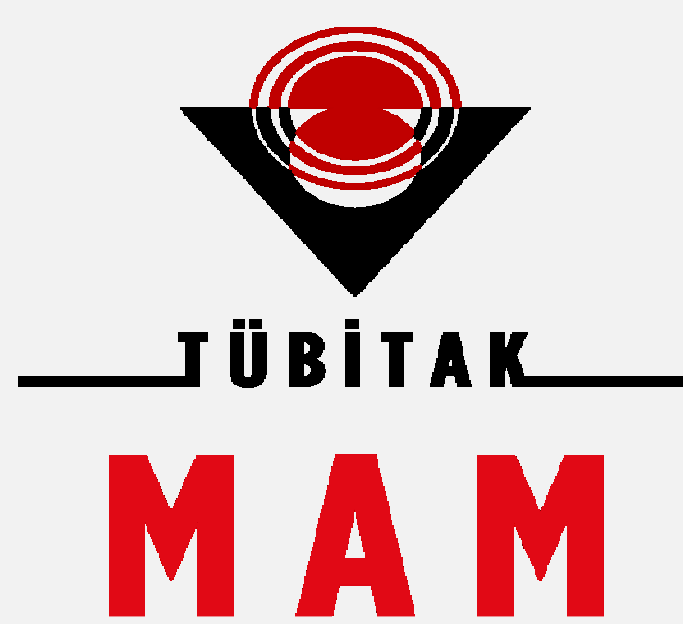


A preliminary genetic analysis of some Turkish horse breeds and implications for breed management studies



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Introduction/objectives

Horse domestication took place in multiple regions and compared to livestock, horse was recently domesticated (6,000 BP) (Vila, C 2001). All extant domestic horses were generated by one founder stallion, or to closely related stallions having the same Y haplotype (Lindgren et. al, 2004). The presence of low patrilineal and high matrilineal diversity indicates the start of horse domestication process after an appropriate male obtained.

The domestication of livestock species, including horses, has been widely affected by the course of civilization. High selection pressure is applied in search of breeds with preferred characteristics. Except for the performance "soundness" and "disease resistance" are two important concerns of horse breeding.

Even though there is high selective breeding in all over the world, there are no breeding studies in Turkey. Moreover, the crossroads between Asia and Europe and it is close to Africa. It received many human migrations through time (Roux, 1997), which were accompanied by livestock species and probably by horses, too. Therefore, genetic analysis of Anatolian horse breeds may have a vital importance as they still carry high genetic diversity due to lack of selection and inbreeding. The results may be used in improving of other horse breeds. They may also shed some more light on the domestication history of the horse and diversification of the present horse breeds.

The current project aims at genotyping of five Anatolian horse breeds by 20 microsatellite loci and analysis of mtDNA D-loop diversity based on 479 bp region. The project also aims at establishing proper conservation and management strategies including establishing of tissue culture banks for conservation purposes.

Materials and methods

For MtDNA D loop sequencing (478 bp region), the primers used are:

Forward: 5'-CCCAAGGACTATCAAGGAAG-3'
Reverse: 5'-GGAATGGCCCTGAAGAAAGA-3'

Genotyping was performed by PCR multiplex groups of 9 and 5 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 and CA425

The results were analyzed with the CEQ8800 Genetic Analysis System.

