The influence of crossbreeding on genetic structure of the Orlov Trotter breed

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Abstract. The aim of the study was to investigate the nature of changes in the genetic structure of the Orlov Trotter breed under the influence of the introductory crossbreeding used in the 60s of the 20th century. We analyzed the results of the genotyping of 6700 trotters registered in the Stud Book of the Orlov Trotter breed on 16 microsatellite loci. The differences between the descendants of purebred and crossbred Orlov Trotter horses were determined by the level of polymorphism and heterozygosity. It was found that in the group of Orlov Trotters pedigree of which did not contain ancestors of other breeds, the expected heterozygosity (He=0.709) was slightly higher than the observed heterozygosity (Ho=0.707) with a positive value of the inbreeding coefficient (Fis=0.004). In all groups of Orlov Trotters, pedigree of which contained ancestors of other breeds in various combinations, the inbreeding coefficient Fis had a negative value. The maximum differences between the Fis values were observed between the group of Orlov Trotters pedigree of which did not contain ancestors of other breeds and the group of Orlov Trotters pedigree of which contained ancestors of 3 other breeds: American Standardbred, Russian Trotter and Thoroughbred.

1. Introduction

Orlov Trotter is the oldest cultural breed created in Russia by the method of complex crossing with horses of many breeds imported from different countries around the world. The breed was named after Count Alexey G. Orlov. The history of Orlov Trotter is associated with Khrenovskoy stud founded by the Count in 1776 in Voronezh province. In the 19th century robust, handsome and elegant Orlov Trotter horses became very popular not only in Russia but also in foreign countries. Many of Orlov Trotters were imported to Western European countries: Austro-Hungary, France, Holland, Germany and Italy. In the 20th century, the great damage to the horse breeding in Russia was caused by the civil war, when a significant part of the breeding stock was lost.

The number of the remained purebred Orlov Trotters was very small and the breed was on the verge of extinction [1]. Because of this by the decree of the Authority of Horse Breeding and the Department for Horse Breeding of the People's Commissariat, all the survived Orlov mares were used only for pure breeding since 1923 [2]. By the mid-twentieth century the restored Orlov Trotter breed became one of the most popular cultural breed of horses in Russia. In the second half of the twentieth century a tendency to a total reduction in the number of breeding horses in the country was provoked by widespread mechanization that resulted in the loss of the former strategic importance of horse breeding.

This led to a significant reduction in the number of breeding animals and the dissolution of a number of studs. According to G. A. Rojdestvenskaya in 1954 there were 3212 Orlov Trotter broodmares at 37 state studs, and by 1981 at 16 studs only 950 broodmares remained that raised serious concerns about the undesirable reduction of the gene pool of the breed [1]. In the 1960s on the task of the Ministry of Agriculture, the attempts of crossbreeding of Orlov Trotter mares with Thoroughbred, Standardbred and
Russian Trotter stallions were made in order to expand the genetic diversity of the breed, as well as to increase the racing performance of horses.

The analysis of crossbreeding results showed that some of crossbred animals showed outstanding performance, but on the whole they provoked a deterioration of the conformation and a significant deviation from the desired type of Orlov Trotter [3].

In 1998 the regulations of the State Stud Book were adopted, according to which only purebred descendants of the registered horses were allowed to be recorded in the Stud Book [4]. It is important to note that in the 1990s, the trend to reduction of the number of Orlov Trotter breeding stock dramatically worsened due to the economic crisis in the country. Only thanks to the undertaken measures on rescue of the oldest national breed, the increase of the mares number used in breeding to 1900 individuals became possible.

Currently, the main breeding stock of the Orlov Trotters is concentrated on the territory of the Russian Federation. Selection is carried out on a complex of traits, the most important of which are: type of the breed, elegance, right conformation and high speed on trot. In order to preserve and improve the breed, it is necessary to work out a breeding program aimed on the preventing reduction of intrabreed diversity. Now investigations aimed at studying changes in the genetic structure of the breed are particularly relevant.

One of the modern effective methods of studying genetic processes in animal populations is based on the use of microsatellite DNA markers [5, 6, 7, 8, 9, 10, 11]. Microsatellite markers are also used for genetic identification and parentage verification in animals. The use of DNA tests became obligatory for all Orlov Trotters in Russia. Genetic studies of cultural and native horse breeds using microsatellite markers showed that the modern Orlov Trotter differs from the most stud breeds by relatively high level of intrabreed polymorphism [12, 13].

In this paper, the differences between the descendants of purebred and crossbred Orlov Trotters at the DNA level were determined for the first time. The aim of our investigation was to study the nature of changes in the genetic structure of the Orlov Trotter breed under the influence of crossbreeding carried out in the 60s of the twentieth century.

2. Materials and methods
Materials for the research were the results of the genotyping of 6700 horses registered in the Stud Book of the Orlov Trotter breed on 16 microsatellite loci: AHT4, AHT5, ASB17, ASB2, ASB23, CA425, HMS1, HMS2, HMS6, HMS7, HTG10, HTG4, HTG6, HTG7, LEX3, VHL20. According to the analysis of 5 pedigree generations all investigated horses were divided into 8 groups:
1. Orlov Trotters pedigree of which does not contain ancestors of other breeds (Orl);
2. Orlov Trotters pedigree of which contains Thoroughbred ancestors (OrlTB);
3. Orlov Trotters pedigree of which contains American Trotter ancestors (OrlAm);
4. Orlov Trotters pedigree of which contains Russian Trotter ancestors (OrlRus);
5. Orlov Trotters pedigree of which contains American and Russian Trotter ancestors (OrlAmRus);
6. Orlov Trotters pedigree of which contains American Trotter and Thoroughbred ancestors (OrlAmTB);
7. Orlov Trotters pedigree of which contains Russian Trotter and Thoroughbred ancestors (OrlRusTB);
8. Orlov Trotters pedigree of which contains American and Russian Trotter and Thoroughbred ancestors (OrlAmRusTB);

The population analysis was carried out to determine the frequency of microsatellite alleles in each of the studied groups of horses, the number of alleles per locus (A), the effective number of alleles (Ae), the observed (Ho) and expected heterozygosity (He) and the inbreeding coefficient Fis.

