

GENETIC DIVERSITY OF POPULATIONS OF AKHAL-TEKE HORSES FROM THE CZECH REPUBLIC, RUSSIA, ESTONIA AND SWITZERLAND

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Abstract

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Our research objective was to evaluate the genetic parameters in the populations of Akhal-Teke horses in 4 countries: Czech Republic, Russia, Estonia and Switzerland. The experiment involved a total of 325 Akhal-Teke horses; 121 horses came from the Czech Republic, 152 were from Russia, 28 were from Estonia and 24 horses came from Switzerland. For the divided database of micro satellites we evaluated the following parameters: effective number of alleles, frequency of alleles for the groups of horses; the observed heterozygosity (H_o); the expected heterozygosity (H_e); the inbreeding coefficient (Fis); and the genetic distance. The researched population is polymorphous. The population in the Czech Republic differs from the other three countries in the numbers of alleles per locus. The Czech population also includes Akhal-Teke horses which are not purebred Akhal-Teke horses. A confirmation of this fact is the effective number of alleles. The population in the Czech Republic exhibits the highest mean number of effective alleles. The Akhal-Teke population in Estonia exhibits the highest mean observed heterozygosity. By contrast, the population in the Czech Republic exhibits the lowest mean observed heterozygosity. In the Czech Republic the mean Fis value is a positive number indicating a reduced number of heterozygotes in the Czech Akhal-Teke population. The genetic distance is the highest between populations of horses bred in Russia and Estonia. The genetic distance is the lowest between populations of Akhal-Teke horses bred in Russia and in the Czech Republic.

Keywords: Akhal-Teke horse, microsatellite variability, genetic diversity, genetic distance

INTRODUCTION

Akhal-Teke horses are thought to be the oldest surviving horse breed in the world. The ancestors of the Akhal-Teke breed date back to animals living in the 3rd millennium B.C. (Shirliyev, 2003; Abramova, 2003). The Akhal-Teke takes its name from the Akhal oasis of Turkmenistan and the Teke tribe which lived in the oasis (Marešová and Skopalová, 2005; Dušek, 2001).

As the breeding of the Akhal-Teke horse developed in the Czech Republic all the breeders integrated in the Czech Akhal-Teke Association

(CATB); later on CATB joined the International Association of Akhal-Teke Breeders (MAAK) in Russia as a regular member. At present there are 200 Akhal-Teke horses in the Czech Republic, of which 100 horses are purebred Akhal-Teke horses (<http://www.achalteke.cz/cschat.asp>). In Russia the population of the Akhal-Teke horse counts 170 stallions and 600 dams and every year ca 300 foals are born. The populations of Akhal-Teke horses in Estonia and Switzerland are smaller; 30 horses in Estonia and 70 purebred Akhal-Teke horses in Switzerland (Babaev and Klimuk, 2010).

In contrast to breeds distributed globally Bowling and Ruvinsky (2000) reported that there are several groups of unique genetic resources which mostly differ in type, conformation and use. One example of such a unique genetic resource is the Akhal-Teke horse (Relichová, 2009).

The Caspian pony is a breed which belongs to the group of Iranian horses just like the Akhal-Teke horse; a number of authors studied its genetic parameters. Seyedabadi *et al.* (2006) analyzed the paternity and genetic parameters of Caspian horses and they saw that the observed heterozygosity ranged between 0.756 and 1, and the expected heterozygosity between 0.617 and 0.741, therefore high. Amirinia *et al.* (2007) discovered that heterozygosity of the population of the Caspian pony in Iran was high (0.9433); they also discovered that the genetic diversity in the breed was significant. Shahsavarani and Rahimi-Mianji (2010) analyzed the genetic diversity and coefficient of inbreeding in 100 horses and 16 micro satellites of the Caspian pony. They found that the average number of alleles per locus was higher than in other Asian, European and other breeds. In their study Szontagh *et al.* (2005) dealt with the genetic diversity of Achal-Teke horses bred in Turkmenistan.

Luís *et al.* (2007) evaluated the genetic diversity and relationship between Portuguese horses and other horse breeds and discovered that the observed and expected heterozygosity in Achal-Teke horses was 0.668 (± 0.016) and 0.715 (± 0.029), respectively. They also discovered that the Lipizzaner horse can be incorporated into the groups of related breeds together with the Akhal-Teke, Arabian and Caspian horses.