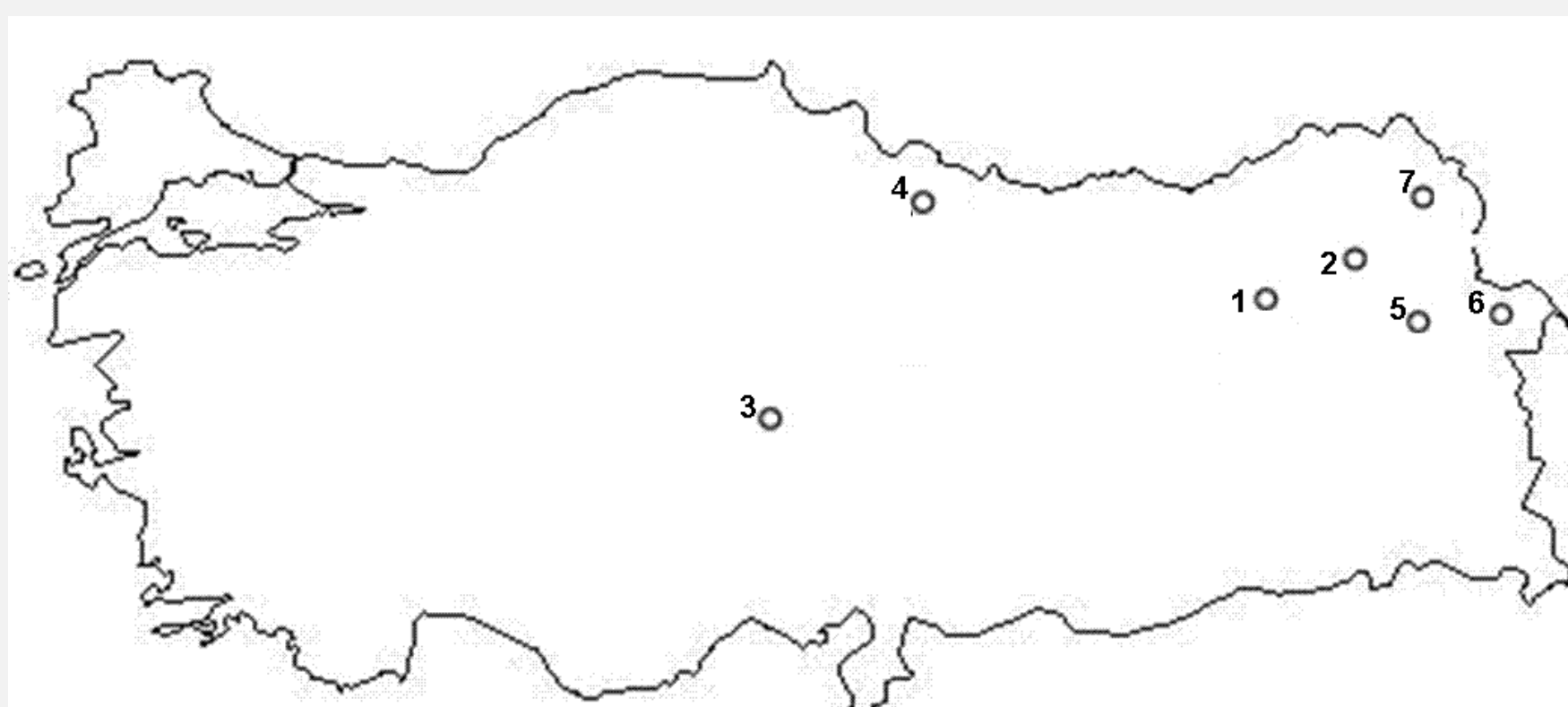


Figure 1: Sampling places of the populations (1) HKK and ERZ, (2) MLK and KRS, (3) UZY and KYS, (4) CNK, (5) AGR, (6) MLK and IGD, (7) MLK and ARD

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

Preliminary results

Table 1: Observed (on the left) and unbiased expected heterozygosity (on the right) estimates based on 14 loci.

