Investigation of the D-loop sequence of mitochondrial DNA of the Volgograd sheep breed

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Abstract. The paper presents the results of studies of the sequence of the D-loop of mitochondrial DNA of the Volgograd sheep breed. Mitochondrial DNA has a number of unique features that make it possible to effectively use markers based on it in phylogenetic studies of a wide range of organisms.

1. Introduction
Genomic selection of animals in the XXI is becoming increasingly important in connection with the need to accelerate the selection process and achieve the desired results as soon as possible [1, 2]. It includes many aspects, the most important of which are: genotyping of animals with reliable values of breeding value, development of a prediction equation for the reference population, assessment of young animals using regression models based on the use of genetic markers (Direct Genomic Value, DGV); selection of assessed juveniles that meet the requirements of the breeding program, etc. [3, 4, 5].

DNA markers are a convenient tool in studies to reconstruct the origin of breeds of domestic animals, in particular sheep. The synthetic origin of domestic sheep breeds, confirmed at the molecular level, limits the information content of nuclear DNA markers when studying their demographic history. Analysis of the sequence of mitochondrial DNA, which has a maternal character of inheritance, serves as an effective way to assess the historical origin of breeds [6].

Various types of DNA markers are used in the study of sheep biodiversity. Mitochondrial DNA develops 5-10 times faster than nuclear DNA, and different parts of its genome change at different rates. This suggests that mitochondrial DNA is the most suitable marker for studying the pro-cesses of micro- and macro-development. The most informative region of mitochondrial DNA for the study of maternal inheritance due to its high variability is the Control region or D-loop, the size of which ranges from 16613 to 16620 BP in domestic sheep, and 16613 to 16696 BP in wild sheep. The difference in the length of mitochondrial DNA is mainly due to the variability in the length of tandem repeats (75-76 BP) and their number in the D-loop (D-loop, control region, CR) of mitochondrial DNA.
Mitochondrial DNA has a number of unique features that make it possible to effectively use markers based on it in phylogenetic studies of a wide range of organisms. Mitochondrial DNA is easily isolated from biological samples, since it is represented in cells by a large number of copies. In most cases, mitochondrial DNA does not recombine and is inherited through the maternal line, which greatly simplifies the study and subsequent analysis of the results obtained.

The aim of this work was to obtain data on the nucleotide sequence of the mitochondrial DNA D-loop of the Volgograd sheep breed. Based on the data obtained, the analysis of mitochondrial DNA D-loop polymorphism was carried out in a comparative aspect with breeds of domestic and foreign selection. The use of this marker in the light of modern ideas about the origin and genetic diversity of sheep expands our understanding of the history of formation and the current state of the gene pool of the Volgograd sheep breed.

2. Materials and methods
Sheep of the Volgograd breed (n = 13) served as the material for research on mitochondrial DNA of Agricultural production cooperative - Plemzavod «Romashkovsky» of the Volgograd region. Mitochondrial DNA was isolated from tissue samples using a set of regents «K-Sorb-100» (Limited Liability Company «Sintol») in accordance with the manufacturer's instructions. The polymerase chain reaction was carried out according to the standard procedure. The following primers were used to amplify fragments of the mitochondrial DNA D-loop:

OA_D-loop_FGGTCTTGTAAAACCAGAGAAGGAG
OA_D-loop_RTGGAGTCAGTAGACTCATCTAGG(GenBankNC_001941.1).

The visualization of the polymerase chain reaction products was carried out in a 2% agarose gel with the addition of ethidium bromide. Specific fragments of the polymerase chain reaction were isolated from the gel using the Cleanup Mini kit for purifying DNA from the gel (Evrogen Limited Liability Company, Russia). Fragment sequencing services were provided by Syntol. Editing and sequence alignment was performed using the Biöedit v 7.2.6 and MEGA 7 programs. The NCBI accession sequence NC_001941.1 was used as a reference.

To determine the belonging of the studied samples to haplogroups from the NCBI database, the sequences of the D-loop of mitochondrial DNA belonging to haplogroups A, B, C, D, and E were selected (table 1).

| Haplogroups | Code | Geographical origin | GenBanka |
|-------------|------|---------------------|----------|
| A           | RA_1 | Turkey              | DQ852286 |
| B           | RB_1 | Turkey              | DQ852282 |
| C           | RC_1 | Turkey              | DQ852284 |
| D           | RD_1 | Turkey              | DQ852288 |
| E           | RE_1 | Israel              | DQ852280 |

To assess the genetic diversity of the Volgograd breed, the number of haplotypes (H), haplotype (HD) and nucleotide (π) diversity, the average number of nucleotide substitutions per site (k), and genetic distances between populations were determined using the DNA SP 5.10 program. Calculations and construction of ML (maximum likelihood) trees were performed using the MEGA 7.0 program.

