

The Comparative Analysis of the Allele Pool of Thoroughbred Horses in Different Countries

Research Article

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ABSTRACT

The aim of the present study was the conducting of comparative analysis of allele pool of Ukrainian population of Thoroughbred horses and the populations from England, USA, Russia and South Korea using microsatellite loci of DNA on the basis of our own researches and literary sources. Comparative analysis of allele pool of Thoroughbred populations from different countries was conducted using 6 microsatellites loci: AHT04, AHT05, HMS03, HMS06, HMS07 and HTG04, which are recommended by International Society for Animals Genetics (ISAG) for parentage verification and identity testing of horses. The number of observed alleles in Ukrainian population was higher than in other countries. The most similar populations by allele frequencies were English and American populations of Thoroughbred horses.

KEY WORDS frequency of allele, heterozygosity, microsatellites of DNA, population, Thoroughbred horses.

INTRODUCTION

The Thoroughbred horse is one of the few breeds of horses in the world, which can be truly called Thoroughbred. Since the ends of the eighteenth century, it has been a closed population, bred strictly within the limits of the breed verified by individuals' pedigrees. From the first edition of the Stud Book for this horse breed in 1791 (for the rare exception when it is given by an association of breeding Thoroughbred horse), only those individuals whose ancestors were recorded within the Stud Book could be registered. The breed is the fastest and most maneuverable among the riding breeds in the world (Frederiko, 2002). The other side of the strictly pure breeding is the increase in homozygosity for certain undesirable alleles, which will

appear in the phenotype as abnormalities in the nervous system, decreased resistance to disease et cetera. The results of genetic-population analysis confirm the high level of genetic consolidation within Thoroughbred horses as a result of the closed Stud Book (Khrabrova *et al.* 2011). Therefore pedigree work with the Thoroughbred horse needs strict control of parentage verification. According to the requirements of the International Society for Animals Genetics (ISAG) and the International Stud Book Committee (ISBC), since 2001 genetic laboratories are obliged to conduct testing of Thoroughbred horses using microsatellite loci of DNA. The microsatellites of DNA (STR) are characterized by the codominant character of inheritance and a high level of polymorphism. Numerous studies indicate a high efficiency of using STR-markers not only

for parentage control, but also in genetic and population analysis (Bigi *et al.* 2012; Blokhina *et al.* 2012). ISAG proposed 9 basic microsatellites (AHT04, AHT05, ASB02, HMS03, HMS06, HMS07, HTG04, HTG10 and VHL20) as an international standard panel of microsatellite markers (Guerin *et al.* 1994; Marklund *et al.* 1994; Van Haeringen *et al.* 1994). Efficiency of parentage control of these horses using this type of genetic markers is higher than the 99.95%, which is recommended by ISBC (Shelyov *et al.* 2009). Nowadays the Thoroughbred breed is widespread in many countries around the world, such as: USA, France, Ireland, Australia, NZ, Romania, Slovakia, although Great Britain remains the world centre in breeding of these horses. Every country has its own features of work with the breed, due to the number of species and their characteristics. The aim of our work was a conduction of comparative analysis of allele pool of Thoroughbred horse populations in Ukraine, England, USA, Russia and South Korea.

MATERIALS AND METHODS

A sample of Ukrainian Thoroughbred horses (n=51) was conducted. Genomic DNA was isolated from peripheral blood by the «DNA-sorb-B» kit («AmpliSens», Russia), according to the manufacturer's instructions. The polymerase chain reaction was conducted in standard conditions (Spyrydonov, 2010). The products of amplification were denaturated by formamide (Sigma, USA) and electrophoresis was performed using a Genetic Analyzer ABI PRISM 3100 (Applied Biosystems, USA). The sizes of alleles were determined by using the size standard GENESCAN-LIZ 500 (Applied Biosystems, USA) and «Gene Mapper software 3.7» (Applied Biosystem, USA). Information about the allele pool's polymorphism of Russian (n=42), American (n=71), English (n=77) (Zaytseva, 2010) and Korean (n=1224) (Choi *et al.* 2012) populations for a comparative analysis was taken from previously published literature. Comparative analysis of the allele pool of microsatellite loci of the Thoroughbred breed from different countries was conducted by using 6 microsatellite loci, according to which horses of all populations were genotyped: AHT04, AHT05, HMS03, HMS06, HMS07 and HTG04.