HOB5												HEXP											
	HKK	ERZ	KRS	MLK	CNK	UZY	KYS	AGR	IGD	ARD	Mean		HKK	ERZ	KRS	MLK	CNK	UZY	KYS	AGR	IGD	ARD	Mean
HTG6	0.67	0.65	0.41	0.67	0.67	0.83	0.50	0.62	0.74	0.25	0.64	HTG6	0.70	0.70	0.71	0.59	0.69	0.65	0.71	0.66	0.68	0.25	0.68
HMS3	0.77	0.76	0.94	0.64	0.78	0.83	0.83	0.50	0.63	0.50	0.74	HMS3	0.86	0.81	0.83	0.78	0.81	0.80	0.88	0.85	0.80	0.86	0.82
HMS5	0.63	0.53	0.76	0.75	0.65	0.50	0.67	0.64	0.45	0.50	0.62	HMS5	0.67	0.67	0.67	0.65	0.69	0.68	0.68	0.65	0.66	0.68	0.67
HMS6	0.77	0.71	0.76	0.54	0.84	1.00	0.67	0.86	0.80	0.75	0.77	HMS6	0.76	0.77	0.80	0.74	0.78	0.77	0.76	0.77	0.77	0.68	0.77
ASB2	0.83	0.94	0.76	0.67	0.84	0.83	0.83	0.71	0.80	0.75	0.80	ASB2	0.84	0.85	0.81	0.87	0.84	0.89	0.83	0.84	0.86	0.82	0.85
I18	0.80	0.76	0.71	0.63	0.71	0.67	0.83	1.00	0.70	0.75	0.76	I18	0.78	0.78	0.75	0.82	0.78	0.62	0.88	0.87	0.78	0.82	0.78
AHT4	0.80	0.76	0.88	0.79	0.86	0.83	0.83	0.93	0.85	1.00	0.84	AHT4	0.84	0.76	0.86	0.84	0.82	0.83	0.73	0.83	0.85	0.86	0.82
LEX33	0.87	0.76	0.71	0.75	0.86	0.67	1.00	0.79	0.80	0.75	0.80	LEX33	0.81	0.78	0.83	0.82	0.83	0.89	0.92	0.76	0.86	0.64	0.83
COR2	0.80	0.65	0.59	0.54	0.68	0.83	0.33	0.86	0.75	0.75	0.67	COR2	0.78	0.71	0.62	0.66	0.68	0.85	0.70	0.74	0.72	0.68	0.72
ASB43	0.80	0.82	0.88	0.57	0.75	0.67	1.00	0.86	0.80	0.75	0.79	ASB43	0.75	0.78	0.81	0.74	0.78	0.79	0.84	0.77	0.76	0.75	0.78
AHT33	0.83	0.88	0.88	0.75	0.89	0.83	0.83	0.64	0.80	0.75	0.82	AHT33	0.89	0.91	0.81	0.88	0.87	0.86	0.86	0.86	0.86	0.89	0.87
NEVHEQ79	0.77	0.82	0.82	0.61	0.84	0.83	0.50	0.50	0.80	0.75	0.72	NEVHEQ79	0.83	0.75	0.77	0.74	0.82	0.79	0.67	0.61	0.78	0.79	0.75
HMS2	0.87	0.88	0.82	0.61	0.76	1.00	1.00	0.79	0.70	0.75	0.83	HMS2	0.82	0.84	0.85	0.80	0.84	0.76	0.89	0.84	0.83	0.86	0.83
CA425	0.73	0.65	0.82	0.68	0.83	0.50	0.67	0.50	0.85	0.75	0.69	CA425	0.81	0.71	0.73	0.75	0.80	0.59	0.56	0.65	0.83	0.75	0.71
Mean	0.78	0.76	0.77	0.66	0.78	0.77	0.75	0.73	0.75	0.70	0.75	Mean	0.80	0.77	0.78	0.76	0.79	0.77	0.78	0.76	0.79	0.74	0.78

Table 2: Allelic diversity estimations based on 14 loci.

	HKK	ERZ	KRS	MLK	CNK	UZY	KYS	AGR	IGD	ARD	TOTAL	MEAN
HTG6	5	5	6	6	8	4	3	4	5	2	10	5.1
HMS3	8	7	8	8	9	5	6	7	6	5	10	7.1
HMS5	3	4	3	3	6	3	3	4	3	3	6	3.6
HMS6	6	5	6	4	7	4	5	6	5	3	7	5.3
ASB2	9	9	8	10	9	6	5	8	12	4	16	8.4
I18	9	7	7	9	10	3	7	8	7	4	12	7.4
AHT4	10	5	10	8	10	7	5	7	8	5	12	7.8
LEX33	8	7	7	8	15	7	8	8	10	4	17	8.7
COR2	5	5	5	6	5	5	4	4	5	3	6	4.9
ASB43	6	5	7	5	5	5	5	6	4	8	8	5.4
AHT33	11	11	8	10	14	6	7	9	11	6	17	9.7
NEV79	8	8	7	8	12	5	5	5	8	4	17	7.3
HMS2	8	7	8	9	8	4	7	9	8	5	10	7.6
CA425	9	6	7	8	13	3	4	6	8	4	13	7.1
MEAN	7.5	6.5	6.9	7.3	9.4	4.8	5.3	6.4	7.3	4.0	11.5	6.8

