Application of modern technologies in identifying distinctive features in the subpopulation of Novoaltaiskaya horses

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Abstract. Novoaltaiskaya meat horse breed was developed in the end of the XX century by crossing heavy draft sires of different breeds with native and crossbred Altai mares. The aim of the study was to evaluate the differences in two geographically separated subpopulations of horses of Novoaltaiskaya breed and their relationship with the Altaiskaya horse using 17 panel microsatellite loci (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1 and CA425). The horses of Novoaltaiskaya breed demonstrated a high level of variability of all 17 STR loci with calculated 148 alleles in total. In the genetic structure of Novoaltaiskaya horse breed we identified the rare alleles VHL20S, ASB23V, ASB2T, HMS1Q, HMS2S, HMS3S and CA425K that are typical of native horse breeds of our country. A comparative analysis of the four groups according to the main genetic and population characteristics showed that the horses of the «Merkit» subpopulation (Altai Republic) are leading in all parameters of genetic diversity, including the maximum level of polymorphism (Ae=4.959), the level of observed heterozygosity Ho (0.776), and the expected heterozygosity of He (0.758). That ensured a negative Fis value. The coefficient of genetic similarity between two subpopulations of Novoaltaiskaya horses was 0.935. Thus, the use of genetic biotechnology allowed us to identify rare and frequently occurring alleles in the subpopulation of Novoaltaiskaya horses.

1. Introduction
Novoaltaiskaya breed of meat horses was found in the end of the XX century by crossing Russian, Soviet and Lithuanian Heavy Draft sires with native Altai mares. The breed was in the State Register of Breeding Achievements in 2000 and now it is in great demand in others regions. The uniqueness of the breed lies in the fact that the horses are on grazing and shade maintenance all year round. Moreover the Novoaltaiskaya horses have a fairly large growth, high live weight and good meat qualities, inherited from heavy breeds and adaptability to harsh year-round conditions from Altai local horses regions [1, 2].

Horses of Novoaltaiskaya breed have a harmonious physique, good productive and adapted quality. The average live weight of sires is 616 kg, mares – 573 kg, young animals at the age of 1.5 year old – 349 kg. In addition to their main purpose, productive horse breeding of various horses of Novoaltaiskaya breed is also valuable improvers in the productive horse breeding in various natural and climatic zones of Altai and Siberia.

At the present stage of studying the genetic structure of horse populations, various DNA markers predominantly are used as an effective tool for assessing the biological uniqueness of various breed
and their microevolution. Currently DNA microsatellites have found wide practical application both in monitoring the origin of horses and in assessing the diversity of populations [3–8].

Evaluation of interbreed diversity is required for planning selection programs of breeds. The study of the genetic characteristics of the genealogical structure of several horse breeds shows certain differences between lines, mare families and subpopulations in the number and frequency of alleles and a degree of heterozygosity of microsatellite loci [9–12].

The aim of this study is to evaluate a genetic variability in two geographically separated subpopulations of horses of Novoaltaiskaya breed and their relationship with the Altaiskaya horse using 17 panel microsatellite loci.

2. Materials and methods

Hair samples were taken from 150 horses of Novoaltaiskaya breed of two farms: «Merkit» (n = 71) and «Novotalitskoë» (n = 79), located in the Altai Territory. For comparison we used the results of DNA testings of Altai horses (n = 39), which participated in their creation. Testing of the total number of horses was carried out in the laboratory of the All-Russian Research Institute of Horse Breeding according to generally accepted methods. At the first stage, DNA was extracted from hair follicles using ExtraGene DNA Prep 200 reagents manufactured in Moscow Isogen Laboratory. At the next stage, the isolated DNA probes amplified by the method of the polymerase chain reaction (PCR) using a commercial 17-plex StockMarks™ kit for the genotyping horses. Amplifications were tested at 17 microsatellite loci: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1 and CA425 using a genetic analyzer AB 3130 (Applied Biosystems). The size of microsatellite markers was determined using Gene Mapper, version 4.0, applying the control of the DNA profile.

The genetic analysis was performed according to basic indicators: the number of alleles at each locus (Na), the number of effective alleles (Ae), observed (Ho) and expected (He) heterozygosity. Genetic distances within populations were calculated according Nei [13]. Coefficients of inbreeding Fis and genetic differentiations were determined by methods of F-statistics [14, 15] using MS Excel 2010, Statistica 12 (www. StatSoftStatistica) and program FSTAT 1.2. (www2.unil.ch/popgen/fstat.htm).