For many centuries the breeders, particularly those from Turkmenistan and Russia, have used inbreeding to preserve the typical conformation and character of the Akhal-Teke horse. That is the reason why it is very important to know the coefficient of inbreeding and the genetic distance among the individual populations in each country where the horse is bred and in dependence on these parameters to set up a suitable breeding plan in order to preserve the breed characteristics of the Akhal-Teke horse. That is why our objective was to find out the genetic diversity of the Akhal-Teke horse in the countries where the horse is bred.

MATERIAL AND METHODS

The objective of our investigation was to determine the microsatellite variability and genetic diversity and distance among populations of purebred Akhal-Teke horses in the Czech Republic, Russia, Estonia and Switzerland.

The experiment involved a total of 325 Akhal-Teke horses; 121 horses came from the Czech Republic, 152 were from Russia, 28 were from Estonia and 24 horses came from Switzerland.

Total genomic DNA was extracted from hairs. We determined the genetic type of Akhal-Teke

horses bred in the CZECH REPUBLIC in the accredited immuno-genetic laboratory in Hradištko pod Medníkem and in the accredited genomics laboratory at Mendel University in Brno. We isolated DNA from hair follicles using the JETQUICK® Tissue DNA Spin Kit in the genomics laboratory of Mendel University in Brno, followed by Multiplex PCR and fragment analysis.

The DNA type of horses from the Akhalt-service Eisk Stud Farm was analyzed in the laboratory of the University of California, Davis. The owners of horses from Estonia provided protocols of paternity tests together with micro-satellites for evaluations of the genetic parameters. DNA tests of horses from Switzerland were provided by a representative from the Association of Breeders. All laboratories completed comparison tests in the International Society for Animal Genetics (ISAG).

For the divided database of micro satellites we evaluated the following parameters: effective number of alleles, frequency of alleles for the groups of horses; the observed heterozygosity (H_o); the expected heterozygosity (H_e); the inbreeding coefficient (Fis); and the genetic distance.

The following markers were researched: VHL20, HTG4, HTG6, HTG7, HTG10, AHT4, AHT5, HMS1, HMS2, HMS3, HMS6, HMS7 and ASB2.

Statistical Analysis

The mean number of alleles per locus (MNA), allele frequency, observed heterozygosity (H_o) and expected heterozygosity (H_e) were obtained across different loci and populations using the Excel Microsatellite Toolkit v. 3.1.1. add-in utility for Microsoft Excel (Park, 2001). Using the POPGENE version 1.31. software (YEH *et al.*, 1999) we evaluated the individual genetic parameters. Graphical representation of the estimated membership fractions of individuals of the breed was analyzed using the program Structure version 2.2. (Pritchard *et al.*, 2000). We constructed a dendrogram based on Nei's (1972) genetic distance: method = UPGMA modified after the NEIGHBOR procedure of PHYLIP version 3.5.

RESULTS AND DISCUSSION

The investigated population is polymorphous; see Tab. I. The number of alleles per locus in the entire population ranges from 3 to 12 alleles. The following loci have 3 alleles: HTG6 and HTG7 in the Russian population, HTG6 in Estonia and HTG7 in Switzerland. The Czech population differs from the other three countries in the number of alleles per locus. Five loci exhibit 10 alleles and locus HMS3 no fewer than 12 alleles. The means also document this fact as the mean is the highest in the Czech Republic followed by the Akhal-Teke populations in Russia; the populations in Switzerland and Estonia exhibit the lowest mean. It is possible that the mean number of alleles per locus in Switzerland and Estonia is low because of the small population size of the horses.

