A genetic study on Turkish horse breeds based on microsatellite and mtDNA markers and inferences for conservation

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Introduction/Objectives

The domestication of horses has been widely affected by the course of civilization (Lister *et al.,* 1998). Two alternative hypothesis were suggested for the origin of domestic horses. A restricted origin hypothesis claims that domestic horses originated through selective breeding of a few loci. The other one, multiple origins scenario, suggests that domestication developed through large number of founders (Vila *et al.*, 2001). In order to shed light on evolutionary history of horse lineages and the domestication process, various studies in different regions have been studied (Jansen *et al*., 2002; Bruford *et al*., 2003; McGahern *et al*., 2006). However, there have not been a genetic study conducted on Anatolian horse before.

As Anatolia is home to many ancient civilizations and it is a land bridge between Europe and Asia, Anatolian horses might be carrying important genetic heritage. Therefore, the present study might have an important contribution to global dataset of horse genetics and may help understanding the domestication history of the horse. In addition, it is the first study on the genetic characterization of Anatolian domestic horse breeds.

The main objective of this project is to present the first molecular characterization of five Anatolian horse breeds by 21 microsatellite loci and analysis of mtDNA D-loop diversity based on 479 bp region. In addition, the project will provide information to develop proper management strategies and cost effective breed conservation programs for Anatolian horse breeds. Here we report the preliminary results from 4 breeds and some degenerate horses.

Materials and Methods

For mtDNA D loop sequencing (479 bp region), the primers used are:
Forward: 5'-CCCAAGGACTATCAAGGAAG-3'
Reverse: 5'-GGAATGGCCCTGAAGAAAGA-3'

Genotyping was performed by PCR multiplex groups of 9, 5 and 7 microsatellite loci primer pairs (Glowatzki-Mullis *et al*, 2005):
First panel: I18, AHT4, LEX33, COR02, HMS5, HMS6, ASB2, HTG6, HMS3.
Second panel: ASB43, AHT33, HMS2, NEVHEQ79, CA425.
Third panel: ASB17, ASB23, TKY301, HMS7, HTG4, VHL20, COR58.

DNA sequencing and genotyping analyses were done on the Beckman CEQ8800 Genetic Analysis System.

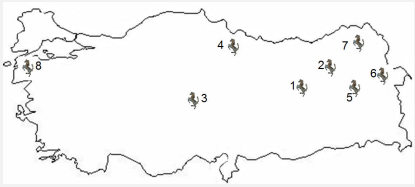

Figure 1: Sampling places of the populations (1) HKK and ERZ, (2) MLK and KRS, (3) UZYand KYS, (4) CNK, (5) AGR, (6) MLK and IGD, (7) MLK and ARD, (8) AMD

MLK, CNK, UZY and HKK are the breeds of known phenotypes, AGR, IGD, ARD, KRS, ERZ and KYS are degenerate horses that do not have defined phenotypic characters.

Preliminary Results

Table 1: Allelic diversity of populations based on 21 microsatellite loci.

	HKK	CNK	UZY	MLK	AMD	ERZ	KRS	KYS	VAN	AGR	IGD	ARD	Mean
HTG6	6	9	4	6	7	5	6	3	6	4	5	2	5,3
HMS3	9	8	5	8	9	7	7	6	8	7	6	5	7,1
HMS5	5	6	3	3	5	4	3	3	4	4	3	3	3,8
HMS6	6	7	4	5	8	5	6	5	5	6	5	3	5,4
ASB2	10	9	6	10	12	9	8	5	10	7	10	4	8,3
I18	9	11	3	9	11	7	7	7	6	8	7	4	7,4
AHT4	10	10	7	9	9	5	10	5	7	7	6	5	7,5
LEX33	10	11	5	9	10	7	7	7	8	6	5	4	7,4
COR2	5	5	5	5	5	5	5	4	6	4	5	3	4,8
ASB43	6	6	5	5	7	5	7	5	4	5	6	4	5,4
AHT33	11	13	6	11	11	11	8	7	11	9	11	6	9,6
NEV79	10	10	5	8	12	8	7	5	8	5	8	4	7,5
HMS2	8	8	4	9	10	7	8	7	7	9	7	5	7,4
CA425	9	13	3	9	8	6	7	4	8	6	8	4	7,1
ASB17	16	18	5	15	14	9	11	5	8	12	10	4	10,6
TKY301	9	8	5	8	7	7	9	5	6	6	6	6	6,8
ASB23	10	10	6	9	12	6	8	6	7	8	6	5	7,8
VHL20	11	12	7	9	9	8	10	6	7	9	8	4	8,3
HTG4	6	7	4	8	7	5	6	5	4	5	6	5	5,7
HMS7	8	9	6	9	9	7	7	6	8	7	6	5	7,3
COR58	11	13	5	12	12	10	9	9	9	10	10	5	9,6
Mean	8,8	9,7	4,9	8,4	9,2	6,8	7,4	5,5	7,0	6,9	6,9	4,3	7,1