To determine the genetic distance between the breeds, the analysis included the sequences of the D-loops of mitochondrial DNA belonging to different breeds of sheep and to determine the belonging of the studied samples to haplogroups from the NCBI database, the sequences of the D-loops of mitochondrial DNA belonging to haplogroups A, B were selected. C, D and E (table 2).
Table 2. Sequences of d-loop haplotypes of different breeds of sheep.

| №  | The name of the breed       | GenBank    |
|----|----------------------------|------------|
| 1  | AUSTRALIAN MERINO          | HM236174.1 |
| 2  | AUSTRALIAN ROMNEY          | HM236175.1 |
| 3  | MERINOLANDSCHAF            | NC_001941.1|
| 4  | KAZAKH                     | KF938333.1 |
| 5  | KULUNDA                    | KF938358.1 |
| 6  | ALTAY                      | KF938320.1 |
| 7  | TEXEL                      | KJ954145.1 |

3. Results and discussion
Among the most popular methods for studying the domestication of farm animals, including sheep, is
the analysis of polymorphism of mitochondrial DNA sequences: either a noncoding control region (D-
loop) or complete mitochondrial genomes [7, 8].

We analyzed the complete sequence of the control region (D-loop) of mitochondrial DNA in 13
individuals of the Volgograd breed.

All nucleotide sequences had a length of 1179 BP, and we also determined the primary structure of
nucleotides between positions 15437-16616. In all studied animals, 4 tandem turns of 75 BP were
established.

Table 3. Indicators of genetic diversity within the breed.

| Volgograd breed | N  | S   | H   | HD   | k  | π   |
|-----------------|----|-----|-----|------|----|-----|
|                 | 13 | 88  | 13  | 1.000| 25.231 | 0.02207 |

Based on the obtained sequences of 13 fragments of the D-loop of mitochondrial DNA, 88
polymorphic sites were identified in the studied group of sheep. As a result, 13 haplotypes were
identified. The variety of haplotypes in sheep of the Volgograd breed was 1,000. The number of
nucleotide substitutions per site was 25,231. Nucleotide diversity in general for the study group is
0.02207.

The results of the study, coming from foreign sources, showed that domestic sheep, wild rams,
mouflons and argali had 4 tandem repeats of 75 BP, in contrast to Urial sheep, which had one repeat of
75 BP and 4 repeats 76 BP long.

Genetic differences between populations make it possible to determine similarities or differences
between breeds. There are different formulas for calculating genetic distances (Wright, Ney, Cavalli-
Sforza and Edwards, some others), but in practice they all give similar results. In our studies, we
calculated the distance according to the Tamura-Ney model, between the merino and fine-wooled breeds
of domestic (Kazakh, Kulunda) and foreign breeding (Altai merino landscape, Australian merino and
Australian romney march, texel) [9, 10].

The phylogenetic relationships between breeds are constructed using the maximum likelihood
method based on the Tamura-Ney model. The analysis included 8 nucleotide sequences of the
mitochondrial DNA D-loop fragment. All items containing spaces and missing data have been excluded.
There were 1177 positions in the final dataset.

According to the results obtained, the following breeds can be distinguished into a separate group -
Volgograd, Kazakh, Texel.

The fine-wooled Volgograd breed of sheep was bred by the method of complex reproductive crossing
of coarse-wooled fat-tailed queens, chosen as a mother line with fine-wooled rams of the Novokaukau and
Prekos breeds, obtained as a result of hybrid queens in the desired type, mainly of the second generation,
were bred in themselves. The offspring from these crosses, in the first place, did not satisfy in terms of
wool productivity. Therefore, simultaneously with the improvement of meat qualities and early maturity,
in order to improve the indicators of shearing and quality of wool, obtained by crossbred queens since
1948, they began to cross with rams of the Caucasian and, to a lesser extent, Grozny breeds.
The Kazakh fine-wool breed of meat and wool direction was created at the Kazakh Scientific Research Institute of Animal Husbandry in 1931-1946. The uterus of local fat-tailed sheep was taken as a basis, which were crossed with rams of the Precos and Rambouillet breed. To improve the quality of the wool during repeated crossing, rams of the Caucasian, Grozny and Askanian breeds were used.

Work on breeding Texel sheep was begun in the middle of the 19th century on Texel Island by crossing low-yielding marching queens with British rams Lincoln, Leicester, Wenleidale and Hampshire. The import of breeding Texel sheep from Holland, Finland and Australia to the territory of the Russian Federation was carried out in 1996-1998. They were used as enhancers of meat productivity and other economically useful traits when creating an early maturing type of meat sheep.

Merino-landscape sheep were bred by crossing the Spanish fine-fleeced sheep with ewes of the local South German breed. This breed is distinguished by problem-free maintenance, high growth rates, excellent meat qualities, endurance, good wool performance.

From the analysis of our data, it follows that the gene pool of the Volgograd sheep breed is represented by variants of haplotypes included in the widespread haplogroup B, which is typical for European sheep breeds (figure 1, 2). Also in haplogroup B, there are breeds: Kulunda, Kazakh, Texel, Merinolandschaf. This circumstance confirms that the ancestral populations, on the basis of which the Volgograd breed was formed, are of European origin.