3. Results and discussion
Analysis of 5 pedigree generations of 6700 Orlov Trotters showed that a wide use of some crossbred stallions in breeding led to the predominance in the modern livestock of descendants of the horses
obtained by the method of crossbreeding, while only 41.8% of modern Orlov Trotters do not contain ancestors of other breeds in their pedigree.

In the analysis of genotypes on 16 loci of DNA microsatellites, 125 alleles were identified in 6700 modern Orlov Trotters of different origin. Allele diversity indices for each group of Orlov Trotters were calculated (Table 1). The average number of alleles per locus ranged from 6.3 in the group of Orlov Trotters pedigree of which contained American and Russian Trotter ancestors (OrlAmRus) to 7.8 in the group of Orlov Trotters pedigree of which did not contain ancestors of other breeds (Orl). The effective number of alleles per locus ranged from 3.4 in the group of Orlov Trotters pedigree of which contained American and Russian Trotter ancestors (OrlAmRus) to 3.9 in the group of Orlov Trotters pedigree of which contained Thoroughbred ancestors (OrlTB). At the same time a rare allele HMS6-N was private for the group of Orlov Trotters, pedigree of which did not contain ancestors of other breeds, i.e. it was not found in other studied groups of Orlov horses. It was determined that crossbreeding did not cause significant changes in the number of microsatellite alleles found in the Orlov Trotter breed. Only two individuals pedigree of which contained not-Orlov ancestors had private allele ASB23-T.

Allele ASB23-T is rare and missing in most studied cultural breeds of horses, but its presence is typical for allele pool of the Russian Trotter breed. Analysis of the origin of the Orlov Trotter individuals carrying this allele showed that they had Russian trotter ancestors within 5 generations of their pedigree.

Table 1. Results of genotyping of the investigated groups of Orlov Trotters on 16 microsatellite loci

| №  | Groups of horses          | Number of identified alleles | Private alleles | Average number of alleles per locus | Average effective number of alleles per locus | Heterozygosity |
|----|---------------------------|------------------------------|-----------------|------------------------------------|---------------------------------------------|----------------|
| 1  | Orl (n=2798)              | 124                          | HMS6-N          | 7.8                                | 3.8                                         | 0.709          |
|    | OrlTB (n=1752)            |                              |                 |                                    |                                             | 0.709          |
| 2  | OrlAm (n=589)             | 123                          | no              | 7.7                                | 3.9                                         | 0.709          |
|    | OrlRus (n=410)            |                              |                 |                                    |                                             | 0.714          |
| 3  | OrlAmRus (n=130)          | 117                          | no              | 7.3                                | 3.7                                         | 0.697          |
| 4  | OrlAmTB (n=472)           | 113                          | ASB23-T         | 7.1                                | 3.6                                         | 0.697          |
| 5  | OrlRusTB (n=400)          | 101                          | no              | 6.3                                | 3.4                                         | 0.677          |
| 6  | OrlAmRusTB (n=149)        | 117                          | no              | 7.3                                | 3.7                                         | 0.696          |
| 7  |                           |                              |                 |                                    |                                             | 0.705          |
| 8  |                           |                              |                 |                                    |                                             | 0.708          |
Figure 1. Ratio of expected heterozygosity and observed heterozygosity in different groups of horses of the Orlov Trotter breed

Observed heterozygosity (Ho) ranged between 0.690 in the group of Orlov Trotters pedigree of which contained American and Russian Trotter ancestors (OrlAmRus) to 0.715 in the group of Orlov Trotters pedigree of which contains Russian Trotter and Thoroughbred ancestors (OrlRusTB).

Significant influence of crossbreeding on changes in the genetic structure of the breed is found out by the analysis of the ratio of expected and observed heterozygosity in the studied groups of Orlov Trotters (figure 1).

Thus, in the group of Orlov Trotters pedigree of which did not contain ancestors of other breeds, the expected heterozygosity (He=0.709) was slightly higher than the observed heterozygosity (Ho=0.707) with a positive value of the inbreeding coefficient (Fis=0.004). The positive Fis indicates a deficit of heterozygosity that might be due to a high level of inbreeding in closed population of purebred animals.

In all 7 groups of Orlov Trotters with pedigrees containing ancestors of other breeds in different ratios, the observed heterozygosity (Ho) exceeded the expected heterozygosity (He). Inbreeding coefficient Fis in these groups had a negative value that meant an excess of heterozygosity, which might be a result of outbreeding. Maximum differences in Fis values were observed between the group of purebred horses pedigree of which did not contain ancestors of other breeds and the group of purebred horses, pedigree of which contained ancestors of 3 other breeds (American Standardbred, Russian Trotter and Thoroughbred) (figure 2). Minimum differences in Fis values were observed between the group of purebred Orlov Trotters, with pedigrees contained no ancestors of other breeds and the group of Orlov horses pedigree of which contained Russian Trotter ancestors.
Figure 2. Values of inbreeding coefficient Fis in the studied groups of horses of the Orlov Trotter breed

4. Conclusion
The Crossbreeding method conducted in the 60s of the twentieth century increased the level of heterozygosity in the Orlov Trotter breed, without causing significant changes in the number of alleles found in the population.

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