

DNA Examination of Polymorphism on Two Lines of the Yakut Breed Stallions using the RAPD-PCR Method



L. G. Kozlova, V. G. Osipov, N. A. Nikolaeva, A. D. Reshetnikov, A. I. Barashkova, Y. L. Shadrina

Abstract: Herd and beef horse breeding are traditional and profitable livestock industries in the Republic of Sakha (Yakutia). Three breeds are propagated in the republic: Yakut, Prilensk and Megezhek. Objective: to evaluate the genetic homogeneity of equine micro-populations in order to determine the degree of their genetic similarity. A DNA isolated from hair follicles of 2 horse lines have been tested using 18 primers. According to obtained data, the primers OPA 07, OPA 09, OPA 10, OPA 18 and ODS 18 are more polymorphic than other 13 primers. Most of the primers amplify fragments from 350 to 1,000 bp in size. As an exception the sequence of primer OPA 02 is absent in 2 lines of horses. Coincidentally, PRA 06 primer fragment of 1200 bp presents only in the mare line of the stallion named Kuobakh. There is a uniform distribution of fragments over the entire range of lengths in the remaining samples of 2 stallion lines. Random Amplification of Polymorphic DNA (RAPD) analysis can serve as a kind of express method – a method of genetic polymorphism, which is especially relevant for poorly studied animal populations, as well as a source of unique locus-specific markers.

Keywords: breed, DNA, horses, polymorphism, primers, RAPD-PCR.

I. INTRODUCTION

Preserving the genetic diversity of local horse breeds is an urgent issue in current times. Based on this, in Greece, the local Macedonian horses have been studied in order to determine the validity of their inclusion in the herdbook as a separate breed. Ioannis A. Giantsis, Nikolaos E. Diakakis and Melpomeni Avdi found that phylogenetic analysis leads to

low genetic distances, confirmed by insignificant values, which indicate a slight genetic discrepancy between different breeds in Macedonia [1].

Mitochondrial DNA genetic variations among four horse populations in Egypt suggest that the populations of horses bred in Egypt have low genetic diversity, and all of them belong to the *Equus caballus* breed [2].

In Brazil held assessment of pedigree information in the Quarter Horse: Population, breeding and genetic diversity. This study aimed to evaluate population parameters and to describe the genetic diversity of Quarter Horse breed (QH) in Brazil. The results demonstrate a large number of founders and ancestors, but a small ancestor group was responsible for the continuity of the QH breed in Brazil. These findings highlight the importance of monitoring genetic diversity, including follow-up by breeding programs, to permit control of next generations [3].

Estonian colleagues Erkki Sild, Krista Rooni, Sirje Värvi, Knut Røed, Haldja Viinlass investigated Genetic diversity of Estonian horse breeds and their genetic affinity to northern European and some Asian breeds. Sixteen microsatellite markers have been used to assess the genetic diversity in native and imported transboundary horse breeds and populations from the Baltic Sea region, North-Eastern Europe and Asia (Mongolia and Yakutia, the Russian Federation), with a special focus on Estonian horse breeds. Testing for within-breed genetic variation, Hardy-Weinberg proportions, linkage disequilibrium between genotypes across loci, molecular variance, genetic relationships and population structure was performed. The local Estonian breeds, together with the Finn horse, Mezen, Mongolian and Yakutian breeds, formed a sparse intermediate aggregate with a low variability, attributed to the differences among the breeds. The population structure analysis showed admixed ancestry in this small-size (pony type) breed group. Their study suggests that the local Nordic (Northern European) breeds ($N = 11$) formed three genetic clusters.

Herd and beef horse breeding are a traditional and profitable livestock industries of the Republic of Sakha (Yakutia). The breeds of herd horses of Yakutia are the most northern that exist nowadays in the world. 3 breeds are propagated in the republic: Yakut, which includes three types: indigenous, Kolyma and Yan, Prilensk and Megezhek.

Revised Manuscript Received on October 30, 2019.

* Correspondence Author

L.G. Kozlova*, FRC YaSC SB RAS, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia.

V.G. Osipov, FRC YaSC SB RAS, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia.

N.A. Nikolaeva, FRC YaSC SB RAS, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia.

A.D. Reshetnikov, FRC YaSC SB RAS, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia.

A.I. Barashkova, FRC YaSC SB RAS, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia.

Y.L. Shadrina, FRC YaSC SB RAS, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia.