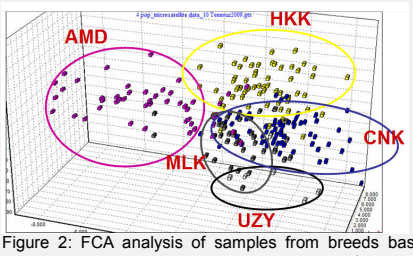

Figure 2: FCA analysis of samples from breeds based on population data with a prior phenotypic breed information.

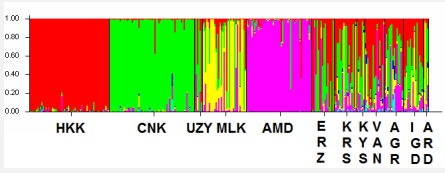

Figure 3: Structure analysis result (K=7) supporting FCA analysis.

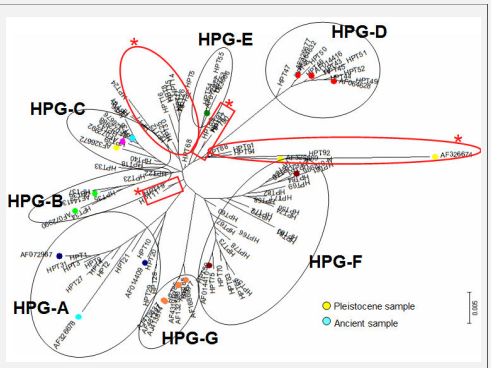

Figure 4: NJ tree constructed using Tamura-Nei model based on mtDNA control region sequences of Anatolian samples. The haplotypes are labelled according to Vila *et al* (2001) using some reference samples. Star labelled lineages are not grouped in defined haplogroups.

Inferences

➢The high heterozygosity (data is not given), allelic diversity and lack of proper resolution between the breeds and degenerate samples (FCA results is not given) reflect high motility of horses, horse trading habits, keeping all the horses of a village in one place over winter without any control on their breeding and lack of proper breeding strategy for horse in the country.

➢However, FCA analysis with prior phenotypic data and structure analysis showed some differentiation between breeds, which may be used in developing breed management and conservation strategies.

➢Neighbour joining tree of Anatolian haplotypes suggests presence of new undefined clades, which may contribute to understand evolutionary history of domestic horse.

Future Plan

➢Allele readings of microsatellite genotypic data will be corrected based on the ISAG horse panel to compare the results with literature data.

➢Unequal sample sizes will be made equal, samples from two foreign breeds (Arabian and Thoroughbred horses) present in Turkey (they might have contributed genetically to Anatolian breeds) will be collected and analysed to compare their results with Anatolian breeds.

➢After completing the analysis of all the breeds, a proper management strategy will be developed for the conservation of the local breeds.

References

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TÜRKHAYGEN-I is a recently initiated national project in Turkey, aimed at genotyping the existing livestock breeds, establishing banks (embryo, sperm, tissue and DNA) to preserve animal genetic resources and to use the knowledge in registration studies, and in developing conservation and management strategies. In context of the study, in situ conservation populations are also being formed. As a part of the study, each species included in the study (horse, goat, sheep, cattle and water buffalo) is analyzed at 21 microsatellite loci and by mtDNA PCR sequence diversity.