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ARTICLES

MITOCHONDRIAL DNA ANALYSIS OF ANCIENT DOMESTIC GOAT IN THE MONGOLIA

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Abstract: *Food and agricultural production sector, especially livestock production is vital for Mongolia's economic and social development. The five breeds of livestock – cattle, horses, sheep, goats and camels, have always been directly related to the history, culture and economy of Mongolia. It is undeniable that these five breeds of livestock are considered as an important asset of the Mongolian economy as well as guarantee of national security. Out of the five breeds, small livestock including sheep and goats account for more than 80% of the total livestock population (according to the National Statistical Office data). It is not hard to retrieve research and recorded materials about the morphology or the economic productivity of these breeds and their sub-breeds. However, the development of society now demands higher yield from the livestock animals. Genetically, the a1, a2, a3 and a4 samples appertaining to some 3,000 years ago are substantially different from the haplogroups. However, judging by the phylogenetic tree the 800 year-old samples, as compared to the phylogroups of the 3rd century BC, are closer to the modern samples. Alongside this, from molecular distance we find that the M1 haplogroup is a sample belonging to the 3rd century BC, which had separated the earliest, while the aforementioned 800 year-old sample separates at a later period, which chronologically is convincing. But the a6 ancient sample conforms to the B haplogroup and the a8 or the sample of 800 years ago is in the same haplogroup as the sample of the 3rd century BC. However, the a6 sample obtained from the 3rd century BC tombs obtained sample was the origin of modern goats that can be included in haplogroup B.*

Keywords: *Capra hircus; mtDNA; 3rd century BCE; haplotypes; 13 century AD haplotypes; haplogroup; D-loop; genetic diversity;*

INTRODUCTION

The domestication of goat (*Capra hircus*) was probably a watershed in the evolution of human modes of subsistence [2][3][10][7]. Besides its valuable dairy produce, its portability has made goat the key element to ensure a stable human settlement in an otherwise inhospitable habitat. The genetic traces of domestication in this species have been first addressed [9] by analyzing the diversity of the mitochondrial D-loop among individuals

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collected worldwide. Mitochondrial DNA (mtDNA) sequence of maternal lines research in eukariotic organism is a cheap and reliable. Mitochondria is genetically passed on only through egg cell. Mitochondria from sperm will not enter the fertilized egg. In other words, mitochondrial DNA data represents mother's genetic line. [1] Mitochondrial genome codes about 37 genes and it's circular DNA molecule is composed of some 17 thousand base pairs. Mitochondrial DNA plays a key role in the study of evolution and phylogeny, and has been widely used to study goat domestication process and origin [5]. Mitochondrial DNA shows maternal lineage inheritance with a relatively rapid evolution rate and no recombination system [6]; therefore, these genetic features have made mtDNA an appropriate tool for the investigation of the origin of the species. Furthermore, it has been commonly used to describe the genetic polymorphism in goats [6][9]. A certain part of mitochondrial DNA (to be referred as mtDNA) is called D-loop. This D–loop part does not contain genetic codes, and its DNA nucleotide sequence varies substantially from each other. This characteristic makes it possible to identify genetic relations of sequence based on mother line. At the international level, there are numerous studies on goat mtDNA. Some of them include studies into Mongolian goat. In this study, 11 goat samples were collected from two different herders of Dörvöljin soum in Zavkhan aimag and Khutug-Öndör soum in Bulgan aimag from 13th century and 3rd century BC tombs.

Objective and output. The objective of the research is to identify genetic relations and origins of the registered goat breeds and sub-breeds based on mtDNA data.

- Achieving the above objective requires implementation of the following outputs, such as:
- Obtained faunal sample containing DNA from 13th and 3rd century BC tomb sites.
- DNA cleaning and mtDNA molecular genetic marker polymerase chain reaction is performed for each sample.
- mtDNA nucleotide sequence is identified, sequence analysis performed by bioinformatics computer application.
- To identify direction of maternal origins used by phylogenetic analysis. A neighbor-joining tree was obtained by using two-parameter model constructed based on the aligned sequences to identify possible phylogenetic clades with the aid of the MEGA X.

MATERIALS AND METHODS

In order obtain ancient goat DNA, goat faunal bone remains were sampled from the 13th century and 3rd century BC tomb sites. These sites are among one of the earliest agricultural settlements in Central Asia and dates back to the 13th century and 3rd centuries BC. 11 bones were selected for DNA analyses from the faunal remains classed as *C.hircus*.