I: Frequency of alleles in the investigated populations of the Akhal-Teke horse

		Czech Republic	Russia	Estonia	Switzerland	Overall
Sample size		242	304	56	48	650
Locus	AHT4	7	6	6	5	8
	AHT5	7	6	5	4	7
	HMS1	6	5	4	4	7
	HMS2	9	7	6	6	10
	HMS3	12	6	4	6	12
	HMS6	9	5	5	5	9
	HMS7	7	6	4	5	7
	HTG4	10	5	4	4	10
	HTG6	7	3	3	4	7
	HTG7	10	3	4	3	10
	HTG10	10	6	4	4	10
	VHL20	10	8	7	6	10
	ASB2	10	8	6	7	10
Average na*		8.769	5.692	4.769	4.846	9.000
St. Dev		1.787	1.548	1.166	1.144	1.6330

na = observed number of alleles

II: Effective number of alleles in the investigated populations of Akhal-Teke horses

	Czech Republic	Russia	Estonia	Switzerland	Overall
Average ne	4.116	3.434	3.161	3.041	3.899
St. Dev	1.448	1.189	1.020	0.878	1.407

* ne = Effective number of alleles (Kimura and Crow (1964))

The high mean number of alleles per locus in the Czech Republic indicates the effect of other genes in the population. At the same time it indicates that the Czech population includes Akhal-Teke horses which are not purebred Akhal-Teke horses.

A proof of this fact is the effective number of alleles – Tab. II. The mean number of effective alleles is the highest in the Czech Republic and this high number is proof of the preservation of a heterozygote population. In the Czech Republic the degree of heterozygosity is the highest what is a positive fact; but at the same time it confirms

that not all individuals in the Czech Republic are purebred.

We discovered that the number of alleles in the Czech Republic is high, but the frequency is low; e.g. there are 5 alleles but they occur only once.

Tab. III shows the heterozygosity in the populations.

Generally it applies that the higher the heterozygosity, the fewer homozygotes in the population. The mean observed heterozygosity was the highest in the Akhal-Teke horse population in Estonia; the mean observed heterozygosity was the lowest in the Czech Republic. In contrast, the expected heterozygosity was the highest in the Czech Republic.

Comparisons of the observed heterozygosity and the expected heterozygosity show that the expected heterozygosity is higher than the observed heterozygosity only in the Czech Republic. In Estonia it is the reverse; in the Russian and Swiss populations it is balanced. This finding is interesting because it appears that in the Czech population a relationship can be expected. The Akhal-Teke population in the Czech Republic is very young and has only a few horses. Large numbers of horses were imported to the Czech Republic, not only from Russia, but also from Dagestan, Kazakhstan, and/or other European countries. Although originally most of the horses were unrelated, the present Czech population shows inbreeding. The efforts of Czech

III: Summary of heterozygosity statistics for all loci (observed heterozygosity (H_o); the expected heterozygosity (H_e))

		H_o	H_e
Czech Republic	Average	0.648	0.731
	St. Dev	0.121	0.094
Russia	Average	0.669	0.668
	St. Dev	0.153	0.142
Estonia	Average	0.720	0.665
	St. Dev	0.153	0.113
Switzerland	Average	0.664	0.656
	St. Dev	0.156	0.114
Overall	Average	0.665	0.709
	St. Dev	0.117	0.113

IV: Wright's (1978) fixation index (*F_{is}*) as a measure of heterozygote deficiency or excess

Locus	Czech Republic	Russia	Estonia	Switzerland	Overall
AHT4	-0.014	0.135	-0.042	-0.177	0.049
AHT5	0.050	-0.048	-0.122	0.107	0.026
HMS1	0.129	0.053	-0.045	-0.198	0.094
HMS2	0.104	-0.147	-0.233	-0.054	-0.022
HMS3	0.034	0.130	-0.129	0.015	0.104
HMS6	0.261	-0.028	0.038	0.047	0.130
HMS7	0.033	0.055	0.043	-0.207	0.058
HTG4	0.231	-0.081	-0.308	-0.074	0.054
HTG6	0.185	-0.012	-0.110	-0.021	0.076
HTG7	0.142	-0.036	-0.354	0.076	0.074
HTG10	0.155	-0.052	0.057	0.099	0.077
VHL20	0.123	-0.035	-0.079	0.200	0.059
ASB2	0.050	0.002	-0.058	-0.197	0.032
Average	0.114	-0.005	-0.103	-0.029	0.062
Min	-0.014	-0.147	-0.354	-0.207	-0.022
Max	0.261	0.135	0.057	0.200	0.130

V: Nei's Unbiased Measures of Genetic Identity and Genetic distance [See Nei (1978) *Genetics* 89:583-590]

Population	Czech Republic	Russia	Estonia	Switzerland
Czech Republic	****	0.9199	0.8788	0.8964
Russia	0.0835	****	0.8439	0.9126
Estonia	0.1292	0.1698	****	0.8467
Switzerland	0.1094	0.0914	0.1664	****