© The Authors. Published by Blue Eyes Intelligence Engineering and Sciences Publication (BEIESP). This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

DNA Examination of Polymorphism on Two Lines of the Yakut Breed Stallions using the RAPD-PCR Method

They are bred by the method of national selection and they are highly adapted to harsh climatic conditions. They graze outside all year round, they almost do not need additional feeding and these breeds of horses are capable of fast fattening-off and fat deposition.

The native Yakut horse as a separate breed was recognized by the order of the State Agricultural Committee of the USSR No. 860 dated 02.09.1987 and the order State Agricultural Committee of the RSFSR No. 871 dated 01.10.1987. Patentee is the Yakutsk Research Institute of Agriculture. On the territory of the Republic of Sakha (Yakutia) the breeding area extends far beyond the Arctic Circle to forest tundra [5]. The Yakut breed is propagated clean, and breeding is enhancing by selection of the desired types. Horses of this type are bred in farms of the Central and Vilyuysk group of districts of the republic. The average measurements and live weight: stallions – 138.7-147.4-180.5-20.1 cm and 464.5 kg; mares – 136.0-143.6-173.3-18.4 cm and 421.0 kg. Genetic potential of live weight of horses of the indigenous type: stallions – 430-490 kg; mares – 415-470 kg. Slaughter yield: foals of 6-7 months – 55.3%; Adult horses – 53.3%. Milk productivity of mares - 1600 l of milk for 6 months of lactation.

The Prilensk breed is bred by admixture blood of the horses of the Orlovsk and the Russian cart breed to the horses of the Yakut breed. The horses of this breed are relatively large, they have well-defined characteristics of beef breed. Average measurements and live weight: stallions – 142.1-150.4-182.6-20.1 cm and 482.1 kg; mares – 138.0-146.2-173.9-18.5 cm and 432.0 kg. Genetic potential of live weight of horses of the Prilensk breed: stallions – 450-560 kg; mares – 430-530 kg. Slaughter yield: foals of 6-7 months – 56.2%, adult horses – 55.6%. Milk productivity of mares – 1660 l of milk for 6 months of lactation. Patentee is the Yakutsk Scientific Research Institute of Agriculture, patent selection achievement No. 5770 dated 02.16.2011 [6]. The Megezhek breed is bred by admixture blood of the horses of the Kuznetsk and the Russian cart breeds to horses of the Yakut breed. The horses of this breed are large, massive, with a long barrel-shaped body and well-developed musculature, which are typical for horse of beef breed. The average measurements and live weight: stallions – 143.8-154.6-190.1-20.6 cm and 514.2 kg; mares – 140.7-150.0-181.5-18.9 cm and 461.7 kg. The genetic potential of live weight of horses of the Megezhek breed: stallions – 470-610 kg; mares – 450-580 kg. Slaughter yield: foals of 6-7 months – 56.15%; adult horses – 55.90%. The milk production of mares – 2393 l of milk for 6 months of lactation. The patent holder is the Yakutsk Scientific Research Institute of Agriculture, the patent of selection achievement No. 5773 dated 16.02.2011 [6].

Currently worldwide an active work on studying and preserving the local breeds is proceeding. Substantially any local breed is a reserve of genetical merit, that may be needed in the future. Such characteristics of indigenous breeds as adaptability to local environmental conditions, high quality products, a sound constitution, high fecundity and long life expectancy can be used in developing of animals that can adapt to new biotechnologies. The longer the animal population is exposed to extreme environmental impact, the higher the probability of the evolution of adaptive traits.

Local breeds of animals can be used in crosses in order to create new forms that are better adapted to extreme environmental conditions [7]. Objective: to evaluate the genetic homogeneity of the micro-populations of the horses in order to determine the degree of their genetic similarity.

The study of the gene pool of local horse breeds is of interest in terms of the originality of a genetic structure and the identification of polygenes responsible for the high adaptive qualities of animals. Local breeds of horses do not only have exceptionally high adaptive qualities, but they are living cultural monuments of the peoples who created them as well. These breeds are of great historical and ethnographic value. So, at the very beginning of study on DNA testing, a huge number of fragments that previously have not been described by foreign researchers are discovered. Akhal-Teke, Bashkir, Tuva and Yakut breeds are leading by this indicator. Each of these fragments may become a new marker of adaptive and economic traits [8]. The highest level of polymorphism is observed in native horse breeds that are well adapted to environmental conditions: Yakut [9], Mezen, Vyatka. The high level of polymorphism of the marker genes of aboriginal horse breeds, as well as their high adaptive potential, make it possible to use their gene pool in the future as a source of potential economic traits [10]. The features that distinguish local breeds from widespread foreign ones must preserve in the breeding farms. Recent studies have been conducted in the laboratory of immunogenetics of the All-Russian Research Institute of Horse Breeding shows that marker genes can be successfully used in identifying the genetic characteristics of the breeds and genetic certification of the breeds and populations.