Methods for ancient DNA extraction. Deoxyribonucleic acid extraction from the the 13th and 3rd century BC faunal bone samples primarily followed the established protocol. First, the exterior surface of the bones was cleaned with sterile scalpels. The cleaned surface was ground to obtain bone powder (0.4g), which was then suspended in 1 ml extraction buffer (0.01M Tris-HCl, 0.5M EDTA (pH=8)), incubated at 37°C for 16 hours and once again incubated under 56°C for 3 hours. The supernatant was transferred in to a 15 ml tube adding 3 ml of binding buffer (5M CuSCN, 0.025M NaCl, 0.01M Tris-HCl (pH=8)) down to mixture pH to pH=4. The mixture was then transferred to "Qiagen filter column" and centrifuged at 8000 r.p.m. for approximately 1 minute. The supernatant was washed with washing buffer (AW1, AW2), centrifuged at 14000 r.p.m. for 1 minute. Finally, the supernatant was washed with ultrapure water created by Thermo

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Scientific™ to obtain DNA extracts that can be used directly for polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP): PCR amplifications were performed in a reaction volume of 50 µl containg two units of Taq DNA Polymerase (Thermoscientific), 5µl of 10x PCR buffer, 3µl of 25mM MgCl2, 4 µl of 2mM dNTP, 1µl of 10µM each primers and 1.1µl of DNA template in sterilised water. The thermal conditions for PCR are as follows: the first denaturation at 95°C for 2 minutes and 35 seconds,followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minutes, 10 cycles and denaturation again at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C 30 seconds and next 30 cycles, and then final extension at 72° C for 10 minutes. The PCR products were checked by electrophoresis with 1.2% agarose gel. mtDNA sequence were identified at the Macro Gene Lab, Korea. Each sequence was compared with GenBank reference sequences and analyzed by Snapgene 2.3.2 and mtDNA profiler.

Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP): Next primes will be used in PCR:

FW: 5'-CAGTCGAACATCCCTACATTATTATTGG-3',

REV: 5'-TTAGTCTTATTGATTTGGAGGGCGTTA-3'

The cycle condition of PCR: 95°C 30 sec, 55°C 30 sec, 72°C 2 min.

Sequence analysis:

DNA fragments will be sequenced in Macrogen Lab, Korea with Forward/Reverse primers. The internal primer sequences were as follows:

GQF1:
5'-TACAATCAATACACTGGTCTT-3',
GQR1:
5'-ATTACGTTTATGCTGGATT-3';
GQF2:
5'-ATAACGCGGACATACAGC-3',
GQR2:
5'-AGAGTGGGCGATTTTAGGTGAGAT-3';
GQF3:
5'-GGGCCATCTCACCTAAAATC-3',
GQR3:
5'-GGCTGGGACCAAACCTATG-3'.

DNA sequence alignment will be accomplished by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and each sequence was compared with GenBank reference senquence and analyzed by Snapgene 2.3.2 and mrDNA profiler.

RESULTS AND DISCUSSION

The sequences of mtDNA D-loop of control region (fragment of mtDNA) was generated successfully, encompassing between 400 and 430 base pairs. The sequences of mtDNA D-loop of control region have been annotated in NCBI GenBank database and the accession numbers were addressed in the paper (MH341120-MH341125, MH603046-MH603051). The comparison of the 3rd century BC and 13th centrury samples D-loop sequences with GenBank D-loop control region sequences revealed high rates of identity (up to 99%) which shows good preservation of DNA in 13th and 3rd century BC samples. Furthermore, 22 reference sequences from GenBank database were chosen for phylogenic comparison with 3rd century BC samples sequences.

The information for reference sequences is shown in Table 2 [8]. These reference sequences belonged to six main well-defined haplogroups named A, B, C, D, F and G, which were identical to those from the reference studies [4][9]. After the construction of the phylogenetic tree, modern haplotypes were clustered in six main haplogroups, while all

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five 3rd century BC sample sequences were grouped in to A haplogroup. The validity of the main haplogroups was strongly approved by the bootstrap values of >90%. The A haplogroup was the largest, including ancient and modern haplotypes with 11 individuals. The other five haplogroups only consisted of modern sequences and lined up thereafter by three, three, four, four and two individuals for D, G, B, C and F haplogroups respectively [6] (see Fig. 1).

mtDNA sequence was identified at the Macro Gene Lab, Korea. Each sequence was compared with the GenBank reference sequences and analyzed by MEGA X and DnaSP. Haplogroups, identified by using mtDNA tree built software MEGA X, and with Arlequin v3.1.5 program, the expected 140bp sequences nitrogenous base containment was A: 28.97%, T: 30.89%, C: 22.19%, G:17.95