3. Results

In the tested horses of Novoaltaiskaya breed using 17 panel STR loci, we found 148 alleles in total. The highest allele’s spectrum being found at the «Merkit» farm located in Altai Republic (142 alleles), while 134 horses at the «Novotalitskoë» farm in the West Altai region had only 134 alleles (Table 1). In total tested Novoaltaiskaya horses had an allele’s pool, representative for European stud breeds [16, 17].

The number of alleles in the studied loci varied in the range from 4 (HTG7) to 15 (ASB17) for the whole group of horses studied. An average MNA value varied from 7,882 to 8,706 (Table 2). The highest allele’s number in all studied groups was observed at the ASB17 locus (13–15), and relatively low polymorphism was noted at the HTG7 (4), HTG6 (5–7), and HMS1 (4–7) loci.

In the genetic structure of Novoaltaiskaya horse breed we identified the rare alleles VHL20S, ASB23V, ASB2T, HMS1Q, HMS2S, HMS3S and CA425K that are typical of native horse breeds of our country. In the horses of the Altai breed (Altai Republic) 134 alleles were founded, among which there were unique rare variants ASB17 V and ASB17W, which were absent in Novoaltaiskaya breed. A characteristic feature of the horses of Novoaltaiskaya breed was the relatively high frequency of occurrence of the HTG6O allele (0.732) in the «Merkit» farm (0.732) and in the «Novotalitskoë» farm (0.732), whereas Altai horses were characterized by the dominance of allele HTG4M (0.667).

An assessment allele’s spectrum of two subpopulations of Novoaltaiskaya breed showed that the horses of the «Merkit» farm had alleles VHL20S, HTG4Q, AHT5P, HMS3S and CA425K, which were absent in the «Novotalitskoë» farm. At the horses «Novotalitskoë» farm alleles HTG6M, HTG6N, ASB23T, ASB2T and LEX3G were found which were typical only of this population.

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Table 1. The range of alleles in Novoaltaiskaya horses of the different subpopulations

| Loci  | Breeds/Subpopulations | Novoaltaiskaya «Merkit» | Novoaltaiskaya «Novotalitskoe» | Novoaltaiskaya | Altaiskaya |
|-------|-----------------------|-------------------------|-------------------------------|---------------|-----------|
|       |                       | p>0.05                  | p>0.05                        | p>0.05        | p>0.05    | p>0.05 |
| VHL20 | I,L,M,N,O,P           | R                       | J,L,M,N,O,P                   | J,S**         | I,L,M,N,P,Q | O       |
|       | K,L,M,O               | R                       | K,L,M,O,P                     | N             | K,L,M,N,O  | N,P,Q   |
| HTG4  | H,J,K,O               | L                       | H,J,K,O                       | J,K           | H,J,K,N,O  | I,L     |
| AHT4  | I,J,L,M,N,O,P         | J                        | I,J,M,N,O,P                   | G,M,N,P       | G,J,O      | I,M     |
| ABT5  | K,L,M,N,O             | J                        | I,L                          | I,L,M,N,P     | I,M       |
| HMG6  | L,M,N,O,P             | K,L,M,N,O,P             | N                            | K,L,M,N,O     | K,L,M,N,O  | P       |
| ASB23 | I,K,M,N,P,Q           | I,K,M,N,Q,T*            | I,K,M,N,Q                     | K,M,N,Q       | I,J,K,L,S  | M,R,T,U |
| ASB2  | I,K,M,N,P,Q           | I,K,M,N,Q,T*            | I,K,M,N,Q                     | K,M,N,Q       | I,J,K,L,S  | M,R,T,U |
| HTG10 | K,L,M,N,R             | R                       | K,L,M,N,O,R                   | K,M,N,O,R     | K,M,N,O    | N,Q     |
| HTG7  | K,N,O                 | M                       | K,N,O                         | M,N,O         | I,M,N,O,Q,P | R       |
| HMG3  | I,M,N,O,P,Q           | R                       | I,M,N,O,P,Q                   | M,N,O,P,Q     | I,M,N,O,P,Q | R       |
| HMG2  | H,J,K,L,R             | J,M,S                   | H,I,K,L,R                     | J,M,S         | H,I,K,L,R  | M,O,T   |
| ASB17 | G,J,M,N,P,Q           | G,J,M,N,P,Q            | G,J,M,N,P,Q                   | G,J,M,N,P,Q   | G,H,I,J,K,S | G,H,I,N,Q,R | F,K,L,M,O,P,S,V** | W** |
| LEX3  | F,H,L,M,N,P           | F                        | F,H,L,M,N,P                   | F,H,L,M,N,P   | F,H,L,M,N  | I,J,O   |
| HMS1  | J,M                   | J                        | J,L,M                         | I,L,M         | J,L,M      |
| CA425 | G,J,M,N,O             | I.L                      | G,J,M,N,O                     | G,J,M,N,O     | F,G,J,M,N,O | I,L     |

Note:* alleles not detected in other compared breeds; ** unique alleles not included in the standard register (16).