While preserving the breeds, the main task is not to lose specific gene complexes, which determine the phenotypic breed characteristics associated with exterior features, productivity, viability, and resistance of animals. The molecular genetic characteristics of the Yakut horse population is an important contribution to the system of poorly investigated knowledge about the gene pools of native Siberian horse populations [11].

II. MATERIALS AND METHODS

The study was carried out in the laboratory of horse breeding of the Yakutsk Scientific Research Institute of Agriculture. Two lines of indigenous horses of the State Unitary Enterprise “The Horse Plant named after Hero Popov” of Megino-Kangalassky district of the Republic of Sakha (Yakutia) have been selected to determine the reliability of the origin of their offspring. A DNA have been isolated from the hair follicles of native-type horses based on the father-mother-son principle by sorbent method using 18 decimal oligonucleotide primers of the AmpliPrime DNA-Sorb-V kit (manufactured by NextBio), according to the manufacturer's recommendations and the following devices: thermal cycler TProfessional (manufactured by Biometra); horizontal electrophoresis chamber SE-1 (manufactured by Bioclon LLC); transilluminator (manufactured by NPF Bioclon) and video system GEL-IMAGER 2. (Table I).



Table I. RAPD primers used for DNA amplification of samples of native Yakut breed horses

Primer	Nucleic acid sequence	Annealing temperature
	5'_3'	
OPA02	TGC CGA GCTG	36 °C
OPA06	GGT CCC TGAC	36 °C
OPA07	GAA ACG GGTG	36 °C
OPA08	GTG ACG TAGG	36 °C
OPA09	GGG TAA CGCC	36 °C
OPA10	GTG ATC GCAG	36 °C
OPA11	CAA TCG CCGT	36 °C
OPA12	TCG GCG ATAG	36 °C
OPA15	TTC CGA ACCC	36 °C
OPA17	GAC CGC TTGT	36 °C
OPA18	AGG TGA CCGT	36 °C
OPB13	TTC CCC CGCT	36 °C
OPB 15	GGA GGG TGTT	36 °C
OPB 18	CCA CAG CAGT	36 °C
OPB20	GGA CCC TTAC	36 °C
OPC02	GTG AGG CGTC	36 °C
OPC03	GGG GGT CTTT	36 °C
OPC 05	GAT GAC CGCC	36 °C

III. RESULTS

DNA samples isolated from the hair follicles of two horse lines were analyzed using 18 primers (Table II). According to obtained data, the primers OPA 07, OPA 09, OPA 10, OPA 18 and ODS 18 are more polymorphic than other 13 primers. Most of the primers amplify fragments from 350 to 1000 bp.

As an exception the sequence of primer OPA 02 is absent in 2 line of horses. Coincidentally, the PRA 06 primer fragment 1200 p. presents only in the mare of the stallion line named Kuobakh. There is a uniform distribution of fragments over the entire range of lengths in the remaining samples of 2 stallion lines.

Table II. The size of the fragments obtained in the course of RAPD-PCR from two lines of Yakut breeding stallions

Primers	Line of stallion «Kuobakh»			Line of stallion «Djollookh»		
	Father	Son	Mother	Father	Son	Mother
	GP-4/1 Born 1996	GP-15-2 Born 2015	GP- 7-6 Born 1996	GP-5-1	GP-5-1 Born 2015	GP-25
OPA02	-	-	-	-	-	-
OPA06	-	-	1200	-	-	-
OPA07	700	600	700	700	700	700
	650	550	650	650	500	650
	550	450	650	550	500	650
OPA08	1300	1000	1400	-	800	850
	900	900	850	-	750	800
	900	700	850	-	600	800
	900	500	850	-	600	800
OPA09	1000	900	1100	1000	1000	650
	650	800	1100	950	900	650

DNA Examination of Polymorphism on Two Lines of the Yakut Breed Stallions using the RAPD-PCR Method

