Comparison of genetic diversity of the Terskaya and Orlov trotter breeds based on the analysis of STR markers

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Abstract. A comparative analysis of the genetic structure of Terskaya and Orlov Trotter horses was performed based on genotyping by 17 microsatellite markers. It was shown that the Terskaya horse breed is characterized by high allelic richness.. Cluster analysis of the genetic profiles of the horses of the studied breeds showed that Terskaya horses and Orlov Trotters can be distinguished quite accurately based on STR genotyping, which should not, however, replace the analysis of phenotypic traits. In both breeds, there is a predominance of outbreeding matings, which leads to a slight superiority of the observed heterozygosity value over the expected heterozygosity. It was found that in the Terskaya breed of horses, separate groups of related animals can be identified due to the distribution of horses among small private horse farms.

1 Introduction

Horse breeding in Russia is rich in domestic breeds, each of which represents a unique biocultural heritage. Created for specific purposes, such as agricultural work, transportation of passengers and cargo, use in military operations, many domestic breeds are losing in the modern world the significance they had 60-70 years ago [1-9].

In present time many horse breeds have disappeared in different countries, and today the horse population worldwide is estimated at about 60 million, of which about 2 million are in Russia [5, 7].

In recent years, not only the areas of use have changed in Russia, but also the number of horses in various forms of ownership. The number of horses in factories has decreased more than twice, while the number of horses in small farms and private owners is growing. Therefore, there is a reduction in breeding herds of horses, testing and training of animals, which causes concern and creates difficulties in breeding work [6].

The problem is especially acute for gene pool breeds of horses, such as the Terskaya. It can rightfully be considered one of the valuable breeds of domestic origin, which have

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excellent adaptability to the heterogeneous climate of our country, are unpretentious to feed and highly resistant to a number of diseases.

Terskaya horses are a unique breed, a cultural heritage of our country, but are currently under threat of extinction.

Therefore, the primary task is to preserve purebred representatives of this breed [**Error! Reference source not found.**].

As is known, the study of genotypes and the creation of a database of gene pool breeds will help improve breeding work, promote the development and support of domestic horse breeding. It will also be possible to independently assess individual horses by genotype, which will provide reliable data to breeders in order to avoid individuals that are phenotypic similar to representatives of the breed, but do not have a confirmed origin, getting into the breeding core.

To create databases of genetic profiles of a specific breed, genotyping of animals by STR loci has become widespread. Therefore, it seems relevant to us to conduct genotyping of the group of horses of the Terskaya breed, to compare its genetic structure with the group of horses of the Orlov trotter breed and to evaluate how significant the differences between these two breeds are. The Orlov trotter breed of horses was chosen because Arabian horses took part in the creation of both analyzed horse breeds..

2 Material and methods

The material for the study was hair follicles of Terskaya horses of different sexes, ages and origins. The samples were obtained from stud farms and from horses of private owners in Stavropol Krai. A total of 23 hair samples were obtained: 17 from the breed under study (Terskaya) and 6 from an additional breed (Orlov Trotter). The Orlov Trotter was chosen as the additional breed, since both breeds were bred on the basis of the Arabian thoroughbred.

General information about the sample is included in Table 1, indicating the total number, age and sex ratio..

Table 1. Characteristics of horse groups of the studied breeds

Breed	N	Year of birth	Number of horses of different sexes		Number of sire, produced studied horses
			stallions	mares	
Terskaya	17	2000-2019	2	15	14
Orlov trotter	6	2004-2021	4	2	6

Hair follicles were taken mainly from the mane, but in some cases - from the root of the tail, in the amount of 25-30 pieces from each individual. Before DNA extraction, the samples were stored in paper envelopes in the refrigerator to prevent DNA degradation. After extraction, the final DNA concentration was determined in the samples.

A set of primers including 17 microsatellite loci recommended by the International Society for Animal Genetics (ISAG) was used for the analysis.

The study was conducted at the Russian State Agrarian University - Moscow Agricultural Academy named after K.A.Timiryazev in the educational and scientific center for collective use "Service Laboratory for Complex Analysis of Chemical Compounds".

Extraction was carried out using the commercial DNA-Extran - 1 kit from Syntol, which is based on the phenol chloroform extraction method. After DNA extraction, the concentration and quality of the samples were determined.

In the analysis, we use the main panel of STR markers recommended by ISAG for horses. The following microsatellite markers were used: VHL20_FAM, HTG4_FAM,

AHT4_FAM, HMS7_FAM, HTG6_VIC, AHT5_VIC, HMS6_VIC, ASB23_VIC, ASB2_VIC, HTG10_NED, HTG7_NED, HMS3_NED, HMS2_NED, ASB17_PET, LEX3_PET, HMS1_PET, CA425_PET.

