C., Frederickson K.M., Flicek P., Florea L., Folkerts O., Groenen M.A.M., Harkins T., Herrero J., Hoffmann S., Megens H.-J., Jiang A., Jong P., Kaiser P., Kim H., Kim K-W., Kim S., Langenberger D., Lee M-K., Lee T., Mane S., Marcais G., Marz M., McElroy A., Modise T., Nefedov M., Notredame C., Paton I.R., Payne W.S., Pertea G., Prickett D., Puiu D., Qioa D., Raineri E., Ruffier M., Salzberg S.L., Schatz M.C., Scheuring C., Schmidt C.J., Schroeder S., Searle S.M.J., Smith E.J., Smith J., Sonstegard T.S., Stadler P.F., Tafer H., Tu Z.J., Tassell C.P., Vilella A.J., Williams K.P., Yorke J.A., Zhang L., Zhang H.-B., Zhang X., Zhang Y., Reed K.M. Multi-platform next-generation sequencing of the domestic turkey (*Meleagris gallopavo)*: Genome Assembly and Analysis. *PLoS Biology*, 8(9): e1000475 (doi: 10.1371/journal.pbio.1000475).