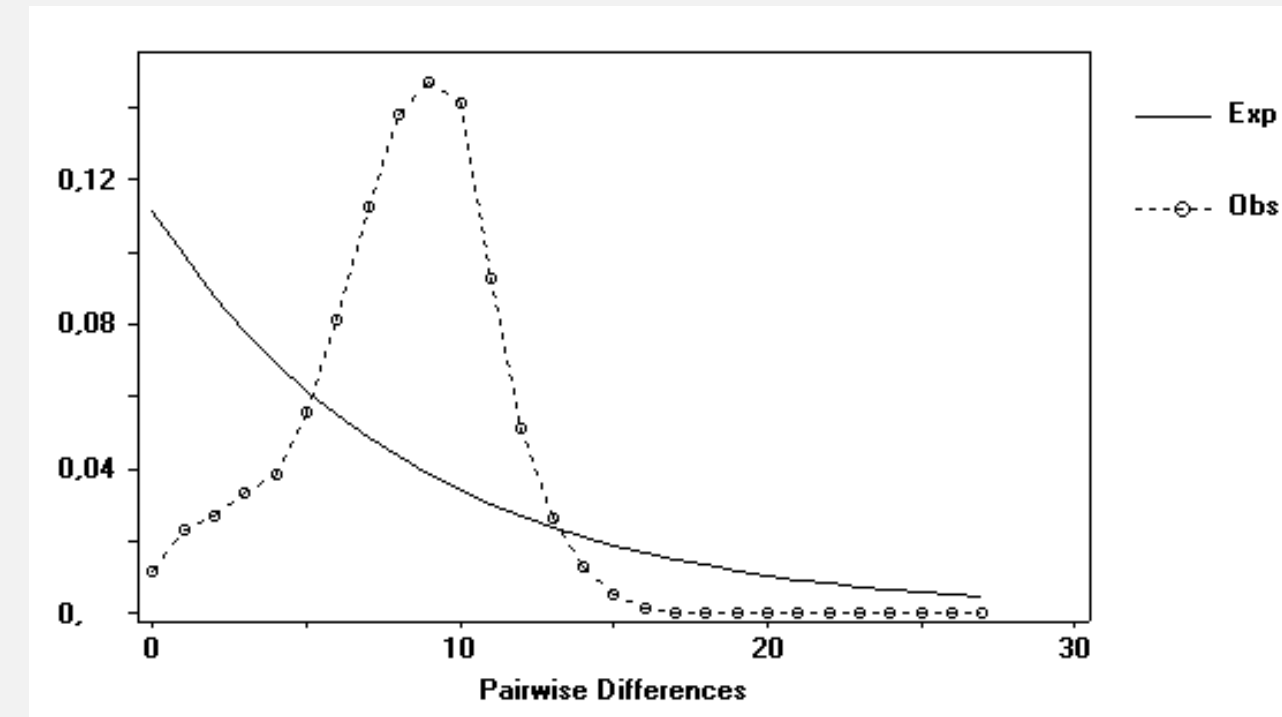


Figure 3: Mismatch distribution graph of 439 bp of mtDNA D-loop sequences (n:165).