At the next stage, we performed a polymerase chain reaction on a Bio-Rad cfx96 amplifier using oligonucleotides with dyes (TAMTRA, R6G, ROX, FAM) synthesized by Syntol. After amplification, a PCR product was obtained for all 17 microsatellites.

Electrophoresis of the amplification products was performed on an Honor 1616 automatic genetic analyzer for sequencing and fragment analysis.

The decoding and documentation of the obtained results was carried out using the software for automatic decoding of the results of fragment analysis Gene Sequencing Analysis. To verify the reliability of the results we obtained, we sent part of the biomaterial to the commercial laboratory Gordiz.

The obtained data were processed using the GenAlex 6.5 macro in MS Excel [**Error! Reference source not found.**. When processing the data, the ISAG allele table was used to convert numerical values into letters [1]. Next, we used the STRUCTURE program to find the genetic difference between the Terskaya and Orlov trotter breeds, and also to check whether the number of microsatellites would allow us to distinguish the genotypes of these two breeds, or this would be insufficient [2, 4].

3 Results and discussion

Analysis of the genetic structure of the selected groups using microsatellites revealed that in 17 loci the number of alleles ranges from 3 to 9. The highest polymorphism is observed in the AHT4, ASB2, and LEX3 loci in the Terskaya group; HMS2, ASB17 and LEX3 are in the Orlov group, and the lowest is in the HTG7 loci of the Terskaya group; HTG2 – Orlov (Table 2).

Table 2. Genetic diversity of 17 STR loci of the Terskaya and Orlov trotting breeds

Locus	Terskaya breed			Orlov trotter		
	Na	Ne	I	Na	Ne	I
VHL20_FAM	5,000	4,042	1,503	4,000	3,429	1,309
HTG4_FAM	4,000	1,801	0,794	3,000	2,182	0,888
AHT4_FAM	8,000	5,898	1,880	4,000	2,667	1,127
HMS7_FAM	6,000	5,070	1,690	3,000	2,000	0,868
HTG6_VIC	5,000	3,284	1,337	2,000	1,385	0,451
AHT5_VIC	5,000	3,635	1,386	4,000	3,130	1,237
HMS6_VIC	5,000	3,440	1,357	3,000	2,571	1,011
ASB23_VIC	7,000	4,281	1,648	3,000	3,000	1,099
ASB2_VIC	8,000	3,803	1,646	4,000	2,880	1,199
HTG10_NED	6,000	4,250	1,577	4,000	2,880	1,199
HTG7_NED	3,000	2,028	0,783	3,000	2,571	1,011
HMS3_NED	6,000	3,905	1,542	4,000	2,880	1,199
HMS2_NED	7,000	2,087	1,171	5,000	3,600	1,424
ASB17_PET	6,000	5,115	1,709	5,000	3,130	1,358
LEX3_PET	8,000	5,780	1,875	5,000	3,789	1,445
HMS1_PET	4,000	1,883	0,909	3,000	1,674	0,721
CA425_PET	5,000	3,322	1,292	3,000	2,182	0,888
Mean	5,765	3,743	1,417	3,647	2,703	1,084
SE	0,359	0,312	0,083	0,209	0,160	0,064

Na = No. of Different Alleles
Ne = No. of Effective Alleles
I = Shannon's Information Index

The average value of heterozygosity is 0.734 and 0.618 in the Terskaya breed and in the Orlov trotters, respectively, which indicates a higher genetic diversity of the Terskaya breed. The analysis of the values of the expected average heterozygosity and the observed heterozygosity (0.694 and 0.734 for the Terskaya breed, 0.604 and 0.618 for the Orlov trotter breed) showed that in the compared groups random mating system prevails over inbreeding, and in the Terskaya breed to a greater extent than in the Orlov trotters (table 3).