RESULTS AND DISCUSSION

Analysis of the locus AHT04 demonstrated the similarity between the genetic structure of Korean, English, American and Russian populations of Thoroughbred horses (Table 1). The Ukrainian population was considered as a specific by the frequencies of distributing allelic variants.

Alleles F, G, I and P were inherent only for Ukrainian population of horses, thus the last appeared with the highest frequency 0.098. Simultaneously alleles H, K and O were found with the least frequency. Allele N was specific for Thoroughbred horses from South Korea and the Ukraine and was identified with the frequency 0.0004 and 0.069, accordingly.

English and American populations were almost identical when the distribution of allele frequencies was compared. Alleles H, J, K and O were common for them. Allelic variants of H, J and K had the highest frequency in the Russian population, with frequencies 0.250, 0.238 and 0.226 respectively.

Locus AHT05 in compared populations was represented by 4-6 allelic variants (Table 2). Allele I was specific only for English and Ukrainian populations with frequencies of 0.014 and 0.078 respectively. Additionally the allelic variant L was identified in the Ukrainian samples (0.020). The Russian population for this locus was the most consolidated with only 4 alleles found (J, K, M and N) besides, other population had the same alleles. Allele O was discovered with a frequency of 0.025, 0.033 and 0.021 in Thoroughbred horses from South Korea, England and USA, accordingly. The number of detected alleles by HMS03 in the population of Thoroughbred horses from different countries ranged from 5 (Bulgaria, England and Russia) to 10 (Ukraine) (Table 3). In the inspected populations from all countries alleles I, M, N, O and P were discovered with different frequencies. Alleles H, J and L were specific only for the Ukrainian population, with frequencies -0.078, 0.078 and 0.010 accordingly. Allele Q was discovered only in South Korea and Ukraine (0.0004 and 0.020, accordingly).

Allele I was the most frequent (0.702) in the Russian sample of horses, whilst in the English and USA samples its frequency was 0.507. It must be noted that other common alleles, discovered in the samples from different countries by this locus were: allele M in the English population of Thoroughbred horses (0.233) and alleles L and P in horses from the USA (0.148 and 0.183, accordingly). Allele R was identified in the horses from South Korea, USA and Ukraine with frequencies 0.013, 0.035 and 0.020, accordingly.

Allele K was specific for the Korean sample with a low frequency (just 0.0004). A comparative analysis by locus HMS06 demonstrated similarities between Korean, English and American Thoroughbred breed populations' genetic structure (Table 4) and were found with 5 allelic variants. Simultaneously the Russian population was the most consolidated (4 alleles) by the locus HMS06. Differences between frequencies in these alleles were detected, although deemed to be unimportant.

Table 1 Allele frequencies of microsatellite locus AHT04 in the samples of Thoroughbred horses from the Ukraine, Russia, USA, England and South Korea

The country	Allele								
	F	G	H	I	J	K	N	O	P
Ukraine	0.010	0.039	0.147	0.118	0.196	0.098	0.069	0.226	0.098
Russia	-	-	0.250	-	0.238	0.226	-	0.286	-
USA	-	-	0.197	-	0.225	0.155	-	0.423	-
England	-	-	0.196	-	0.196	0.176	-	0.432	-
South Korea	-	-	0.182	-	0.221	0.201	0.0004	0.396	-

Table 2 Allele frequencies of microsatellite locus AHT05 in the samples of Thoroughbred horses from the Ukraine, Russia, USA, England and South Korea

The country	Allele						
	I	J	K	L	M	N	O
Ukraine	0.078	0.412	0.186	0.020	0.147	0.157	-
Russia	-	0.214	0.452	-	0.19	0.143	-
USA	-	0.232	0.430	-	0.197	0.12	0.021
England	0.014	0.213	0.420	-	0.147	0.173	0.033
South Korea	-	0.157	0.491	-	0.209	0.118	0.025