	550	600	950	650	700	600
		500		600	500	
OPA10	1100	1000	1100		1000	
	700	900	1000	1200	700	1200
	450	800	850	750	400	950
		700	750			
OPA11	1100	900		1200	1000	
	600	500		1000	700	1000
	350	250	-	350	650	350
					250	
OPA12	1200	1200	1400		1300	1300
		800				1100
	850	700	730	1400	700	750
	420	380	450		400	350
OPA15			1000		900	1000
	700	800	900	900	800	850
		700	750	700	700	620
			700			
OPA17			1100			1100
	1200	850	950	1100	1100	900
	900	700	850		900	850
OPA18	1300	700	900	1100	900	1000
	1000	400	600	600	600	600
	620					
OPB13	1200	800		950	1100	1100
	600	380	-	750	900	600
					600	
OPB15	1000			1000		900
	800	-	-	700	-	550
OPB 18	1100			1000	900	1000
	900	-	-	900		850
OPB20	1500	1000	1500		1200	1500
		900		1400		1200
	1000	700	1100	1000	1000	900
	800	400	850		800	750
OPC02		600	1500		1000	
	900	400	1100	500	700	1000

	500	300	900		500	
OPC03	900	500	800	800		850
	850				-	
OPC 05	850	800	900	900	1400	
	700	700	750	700	1000	700
		450			800	
					700	

IV. DISCUSSION

Obtained data shows that the difference between DNA fragments of two breeds of indigenous types of horses of the Yakut breed is not huge. From our point of view, this may indicate both the consolidation of two horse breeds and a single breeding strategy, as well as a close relationship between the groups of studied horse breeds.

While preserving the breeds, the main task is not to lose specific gene complexes (or a balanced system of genes), which determine phenotypic breed characteristics associated with exterior features, productivity, viability, and resistance of animals. It is these features that distinguish local breeds from other widespread horse breeds that must be preserved in breeding horse farms.

V. CONCLUSION

The obtained data will allow to evaluate the genetic homogeneity of the studied micro-populations and the genetic association between them and it will allow to assess the degree of genetic similarity between the micro-populations of horses. Thus, RAPD analysis can serve as a kind of express method for identifying genetic polymorphism, which is especially important for poorly studied animal populations, and can also serve as a source of unique locus - specific markers.

REFERENCES

1. A.Io. Giantsis, and N.E. Diakakis, A. Melpomeni. "Genetic Composition and Evaluation of the Status of a Non-descript Indigenous Horse Population from Greece, the Macedonian Pacer". *Journal of Equine Veterinary Science*, vol. 71, 2018, pp. 64-70.
2. E.O. Otman, F.K. Mahrous, and H.I. Shafey, "Mitochondrial DNA genetic variations among four horse populations in Egypt". *Journal of Genetic Engineering and Biotechnology*, vol. 15(2), 2017, pp. 469-474.
3. R.A.S. Faria, A.M. Maiorano, P.A. Bernardes, G.L. Pereira, and J.Au. II V. Silva, "Assessment of pedigree information in the Quarter Horse: Population, breeding and genetic diversity". *Livestock Science*, vol. 214, 2018, pp. 135-141.
4. E. Sild, K. Rooni, S. Värv, K. Røed, and H. Viinlass, "Genetic diversity of Estonian horse breeds and their genetic affinity to northern European and some Asian breeds". *Livestock Science*, vol. 220, 2019, pp. 57-66.
5. N.D. Alekseev, and N.P. Stepanov, "Horse of the Yakut breed: intra breed species, economic and biological features". *Achievements of science and technology of agriculture*, vol. 5, 2006, pp. 8-10.
6. M.G. Safronov, "60 years in the service of scientific support of agriculture of Yakutia". Yakutsk: Yakutia Media Holding OJSC, 2016.
7. Yu.A. Stolpovsky, and I.A. Zakharov-Gezekhus, "The problem of preserving the gene pools of domesticated animals". *Vavilovsky Journal of Genetics and Breeding*, vol. 21(4), 2017, pp. 477-486.
8. V.V. Kalashnikov, M.M. Dergunova, A.M. Zaitsev, L.A. Khrabrova, and M.A. Zaitsev, "Additional features of the method of DNA analysis in horse breeding". *Farm Animals*, vol. 3(4), 2013, pp. 72 - 74.

9. I.P. Guryev, "Genetic characteristics of Kolyma-type horses of the Yakut breed". *Collection of scientific works of the All-Russian Research Institute for Sheep and Goat Breeding*. Stavropol, vol. 2(2-2), 2009, pp. 29-32.
10. A.V. Gorodnaya, and V.I. Glazko, "Polymorphism of structural genes and ISSR-PCR markers in population genetic studies of some cattle breeds". *Cytology and Genetics*, vol. 1. 2006, pp. 49 - 56.
11. M.M. Kuznetsova, "Genetic structure and phylogenetic relationships of indigenous horse breeds in Western Siberia". Ph.D. thesis. Dubrovitsy, 2011.