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Original Contribution

DNA MICROSATELLITE ANALYSIS OF THE THOROUGHBRED HORSE POPULATION IN BULGARIA. GENETIC RELATIONSHIPS BETWEEN THE STUDIED SIRELINES

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ABSTRACT

The genetic relationships in the Thoroughbred horse population in Bulgaria were studied based on the information of 13 microsatellite loci. The study includes 157 horses with affiliation to the five of the most influential sirelines in the breed. Obtained values for the genetic distances range from 0,048 to 0,302 and genetic identity range from 0,739 to 0,953. Most closely related are the lines of Blacklock and Touchstone which is corresponding with the results from the cluster analysis. In the UPGMA dendogram the lines of Blacklock, Touchstone and Birdcatcher formed a separate cluster. The rates of inbreeding for individuals within populations (**Fis**) are all negative which indicate a complete lack of heterozygote deficiency in the studied population. The within population index of inbreeding (**Fst**) indicate moderate genetic differentiation in the studied population.

Key words: Thoroughbred horse population, microsatellites, genetic relationships, inbreeding.

INTRODUCTION

In present day the DNA markers are used for genetic analysis of different livestock breeds including horses. They are widely applied for analysis of genetic diversity within population and genetic relationships among populations. One of the registration requirements of the Thoroughbred studbooks worldwide is parentage testing of the horses based on the DNA markers. One of the most popular DNA markers for genetic analysis are the microsatellite markers [1, 2]. Their high mutation ability and codominant inheritance are some of the reasons for their widespread use. Many authors are studying the genetic diversity and relationships within different horse populations. The genetic diversity of different horse breeds in Europe has been studied [3, 4] together with recent researches made of thoroughbred horses [5-12]. Using DNA markers some authors disprove the thesis of the eastern origin of some of the foundation mares in the Thoroughbred horse breed [13]. Based on the microsatellite markers others examine the genetic relationships of different horse breeds worldwide [14-16]. In our country earlier studies on the genetic diversity of the Thoroughbreds are based on 13 SSR loci [17].

The aim of the recent study is to estimate the genetic identity and distance within the Thoroughbred horse population in Bulgaria. In connection with that the results of the DNA analysis for five of the most influential sirelines in the population were used.

MATERIALS AND METHODS

The study includes 157 Thoroughbred horses enrolled either in the Bulgarian Thoroughbred Stud Book or in the Non-Thoroughbred Register of the breed. A hair sample has been taken from each horse (either from the mane or tail). The DNA microsatellite analysis was carried out by GeneControl GmbH laboratory in Germany by a team of experts. The examined 13 SSR loci are as follow: AHT4, AHT5, ASB2, HMS1, HMS2,

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HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10 и VHL20. POPGENE software, version 1.31 [18] was used to estimate 3 fixation indices: inbreeding coefficient within population (Fis), coefficient of gene differentiation between population (Fst) and inbreeding coefficient for all sub-populations (Fit) [19].

ARLEQUIN software, version 3.5.1.3 was used for population data analysis [20]. The number of alleles of each locus heterozygosities values, Fst between sub-populations and gene diversity were calculated for each sireline. Genetic distances and genetic identity between the five sub-populations were estimated using POPGENE software version 1.31 according to Nei (1978) method [21].

RESULTS AND DISSCUSION

Establishing the genetic identity and distances amongst the main sirelines in the recent study is necessary in order to determine the kinship between them. In **Table 1** are presented the rates of genetic identity and distances [21] between the five main sirelins in our country. The obtained values for the genetic distances range from 0,048 to 0,302. The greatest genetic distance is observed between Tourbillon and Hurry On sirelines and the least between the lines of Blacklock and Touchstone. On other hand the genetic identity range from 0,793 to 0,953. As expected the greatest genetic affinity is settled between Blacklock and Touchstone and the least between Tourbillon and Hurry On sirelines. As is well known the most influential of the three foundation sires in the breed is Darley Arabian out of whose sireline are branching out the lines of Blacklock, Birdcatcher and Touchstone. According to us the common origin of those three sirelines is the main reason for the low values of the genetic distances between them.

Table 1. *Genetic identity and distances between the studied sirelines in the Thoroughbred horse population in Bulgaria*

SIRE LINES	Blacklock	Birdcatcher	Touchstone	Tourbillon	Hurry On
Blacklock	***0,000****	0,950	0,953	0,781	0,805
Birdcatcher	0,051	***0,000***	0,941	0,807	0,781
Touchstone	0,048	0,060	***0,000***	0,759	0,790
Tourbillon	0,247	0,215	0,276	***0,000***	0,739
Hurry On	0,216	0,247	0,236	0,302	***0,000***

* values for genetic identity are placed over the diagonal, values for genetic distances are placed below the diagonal

The kinship between the five main sirelines in Bulgaria are graphically presented in UPGMA dendrogram **(Figure 1)** generated as a result of the cluster analysis. The graphical result also show close relationship between the three sirelines branching out of Darley Arabian line – Blacklock, Birdcatcher and Touchstone and distant relationships between them and the sirelines of Tourbillon and Hurry On. Despite the fact that Hurry On and Tourbillon originate by the sirelines of two different foundation stallions (Godolphin Barb and Beyrley Turk) they have closer relationship between each other than with the other three sirelines.