Table 3 Allele frequencies of microsatellite locus HMS03 in the samples of Thoroughbred horses from the Ukraine, Russia, USA, England and South Korea

The country	Allele										
	H	I	J	K	L	M	N	O	P	Q	R
Ukraine	0.078	0.510	0.078	-	0.010	0.069	0.029	0.118	0.069	0.020	0.020
Russia	-	0.702	-	-	-	0.060	0.036	0.071	0.131	-	-
USA	-	0.507	-	-	-	0.113	0.014	0.148	0.183	-	0.035
England	-	0.507	-	-	-	0.233	0.027	0.089	0.144	-	-
South Korea	-	0.545	-	0.0004	-	0.147	0.024	0.124	0.147	0.0004	0.013

Table 4 Allele frequencies of microsatellite locus HMS06 in the samples of Thoroughbred horses from the Ukraine, Russia, USA, England and South Korea

The country	Allele						
	J	K	L	M	O	P	Q
Ukraine	0.010	0.118	0.069	0.177	0.010	0.578	0.039
Russia	-	0.024	0.036	0.262	-	0.678	-
USA	-	0.134	0.042	0.296	0.007	0.521	-
England	-	0.079	0.066	0.289	0.007	0.559	-
South Korea	-	0.136	0.029	0.323	0.009	0.503	-

For example, allele K appeared with frequency from 0.024 (Russia) to 0.136 (South Korea), allele L from 0.024 (Russia) to 0.069 (Ukraine), allele M from 0.177 (Ukraine) to 0.323 (South Korea), allele P from 0.503 (South Korea) to 0.678 (Russia). Alleles J and Q were specific only for the Ukrainian sample with frequencies 0.010 and 0.039, accordingly. Locus HMS07 was the most polymorphic in the Ukrainian sample of the Thoroughbred breed, with 8 alleles identified (Table 5).

Alleles J, L, M, N and O were common for all tested samples. The most similar by the distribution of frequencies of detected alleles were the Korean and English samples. Allele K was found in Russian and Ukrainian samples of horses, thus in Ukrainian horses the incidence was higher in comparison with Russian at 0.069 and 0.024 respectively.

Just two alleles were specific for USA (Q, R) and Ukrainian (I, P) populations. The distribution of allele frequencies by the locus HTG04 of the Thoroughbred horses from different countries showed similarity between the Korean, English and American samples and between the Russian and Ukrainian samples (Table 6).

Alleles K, M and N were found in horses of all samples, only allele P wasn't found in Ukrainian population. Thus, in South Korea, USA and Russia the difference between frequencies of allele P were insignificant. Allele L was more frequently discovered in Russian (0.143) and Ukrainian (0.118) populations and for the Korean Thoroughbred horses its frequency was just 0.001. Specific only for Ukrainian and American populations were alleles J (0.039) and R (0.007), accordingly.

The largest number of identified alleles by the locus HTG04 was detected in the Ukrainian sample of horses (6.652), while the least polymorphic by this locus was the English population, where the number of effective alleles was 3.390.

A similar situation was observed for expected heterozygosity. The American and Korean populations were almost identical (0.720 and 0.721, accordingly) and far lower than the value of the Ukrainian sample (0.858).

The Ukrainian sample of horses was the least polymorphic by the locus AHT05, with a frequency of detected alleles of 3.891 and it was the highest index among the tested samples (Table 7).

The difference between heterozygosity in the Ukrainian, English and USA samples was insignificant, viz. 0.750, 0.760 and 0.770 accordingly. The lowest index of heterozygosity was in South Korea.

The numbers of detected alleles by the locus HMS03 range from 5 (Russia and England) to 10 (Ukraine). The least level of polymorphism (1.923) was in the Russian horses and the greatest level (3.365) in the Ukrainian. The least level of the expected heterozygosity (0.380) was observed in the Russian population of horses and in the Ukrainian population it was the highest (0.710). In England, USA and South Korea heterozygosity were approximately identical (0.680, 0.650 and 0.644, accordingly).