Table 3. Characteristics of heterozygosity of 17 STR-locus in the Terskaya and Orlov trotting breeds

Locus	**Terskaya breed**				**Orlov trotter**			
	Ho	**He**	**uHe**	**F**	**Ho**	**He**	**uHe**	**F**
VHL20_FAM	0,882	0,753	0,775	-0,172	0,667	0,708	0,773	0,059
HTG4_FAM	0,353	0,445	0,458	0,206	0,500	0,542	0,591	0,077
AHT4_FAM	0,824	0,830	0,856	0,008	0,500	0,625	0,682	0,200
HMS7_FAM	**0,941**	0,803	0,827	-0,172	0,500	0,500	0,545	0,000
HTG6_VIC	0,647	0,696	0,717	0,070	0,333	0,278	0,303	-0,200
AHT5_VIC	0,706	0,725	0,747	0,026	**1,000**	0,681	0,742	-0,469
HMS6_VIC	0,765	0,709	0,731	-0,078	0,500	0,611	0,667	0,182
ASB23_VIC	0,824	0,766	0,790	-0,074	0,667	0,667	0,727	0,000
ASB2_VIC	0,824	0,737	0,759	-0,117	0,833	0,653	0,712	-0,277
HTG10_NED	**0,941**	0,765	0,788	-0,231	0,833	0,653	0,712	-0,277
HTG7_NED	0,412	0,507	0,522	0,188	0,333	0,611	0,667	0,455
HMS3_NED	0,882	0,744	0,766	-0,186	0,667	0,653	0,712	-0,021
HMS2_NED	0,588	0,521	0,537	-0,130	0,833	0,722	0,788	-0,154
ASB17_PET	0,882	0,804	0,829	-0,097	**1,000**	0,681	0,742	-0,469
LEX3_PET	0,765	0,827	0,852	0,075	0,333	0,736	0,803	0,547
HMS1_PET	0,471	0,469	0,483	-0,004	0,500	0,403	0,439	-0,241
CA425_PET	0,765	0,699	0,720	-0,094	0,500	0,542	0,591	0,077
Mean	0,734	0,694	0,715	-0,046	0,618	0,604	0,659	-0,030
SE	0,044	0,031	0,032	0,031	0,053	0,029	0,032	0,069

Ho = Observed Heterozygosity
He = Expected Heterozygosity
uHe = Unbiased Expected Heterozygosity
F = Fixation Index

As recent studies show, the increase in the level of genetic diversity in the Orlov trotter has led to the formation of selection for a complex of economically useful traits [1].

Figure 1 shows the results of cluster analysis for k=2. There is a division into two groups with different genetic information. The first contains samples of Terskaya horses, the second – Orlov trotters. The observed picture is natural, since the Arabian purebred breed was used in both breeds studied. But the development of each of the breeds follows its own path, so the genetic profiles of horses are quite homogeneous within their breed and differ from another breed.

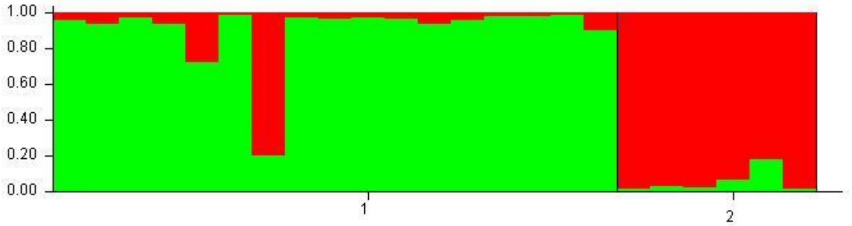

Fig. 1. Results of cluster analysis: 1 group – Terskaya horse breed, 2 group – Orlov trotting breed.

However, it is obvious that in the Terskaya breed group there are individuals whose genotype is noticeably different from their group, but has similarities with the genetic picture of the Orlov trotter profiles.

In order to find an explanation for this phenomenon, it is necessary to find out which individuals stand out. Therefore, we redistributed the results of the cluster analysis by individual animals (Figure 2).

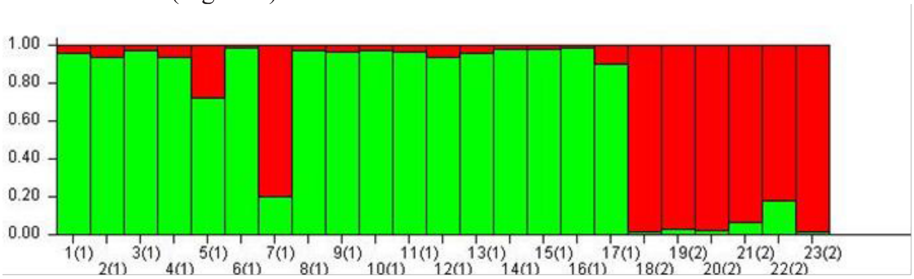

Fig. 2. Results of cluster analysis by individuals (k=2): (1) – Terskaya horse breed, (2) – Orlov trotting breed.