Table 1. A mapping of goat mtDNA 140 bp of nucleotides sequence D-loop control region haplotypes 105 polymorphic sites

Sample number	15604	15605	15607	15608	15610	15611	15613	15614	15615	15616	15617	15618	15619	15620	15621	15622	15623	15625	15626	15627	15628	15629	15631	15632	15633	15634	15635	15636	15637	15638	15640	15641	15642	15643	15644	Haplotype
a1, a2, a3, a4,a8		T	T	T	A	T	T	T	T	T	T	G	G	G	G	G	T	T		T	-	-	A	A	T	A	G	A	T		G	G	T	T		H1
a6	T	G			A	T	T	T	C		T		T	T		A		G	T	A	C		A	G	T	A	C	A	T	T	T	A	C	T	G	H2
a7, a9, a10																							G	T	T	A	C	G	T							H3
a11																																				H4
a12						C																														H5
NC 005044.2	C	C	A	C	C	A	A	C	G	C	C	A	A	C	A	C	C	C	A	C	A	A	G	T	T	A	C	G	C	G	A	T	G	C	A	Reference sequence

Sample number	15645	15646	15647	15648	15649	15650	15651	15652	15654	15655	15656	15657	15658	15659	15660	15661	15662	15663	15664	15665	15666	15668	15669	15670	15672	15674	15675	15677	15678	15679	15680	15681	15683	15684	15685	Haplotype
a1, a2, a3, a4,a8		T	A	G	T	G	G	T	C	A	G	G	G	A	A		A		T	T		A		T	G		T	T			G	C	T	T	G	H1
a6	T	A				C	C	G				T	A			A		A	T	A	A	G	T		A	T	C	A	T	T	C		G	T	C	H2
a7, a9, a10								-																												H3
a11								-																												H4
a12								-													C		-													H5
NC 005044.2	A	G	T	A	C	A	T	-	A		A	C	C	G	C	T	C	G	C	C	T	C	A	C	C	A	A	A	C	A	T	T	A	C	T	Reference sequence

Sample number	15686	15687	15688	15689	15690	15691	15692	15693	15696	15697	15698	15699	15701	15702	15703	15705	15706	15707	15708	15709	15710	15711	15712	15714	15715	15716	15718	15720	15721	15722	15723	15724	15725	15726	15728	Haplotype
a1, a2, a3, a4,a8	G	G	T	G	C	T	G		G	T	G	A	G	C	T	T	-	-	G	G		T	T	T		C	T	G	A	G	T	C			-	H1
a6						G		G		C	C			C	C		T		G		T	C	A	G	A	G	T	G		C		A	C	C	T	H2
a7, a9, a10																	-																				H3
a11																	-																				H4
a12																	-				T		C								C	C					H5
NC 005044.2	A	A	C	A	T	C	C	A	T	A	A	C	C	G	G	C	-	A	T	A	C	A	G	C	T	T	A	A	T	A	G	T	T	T	C	Reference sequence

The nucleotides divesity was 0.4224, Tajima test statistic [10] was 2.495497 and number of polymorphic sites was 105 for the 5 haplotypes that were identified in this study.

Table 2. Described result of Mongolia's genetic diversity

№	N	H	P	D	Hd	Π
1	11	5	105	2.4954	0.7636	04224

N-sample number; H-Haplotype number; P-Number of segregating site; Hd-Haplotype diversitynumber; π-Nucleotide diversity number; D- Tajima test statistic

In our study from 11 samples, 5 haplotypes were identified and haplotype diversity was 0.7636 totally divided into 3 haplogroup suddenly a6 sample only included in haplogroup B and our identified other haplogroup were not included recent studies 7 haplogroup (Table.1) so we signed new haplogroup (M1,M2) (Fig.2). Also haplotypes (H1, H2, H3, H4, H5) were newly identified.

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Table 3. The haplogroup specification of 22 modern reference goats [8]