Nei's genetic identity (upper diagonal) and genetic distance (lower diagonal)

breeders of Akhal-Teke horses to apply inbreeding is obvious

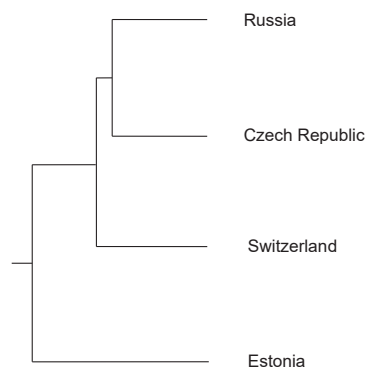
Wright's fixation index (*F_{is}*) – see Tab. IV confirms the above findings. With the exception of locus AHT4, all the other loci in the Czech Republic are positive. Likewise the mean *F_{is}* value in the Czech Republic is positive showing a lower number of heterozygotes in the Czech Akhal-Teke population. In the other three countries *F_{is}* is a negative figure and is the lowest in horses in Estonia. The above proves that there is virtually no relationship in the Estonian population which is a very small population counting only 28 horses. The same can be said of the population in Switzerland which is equally small, although *F_{is}* approaches zero. The *F_{is}* value in Russia (-0.005) indicates that this large population contains a sufficient amount of heterozygotes.

Tab. V compares the genetic distance among the respective populations. The genetic distance was the highest between populations of horses bred in Russia and Estonia. It is apparent that the effect of genes of horses from Turkmenistan and genetic indicators of horses from Turkmenistan imported to Estonia are a far cry from the horse population in Russia. The genetic distance was found to be the lowest between the Akhal-Teke populations bred in Russia and the Czech Republic and is due to the fact that the greater part of the Czech population is

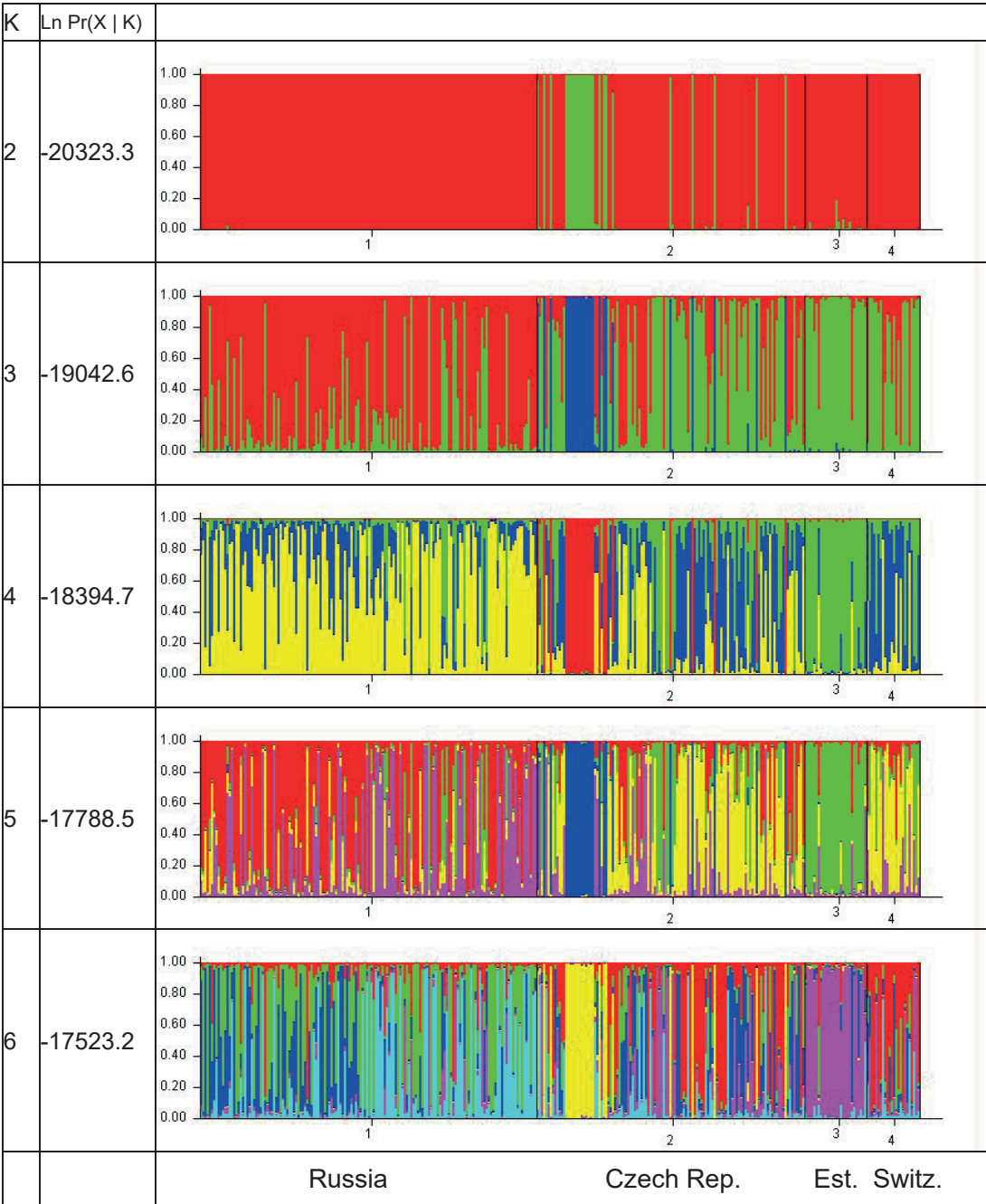
based on horses imported from Russia. The same holds true for the populations of horses from Russia and Switzerland.