Now we can say for sure that two individuals are the most different from the rest of the group: horse No. 5 and horse No.7. Horse No. 7 from the Terskaya breed group stands out, whose genetic information is most similar to the genetic picture of Orlov trotting horses. Having looked through the pedigree of this horse, we found out that its paternal line is represented mainly by purebred horses, and in the maternal part of the pedigree there are also crosses of thoroughbred and ahalteke breeds.

Among the ancestors of horse number 5, representatives of the Trakehner breed are quite common, so that the proband himself is a quarter blood of this breed. Thus, in the group of Terskaya horses there are horses with a high proportion of blood of other breeds, which is not observed in the group of Orlov trotters.

We evaluated the obtained clustering results using the Clumpack program, which showed that the most optimal division of the presented array of animals is into 3 groups (Figure 3).

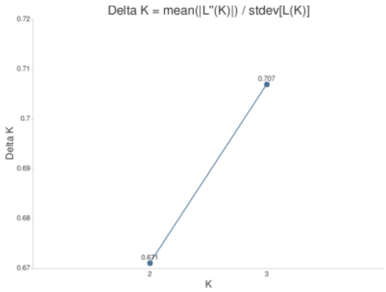

Fig. 3. Optimal number of groups for cluster analysis based on Clumpack processing results.

Figure 4 shows the results of data processing using the STRUCTURE software for 3 groups of genetic information.

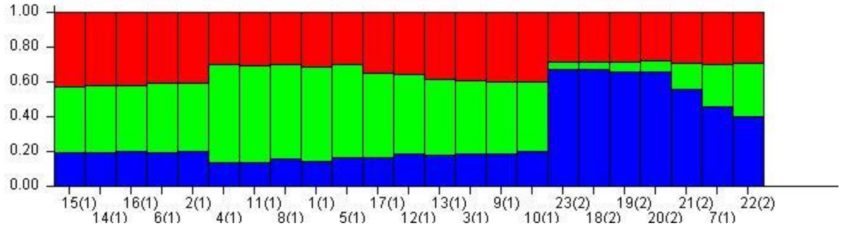

Fig. 4. Results of cluster analysis by individuals (k=3): (1) – Terskaya horse breed, (2) – Orlov trotting breed

The data in Figure 4 show that the third group of animals appeared among the animals of the Terskaya breed of horses, the array of which turned out to be represented by two sets that differ in the structure of genetic information. Horses numbered 14-16, 2 and 6 were included in a separate group, so we decided to compare the pedigrees of these animals.

Analysis of the origin of this group of horses showed that stallions Zeitnot and Cyclotron are found among the ancestors of all 5 animals, and not further than the 3-4 row of the pedigree. This means that cluster analysis separated this group of related animals of the Terskaya breed from all other Terskaya horses that are not related to each other.

Thus, due to the fact that the Terskaya breed of horses is currently kept mainly by owners of private horse farms with a small herd, this can lead to the formation of separate subpopulations in which the degree of relationship is higher than the average for the population. This shows the need for additional research to compare different herds of Terskaya horses, which are geographically isolated from each other. As for the Orlov trotter breed, the structure of the genetic profiles of its representatives is quite uniform, but additional research is also required to compare the genetic structure of the population of different stud farms. This will make it possible to draw up plans for selection work with both unique domestic horse breeds, preserving their genetic diversity.

4 Conclusion

The Terskaya and Orlov Trotter horse breeds have a specific genetic structure and can be reliably distinguished from each other based on cluster analysis. It is also necessary to pay attention to the fact that within the breed there may be separate groups of animals that have a high degree of kinship with each other and will differ in this indicator from the total population of the breed. Based on the obtained results, arrays of loci with the alleles presented in them can be formed as a prototype of the Orlov Trotter and Terskaya horse database, with the help of which it is possible to compare individuals of a particular breed and check for belonging to it and assess the reliability of origin. The created pilot version of the database should be constantly replenished by increasing the population of the horses studied, as well as adding genetic information from other domestic breeds, which will help to increase the accuracy of assessing the origin of animals. All these steps will improve breeding work with domestic horse breeds and contribute to their preservation.

In addition, the creation of databases of STR profiles for identification of a specific breed should not replace the estimation of the compliance of phenotypic characteristics with the breed standard and information on the origin of the horse for at least 5 last generations. Information on the phenotype of the animal and its own productivity are fundamental facts that must be taken into account together with molecular genetic data. It is necessary to remember that the genetic structure of the breed and its structural elements change much faster than the characteristics of productivity and exterior, depends on the geographical isolation of individual intrabreed groups and the principles of selection work with them.

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