Accession no.	H*	Region	Original work	Ref. 1	Ref. 2
AY155721	A	India	Joshi et al., 2004	Naderi et al., 2007	Han et al., 2010
EF618134	A	Italy	Naderi et al., 2007	NA	Han et al., 2010
EF617779	A	France	Naderi et al., 2007	NA	Han et al., 2010
EF618200	A	Jordan	Naderi et al., 2007	NA	Han et al., 2010
EF617945	A	Iran	Naderi et al., 2007	NA	Han et al., 2010
EF617965	A	Iran	Naderi et al., 2007	NA	Han et al., 2010
AB044303	B	Laos	Mannen et al., 2001	Naderi et al., 2007	Han et al., 2010
EF617706	B	Azerbaijan	Naderi et al., 2007	NA	Han et al., 2010
AJ317833	B	Mongolia	Luikart et al., 2001	Naderi et al., 2007	Han et al., 2010
DQ121578	B	China	Liu et al.,2006	Naderi et al., 2007	Han et al., 2010
AY155708	C	India	Joshi et al., 2004	Naderi et al., 2007	Han et al., 2010
AJ317838	C	Switzerland	Luikart et al., 2001	Naderi et al., 2007	Han et al., 2010
EF618413	C	Spain	Naderi et al., 2007	NA	Han et al., 2010
DQ188892	C	China	Liu et al., 2005	Naderi et al., 2007	Han et al., 2010
AY155952	D	India	Joshi et al., 2004	Naderi et al., 2007	Han et al., 2010
EF617701	D	Austria	Naderi et al., 2007	NA	Han et al., 2010
DQ188893	D	China	Liu et al., 2005	Naderi et al., 2007	Han et al., 2010
DQ241349	F	Sicily, Italy	Sardina et al., 2006	Naderi et al., 2007	Han et al., 2010
DQ241351	F	Sicily, Italy	Sardina et al., 2006	Naderi et al., 2007	Han et al., 2010
EF618084	G	Iran	Naderi et al., 2007	NA	Han et al., 2010
EF618535	G	Turkey	Naderi et al., 2007	NA	Han et al., 2010
EF617727	G	Egypt	Naderi et al., 2007	NA	Han et al., 2010

**H, haplogroup; NA, not announced or applicable*

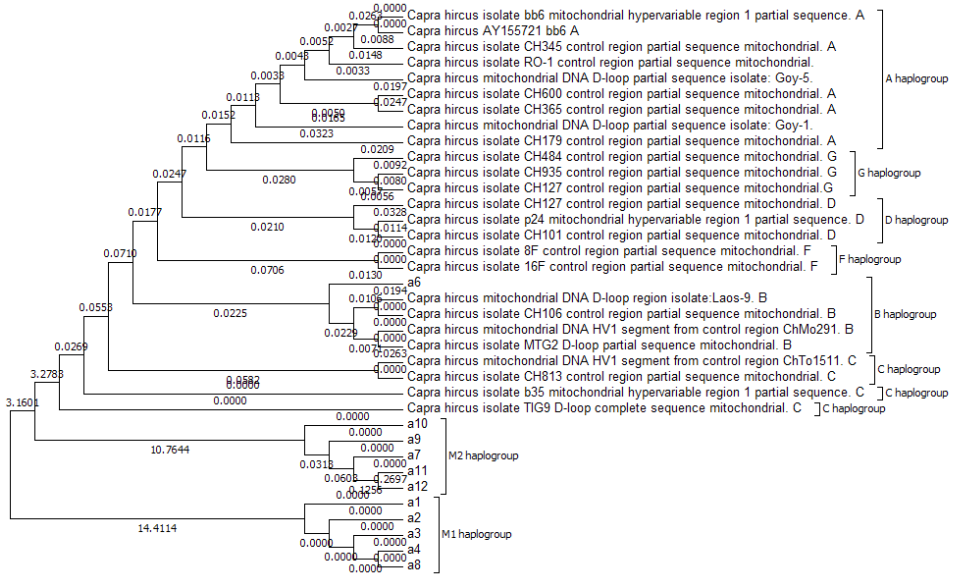

Figure 1. Phylogenetic tree of domestic goat (C. hircus) mtDNA D-loop of control region sequences, constructed by MEGA X software v10 using neighbor-joining method. Bootstrap resampling was calculated 1000.

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CONCLUSIONS

In phylogenetic tree, M1 haplogroup and modern haplogroup, the genetic distance was 14.41, while in the M2 haplogroup and modern haplogroup, the genetic distance was 10.76. From this point of view a1, a2, a3, a4 were 3rd century BC samples. which are at much larger genetic distance than modern samples.

The 13th century samples were relatively close to modern samples and it is clear that they are closer to the 3rd century BC samples in the phylogenetic tree (Fig.1). In addition, from molecular distance, included in the M1 haplogoup are the 3rd century BC samples, so first it's separated and c the fact that 13th centuries before it's separated from time to time. For haplogroup creating chronologically separate new haplogroups but a8 is 13th centuries sample was included in a haplogroup sample of 3rd century BCE. However, the a63rd century BC sample was the origin of modern goat that can be included in the haplogroup B.

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