In **Table 2** are presented the summarized values of F-statistics for the Thoroughbred horse population in Bulgaria based on the results from the 13 examined loci. F-statistics for individuals within population (**Fis**) indicates the rates of inbreeding in the population and range from -1 to + 1. The rates obtained for this fixation index in our study are all negative, which indicate for a complete lack of heterozygote deficiency. The lowest values are observed in locus VHL20 (-0,287) and the highest in locus HTG4 (-0,055). Mean value for **Fis** is -0,154. Some authors also report negative values for **Fis** in their studies: –0,01, –0,023, –0,025 [4, 13, 15]. Others report positive rates as follow 0,009 and 0,058 [14, 16].

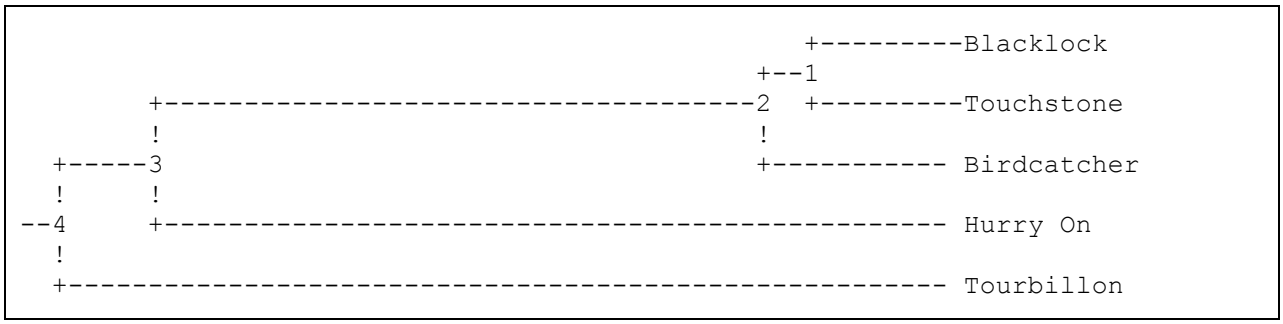

Figure 1. UPGMA dendogram of the studied Thoroughbred sirelines in Bulgaria

The inbreeding coefficient among populations (**Fit**) in our study range from –0,244 in locus VHL20 to 0,090 in locus HMS7 and mean value of -0,055. The within population index of inbreeding (**Fst**) indicate the level of genetic differentiation among sub-populations (in this case among the different sirelines in the studied Thoroughbred horse population). **Fst** always has a positive values that range from 0 to 1. The rates of **Fst** in our survey range from 0,025 in locus HTG4 to 0,143 in locus ASB2 and mean value of 0,086 which indicates moderate genetic differentiation. The substantial difference between the values of **Fis** and **Fst** (-0,154 < 0,086) indicates for the complete lack of heterozygote deficiency.

The process of passing genes from one population to another due to migration or crossing is known as gene flow. Having in mind that the Thoroughbred is a breed of pure bred horses when we talk about crossing we mean crosses between different sirelines. The observed values for gene flow widely varied ranging from 1,500 in locus ASB2 to 9,740 in locus HTG4. Relatively low is the mean value for the population 2,656.

Table 2. *Estimated values for F-statistics (Fis, Fit, Fst) and gene flow (Nm) for the Thoroughbred horse population in Bulgaria*

Loci	Sample size	Fis	Fit	Fst	Gene flow/Nm*
AHT4	312	-0,242	-0,178	0,051	4,640
AHT5	314	-0,072	0,036	0,101	2,217
ASB2	314	-0,132	0.030	0,143	1,500
HMS1	314	-0,173	-0,048	0,107	2,094
HMS2	314	-0,061	-0,012	0,046	5,122
HMS3	314	-0,203	-0,075	0,106	2,107
HMS6	314	-0,195	-0,104	0,076	3,044
HMS7	314	-0,061	0,090	0,143	1,502
HTG4	314	-0,055	-0,029	0,025	9,740
HTG6	314	-0,224	-0,085	0,113	1,952
HTG7	314	-0,240	-0,157	0,066	3,509
HTG10	314	-0,061	0,023	0,078	2,934
VHL20	314	-0,287	-0,244	0,033	7,391
Mean	**314**	**-0,154**	**-0,055**	**0,086**	**2,656**

*Nm – Gene Flow: Fst =0.25(1 – Fst) / Fst.

CONCLUSION

The observed values for genetic relationships between the studied sirelines show that most distant apart are the sirelines of Tourbillon and Hurry On and most related are the lines of Blacklock and Touchstone.

Sirelines of Blacklock, Touchstone and Birdcatcher formed a separate cluster in the dendrogram which correspond with their common origin.

The rates obtained for **Fis** are all negative, which indicate for a complete lack of heterozygote deficiency. The within population index of inbreeding (**Fst**) indicate moderate genetic differentiation in the studied population.

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