Table 2. Assessment of polymorphism of STR loci in horses of the Novoaltaiskaya and Altai breeds

| Breed/population | N   | Na  | A_e | H_e | H_o | F_is | MNA |
|------------------|-----|-----|-----|-----|-----|------|-----|
| Novoaltaiskaya «Merkit» | 71  | 142 | 4.959 | 0.776 | 0.758 | -0.025 | 8.353 |
| Novoaltaiskaya «Novotalitskoe» | 79  | 134 | 4.548 | 0.755 | 0.744 | -0.010 | 7.882 |
| Novoaltaiskaya | 150 | 148 | 4.904 | 0.764 | 0.757 | -0.009 | 8.706 |
| Altaiskaya | 39  | 134 | 4.750 | 0.772 | 0.759 | -0.020 | 7.882 |

Note: N – number of horses; N_a – number of alleles; A_e – effective number of alleles; H_e – expected heterozygosity; H_o – observed heterozygosity; F_is – population inbreeding level; MNA – average amount alleles per locus.

A comparative analysis of the four groups according to the main genetic and population characteristics (Table 2) showed that the horses of the «Merkit» subpopulation are leading in all parameters of genetic diversity, including the maximum level of polymorphism (A_e = 4.959), the observed level of Ho heterozygosity (0.776), and the expected level heterozygosity of He (0.758).
The horses of Novoaltaiskaya breed had in total higher values of the analyzed parameters in genetic diversity than Altaiskaya horses did thanks to use of the allele pool of several draft horse breeds. In all studied populations the observed heterozygosity Ho exceeded He that ensured a negative Fis value and suggested an excess of heterozygous genotypes.

The coefficient of genetic similarity between two subpopulations of Novoaltaiskaya horses was 0.935. In previous studies it was found that in the Thoroughbred horses the coefficient of genetic similarity between stud subpopulation varied in the interval of 0.925–0.958 [9]. Evaluation of interbreed differentiation at the level of subpopulations, male and female lines is important for the genetic characteristics of the breed’s biodiversity.

The established genetic differences between subpopulations of the horses of 17 STR loci are presented in the dendrogram (Figure 1). Cluster analysis conducted on the basis of the matrix of genetic distances demonstrates a high level genetic similarity of relationship horses from «Merkit» and «Novotalitskoy» farms, as well as significant relationship between horses from the «Merkit» farm and Altaiskaya breed, located in the same region. Our data, obtained using a large number of horses, confirm a close relationship between Novoaltaiskaya and Altaiskaya breeds [18].

4. Conclusion
The horses of Novoaltaiskaya breed demonstrated the high level variability of all 17 STR loci, calculated 148 alleles, representatives of European horse breeds. A comparative assessment allele’s spectrum of two subpopulations of Novoaltaiskaya breed showed that the horses of the «Merkit» farm had alleles VHL20S, HTG4Q, AHT5P, HMS3S and CA425K, which were absent in Novotalitskoe farm. Among the horses of the «Novotalitskoe» farm we found alleles HTG6M, HTG6N, ASB23T, ASB2T and LEX3G which were typical only of this population. The horses of the «Merkit» subpopulation are leading in all parameters of genetic diversity, including the maximum level of polymorphism (Ae = 4.959), the observed level of Ho heterozygosity (0.776), and the expected level of heterozygosity of He (0.758).
The horses of Novoaltaiskaya breed had in total higher values of analyzed parameters of genetic diversity than Altaiskaya horses did, thanks to using the allele pool of several draft horse breeds. In all studied populations the observed heterozygosity $H_o$ exceeded $H_e$ that ensured a negative $F_{is}$ value.

The coefficient of genetic similarity between two subpopulations of Novoaltaiskaya horses was 0.935. The genetic relationship of Novoaltaiskaya and Altaiskaya horse breeds was slightly less (0.896). The cluster analysis shows the presence of intra-breed genetic differences between horse subpopulations of Novoaltaiskaya breed, which indicates the promising use of STR’s markers in breeding programs.

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