The dendrogram in Fig. 1 constructed from Nei's genetic distances using the unweighted pair group method with arithmetic mean algorithm (UPGMA) shows the genetic relationships among four groups of horses by country of origin and illustrates the above conclusions.

The UPGMA (unweighted pair group method with arithmetic mean algorithm) dendrogram clarifies the genetic relationship among populations of Akhal-Teke horses. Fig. 1 shows that Russian



1: Dendrogram



2: Graphical representation of the estimated membership fractions of individuals of the breed analyzed in each of the K inferred clusters, for K=2 to K=6

and Czech populations of horses are the closest; the Swiss horse population is more distant and the Estonian horse population of Akhal-Teke horses is the most distant.

Fig. 2 illustrates the differences among populations of Akhal-Teke horses in the respective countries. Cluster K2 shows that the Russian population is balanced and is also evenly distributed

in clusters K3–K6. In all clusters we see a clearly separated **compact strip**. We assume that this means the presence of non-purebred Akhal-Teke horses. We also see that the Estonian population is different and is exhibited particularly in clusters K4–K6. It is manifested in a different but uniform color of the Estonian part of the population.

CONCLUSION

Basing on our results it is evident that the population we investigated is polymorphous. The population in the Czech Republic differs from the other three countries in the number of alleles per locus. The high mean number of alleles per locus in the Czech Republic indicates the influence of other genes in the population; i.e. that the Czech population includes Akhal-Teke horses which are not purebred Akhal-Teke horses, a fact which can be proved by the effective number of alleles. The mean number of effective alleles is the highest in the Czech Republic confirming that not all individuals in the Czech Republic are purebred.

The highest mean observed heterozygosity was detected in the Estonian population of Akhal-Teke horses, while the lowest mean observed heterozygosity was seen in the Czech Republic. On the other hand the expected heterozygosity is the highest in the Czech Republic. We discovered that the expected heterozygosity is higher than the observed heterozygosity only in the Czech Republic. In Estonia it is the reverse; in the Russian and Swiss populations it is balanced. A relationship can be observed in the Czech Republic where we see obvious efforts of Czech breeders of Akhal-Teke horses to use inbreeding. Wright's fixation index (Fis) confirms this finding. In the Czech Republic the mean Fis value is a positive figure indicating a reduced number of heterozygotes in the Czech population of Akhal-Teke horses. In the other three countries the Fis value is negative and is the lowest in horses in Estonia. In Russia the Fis value indicates that this large population contains enough heterozygotes. The genetic distance was the highest between populations of horses bred in Russia and Estonia. The genetic distance was the lowest between populations of Akhal-Teke horse bred in Russia and the Czech Republic.

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