Merino Australian breeds belong to haplogroup A, and the Altai breed belongs to haplogroup C, which is characteristic of Chinese sheep breeds.

**Table 4. Genetic distances between breeds.**

| BREED          | Merinolandschaf | Australian merino | Australian romney | Altay  | Texel  | Kazakh | Kulunda |
|----------------|------------------|-------------------|-------------------|--------|--------|--------|---------|
| Australian merino | 0.03836          | -                  | -                  | -      | -      | -      | -       |
| Australian romney    | 0.03733          | 0.00284           | -                  | -      | -      | -      | -       |
| Altay                  | 0.03429          | 0.04143           | 0.04040           | -      | -      | -      | -       |
| Texel                  | 0.00858          | 0.03529           | 0.03427           | 0.03327| -      | -      | -       |
| Kazakh                 | 0.00954          | 0.04041           | 0.03938           | 0.03632| 0.00858| -      | -       |
| Kulunda                | 0.00858          | 0.03733           | 0.03631           | 0.03530| 0.00954| 0.00858| -       |
| Volgograd              | 0.00954          | 0.04041           | 0.03938           | 0.03632| 0.00858| 0.00762| 0.00858|

**Figure 1.** Phylogenetic relationships there are 8 nucleotide sequences between cities fragment of the mitochondrial DNA D-loop.
Based on the study of the variability of the D-loop of various sheep breeds, three haplogroups A, B, and C were identified.

In further studies, two more haplogroups B and E were identified. Haplogroup B is observed mainly in mouflon and in European domestic sheep, as for Asian sheep, haplogroup A dominates here. A high frequency of haplogroup A was also established in sheep in New Zealand due to the early imports of Indian animals to Australia. Haplogroup C is less common and is found in domestic sheep in Portugal, Turkey, China, and the Caucasus. Haplogroup D is found in sheep in Romania. The rarest haplogroup E has been identified in Turkish sheep breeds.

4. Conclusions
Thus, the data on the nucleotide sequence of the D-loop of mitochondrial DNA of the Volgograd sheep breed were obtained and a comparative analysis with some breeds of the world gene pool was carried out. Our analysis also showed that all evaluated fine-wool breeds of domestic selection, except for Altai, belong to haplogroup B, merino Australian breeds - to haplogroup A, and Altai sheep breed belongs to haplogroup C.

References
[1] Othman L A, Althwani A N and Alkhazraji A J A H 2016 Growth hormone gene in Iraqi and Turkish Awassi sheep using PCR-RFLP World Journal of Pharmaceutical Research 5(1) 87-93
[2] Saleha Y and Alakilli M 2015 Analysis of polymorphism of Caplstatin and Callipyge genes in Saudi sheep breeds using PCR-RFLP technique Int. J. Pharm. Sci. Rev. Res. 30(1) 340-4
[3] Mamontova T V and Aybazov M M 2016 Genetic markers in animal breeding: experience and prospects (review) Collection of Scientific Papers of the All-Russian Research Institute of Sheep and Goat Breeding 1(9) 480-4
[4] Cannon M V, Brandebourg T D, Kohn M C, Ethikic D, Irwin M H and Pinkert C A 2015 Mitochondrial DNA sequence and phylogenetic evaluation of geographically disparate Sus scrofa breeds Anim. Biotechnol. 26(1) 17-28
[5] Gorlov I F, Kolosov Yu A, Shirokova N V, Getmantseva L V, Slozhenkina M I, Mosolova N I, Bakoev N F, Leonova M A, Kolosov A Yu and Zlobina E Yu 2017 Association of the growth hor-mone gene polymorphism with growth traits in Salsk sheep breed Small Ruminant Research 150 11-4
[6] Deikin A V, Selionova M I, Krivoruchko A Yu, Kovalenko D V and Trukhachev V I 2016 Genetic
markers in beef sheep breeding *Vavilovsky Journal of Genetics and Breeding* 20(5) 576-583

[7] Deniskova T E, Selionova M I, Gladyr E A, Dotsev A V, Bobryshova G T, Kostyunina O V, Brem G and Zinovieva N A 2016 Variability of microsatellites in sheep breeds bred in Russia *Agricultural Biology* 51(6) 801-10

[8] Amerkhanov Kh A, Trukhachev V I and Selionova M I 2017 *From the History of Russian Sheep Breeding* (Stavropol: IP Mokrinsky N S) p 408

[9] Aboneev V V, Chamurliev N G, Kolosov Yu A, Marchenko V V, Aboneev D V and Larionov R P 2018 Wool productivity of young sheep of different origins *Proc. of the Lower Volga Agro-University Comp.* 3(51) 230-6

[10] Kolosov Yu A and Chamurliev N G 2018 Stages of education and development prospects of the Salsk sheep breed *Proc. of the Lower Volga Agro-University Comp.* 1(49) 188-94