Table 5 Allele frequencies of microsatellite locus HMS07 in the samples of Thoroughbred horses from the Ukraine, Russia, USA, England and South Korea

The country	Allele									
	I	J	K	L	M	N	O	P	Q	R
Ukraine	0.010	0.078	0.069	0.147	0.206	0.235	0.226	0.029	-	-
Russia	-	0.095	0.024	0.262	0.262	0.19	0.167	-	-	-
USA	-	0.12	-	0.12	0.232	0.246	0.268	-	0.007	0.007
England	-	0.128	-	0.135	0.244	0.189	0.304	-	-	-
South Korea	-	0.074	-	0.137	0.243	0.231	0.315	-	-	-

Table 6 Allele frequencies of microsatellite locus HTG04 in the samples of Thoroughbred horses from the Ukraine, Russia, USA, England and South Korea

The country	Allele							
	J	K	L	M	N	P	R	
Ukraine	0.039	0.392	0.118	0.402	0.049	-	-	
Russia	-	0.405	0.143	0.405	0.012	0.035	-	
USA	-	0.521	-	0.444	0.007	0.021	0.007	
England	-	0.494	-	0.468	0.032	0.006	-	
South Korea	-	0.545	0.001	0.403	0.021	0.030	-	

Table 7 The number of detected alleles (Na), effective number of alleles (Ne) and expected heterozygosity of the studied samples

Locus	Index	Populations of Thoroughbred breed				
		Ukraine	Russia	England	USA	South Korea
AHT04	Na	9	4	4	4	5
	Ne	6.652	3.970	3.390	3.420	3.585
	He	0.858	0.860	0.770	0.720	0.721
AHT05	Na	6	4	6	5	5
	Ne	3.891	3.261	3.642	3.420	3.087
	He	0.750	0.710	0.760	0.770	0.676
HMS03	Na	10	5	5	6	8
	Ne	3.365	1.923	2.935	3.062	2.811
	He	0.710	0.380	0.680	0.650	0.644
HMS06	Na	7	4	5	5	5
	Ne	2.591	1.886	2.459	2.640	2.656
	He	0.620	0.520	0.620	0.650	0.624
HMS07	Na	8	6	5	7	5
	Ne	5.493	4.742	4.999	4.650	4.245
	He	0.826	0.780	0.720	0.730	0.765
HTG04	Na	5	5	4	5	5
	Ne	3.002	2.858	2.155	2.132	2.168
	He	0.673	0.550	0.530	0.460	0.539

Allelic variants detected by locus HMS06 ranged from 4 (Russia) to 7 (Ukraine). Five alleles were detected in each of the samples of horses from England, USA and South Korea. The analysis of the quantity of the effective alleles showed that Korean and American populations were the most polymorphic. As for the Ukrainian sample, the polymorphic level was 2.591. The greatest heterozygosity in the sample of Thoroughbred horses was in the USA (0.650). In the Ukrainian, English and South Korean samples its value was almost identical (0.620, 0.620 and 0.624, accordingly).

The highest level of effective alleles detected by the locus HMS07 was found in Ukrainian populations, a bit less than in English population of horses (4.999). The horses from South Korea had the least level of polymorphism (4.245). The greatest number of detected alleles per locus was in the Ukrainian sample. In the English and South Korean samples this value was 5. The maximum expected heterozygosity was shown by the Ukrainian sample (0.826) with the Russian sample a bit less (0.780). The heterozygosity of Thoroughbred horses from England and USA was almost identical (0.720 and 0.730, accordingly).

The number of identified alleles detected by the locus HTG04 in all samples were between 4 and 5. The effective number of alleles ranged from 2.132 (USA) to 3.002 (Ukraine). The greatest heterozygosity was in the Ukrainian sample, while in the American sample this index was the lowest (0.460).

CONCLUSION

The results of the comparative analysis demonstrated differences between the allele pools of Thoroughbred horses from different countries. The Ukrainian appears to be the most polymorphic in comparison with the other populations. Specific alleles for the Thoroughbred horses from different countries were found. Differences in the structure of the allelic pool in the studied populations of Thoroughbred horses could be as a result of the breeding strategies adopted within the breed. The mass import of Thoroughbred horses into the Ukraine has indisputably influenced on the genetic structure of breed. These results con-

firm the higher level of the allele pool's polymorphism in the Ukrainian population of Thoroughbred horses.

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