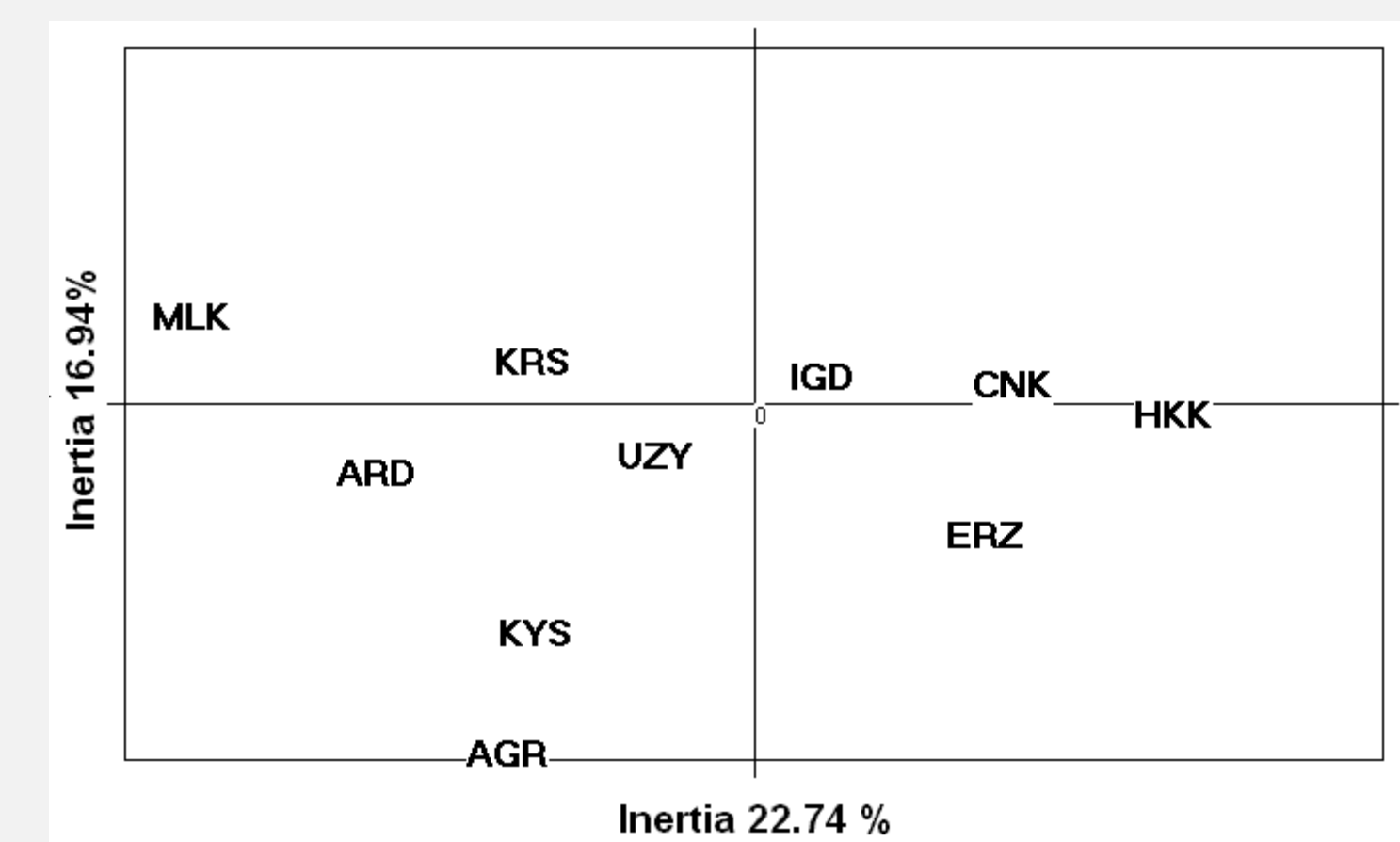


Figure 2: Principle component analysis of the populations based on 14 loci.

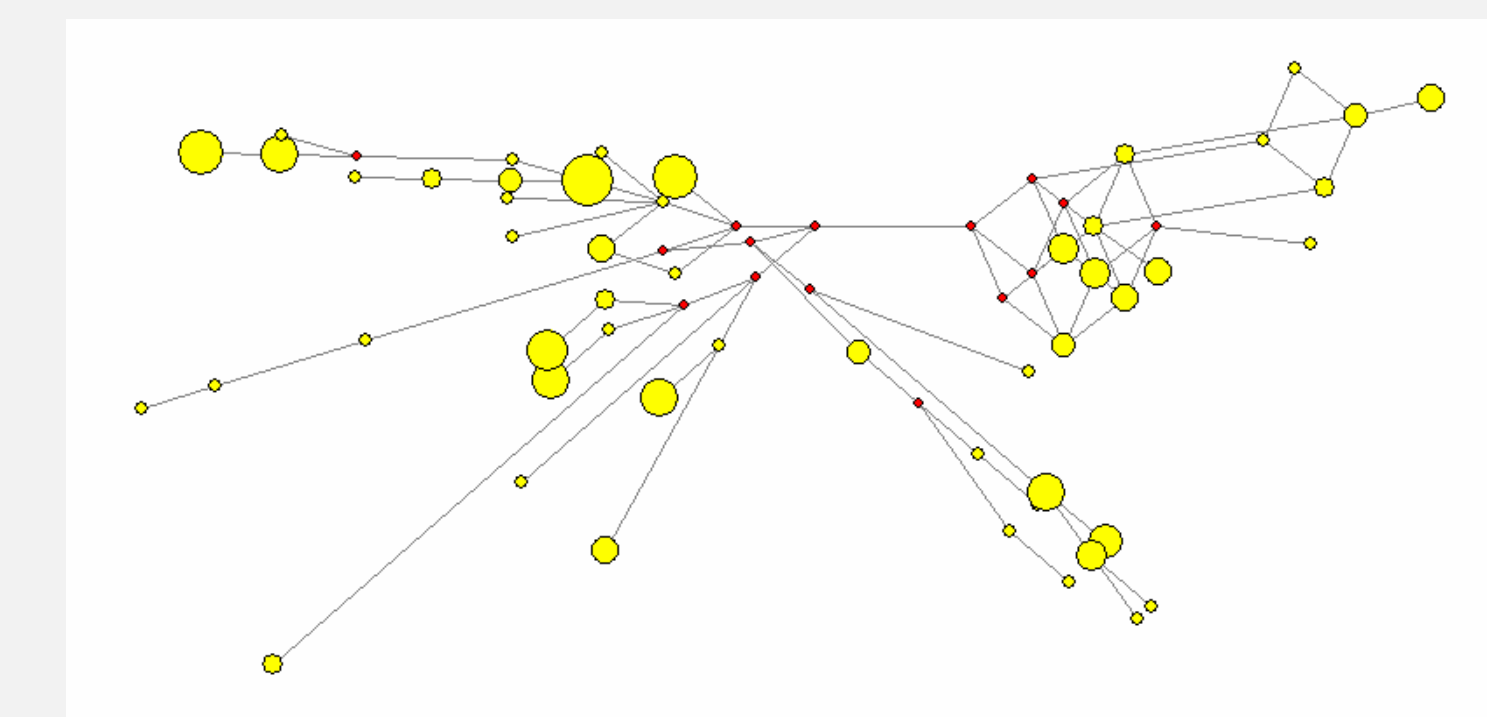


Figure 4: Median Joining Network of 439 bp of mtDNA D-loop sequences (n:165). There are 51 haplotypes after star contraction.

Inferences

- The high heterozygosity (Table 1) and allelic diversity (Table 2) Turkish horses have are due to high motility, dynamic horse trade sector, and lack of breeding strategy for the horse in Turkey.
- Factorial Correspondence Analysis of the individuals, assignment test and pairwise Fst analysis (results are not shown) did not indicate significant population differentiation based on 14 microsatellite loci.
- The PCA analysis explained the variation by the first two axes about 40%; separation of the populations was not in accordance with geographic origin or phenotypic differences.
- Results indicate admixture, but the number of samples within populations are unequal and some are quite low, yet.
- Mismatch distribution graph resulted in unimodal peak with a mean of 7.99 bp difference. The shape of the graph and not significant Tajima's D estimate (-0.82218, p>0.1) estimate did not suggest population expansion.
- Median joining network graph does not support any population expansion, too. There is no central haplotype as seen in livestock populations. The sequences are quite diverse from each other.

Project plan

- Current unequal populations samples sizes (n: 4-63) will be completed to 50.
- 2 more Anatolian breeds will be sampled.
- All the samples will be analyzed at 20 microsatellite loci and for mtDNA control region diversity.
- The microsatellite allele readings will be corrected for ISAG alleles.
- Within and between population genetic diversity will be analyzed and results will be used for breed management.
- mtDNA results will be analyzed using known haplotypes in search for the presence of new haplotypes and for better understanding of the origin of Turkish 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 20 microsatellite loci and by mtDNA sequence diversity.