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PHYLOGENY OF 19 INDIGENOUS SHEEP BREEDS IN XINJIANG INFERRED FROM CYTOCHROME B

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ABSTRACT

Xinjiang has large sheep breeds, but their origins, evolution, genetic diversity of systems are still unclear. In this study, the genetic diversity and phylogeny of 19 indigenous sheep breeds in Xinjiang, China and one western breed, Texel were analyzed. Single-strand conformation polymorphism (SSCP) was used to detect polymorphisms of the sheep mitochondrial DNA fragment and based on SSCP results, individuals from the studied sheep breeds were randomly selected for sequencing entire Cytochrome b gene (Cyt b). Neighbor joining (NJ) and Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) methods were used to constructing the phylogenetic tree. The network analysis and population expansion were carried out with entire sequences of mitochondria Cyt b gene. The length of the Cyt b sequences were considerably variable between 1082 and 1149bp. The phylogenetic analysis revealed that three mtDNA lineages A, B and C were detected in fourteen Xinjiang sheep breeds, except for the absence of lineage C in five breeds. Phylogenetic tree and median joining network were constructed with the sequenced Cyt b gene, and there were three independent clusters with some sequences deviated from cluster A and C based on the phylogenetic tree and network analysis. The genetic distances were showed a multi-maternal originality of the nineteen Xinjiang local sheep breeds, which were clustered with the sheep groups in other parts of China. The genetic diversity of Xinjiang breeds was higher than the other Chinese and foreign sheep breeds. The results of Cyt b sequences analysis revealed that Xinjiang local sheep breeds may have distinct maternal origins than the other sheep breeds of China. These are not only influenced by Mongolian sheep, Tibetan sheep and Kazakh sheep of China, but also had the genetic relationship with the breeds of other neighbor countries. This study will provide valuable information of sophisticated phylogenetic history of Xinjiang local sheep.

Key words: sheep; phylogeny diversity; China, Xinjiang, Cytochrome b.

INTRODUCTION

Presently the control region (D-Loop) and Cytochrome b (Cyt b) of Mitochondrial DNA (mtDNA) has received much attention because of its commonly used genetic markers, which offer important phylogenetic inference, to explore animal genetic diversities. The mtDNA data has many possible uses in phylogenetic field and also been investigated as a potential maternal ancestors. The mtDNA data from wild and domestic sheep has shown that there were no contributions from urial and argali species during domestication process of sheep origin and were identified with three maternal lineages as A, B and C in domestic sheep breeds (Hiendleder *et al*., 1998, 2002; Meadows *et al*., 2007; Wood and Phua, 1996, Guo *et al*., 2005; Pedrosa *et al*., 2005, Hassanin *et al*, 1998; Bunch *et al*, 2006; Tsunada *et al*., 2009).

Xinjiang Uyghur Autonomous Region lying on the northwest of China, have various livestock breeds (Sulaiman *et al*, 2011; ACA, 1964) but very little work is done about their genetic information and phylogenetic position in relation to the other provinces of China and neighbor countries.

This study was designed to describe and examine the genetic diversity and phylogeny of indigenous sheep breeds domesticated in Xinjiang. The available sheep sequences of cytochrome b in previous studies were obtained from Genbank database (Wang.*et al*., 2006; Hiendleder, Mainz *et al*. 1998; Chen *et al*., 2006; Pedrosa *et al*., 2005; Meadows *et al*., 2007) and compared.

MATERIALS AND METHODS

Sample Collection and Genomic DNA extraction: A total of 1035 ear tissues and blood samples of nineteen sheep breeds were collected from remote villages in Xinjiang and for Texel, from European breed, farm in Beijing (Aoxin Stud Farm, Ltd). The individuals were

randomly collected from female populations of each breed depending on the pedigree information provided by the owners. Ear tissues were stored at –20ºC in 75% ethanol and blood samples were stored at –20ºC in Vacutainer, filled with 2ml of Sodium Citrate. Total Genomic DNA was extracted from ear tissue and blood samples using revised version of standard phenol-chloroform extraction method (Sambrook and Russels 2000). The DNA samples were tested with 1% Agarose gel and stored at –20ºC. Geographical distribution of the sampled breeds was described previously (Sulaiman *et al*., 2011).

DNA amplification and sequencing: A total of 1035 genomic DNA samples were analyzed by single-strand conformation polymorphism (SSCP) analysis and two pairs of primers were used described previously (Guo *et al*., 2005). The Polymerase Chain Reaction (PCR) amplification for SSCP analysis was carried out in a 25µl reaction mixture containing 20–100ng DNA template, 1.0µM of each primer, 200µmol of dNTPs, 1x PCR buffer (including 1.5mM $MgCl_2$, and one unit of Taq DNA polymerase (Huitian Dongfang Co., Beijing, China). The PCR conditions consisted, initial denaturation at 94ºC for 5min, followed by 35 cycles at 95ºC for 30s, 58ºC for 45s, 72ºC for 60s and a final extension of 8min at 72ºC. PCR Amplification was performed in an Effondorf Master Gradient Programmable Thermal Controller (Effondorf Inc., Germany).

The SSCP vertical electrophoresis was conducted as reported previously (Sulaiman *et al*, 2011). The gel was silver stained and different lineages of SSCP results were visually determined and scored as described previously (Guo. *et al*., 2005). Individuals of each breed for sequencing Cytochrome b were randomly selected based on the scores of each lineage, and amplified cytochrome b gene using the primers described previously (Meadow *et al*., 2007). The purified PCR products were subsequently shipped to SinoGenoMax Co., Ltd (Beijing) for bidirectly sequencing by BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) on ABI PRISM 3700 DNA Analyzer. Two following internal primers were used for entire sequencing cytochrome b gene as described previously (Chen *et al*., 2005):

CYTB-FI (5′-CGCCTTCCACTTTATCCT CCC-3′) and CYTB-RI (5′-GTCTGATGGAATT CCTGTGGG-3′). The Cytochrome b sequences were edited using DNAstar 5.0 package (DNASTAR Inc.).

Data analysis: A total of 167 individuals from 1035 samples of the nineteen sheep breeds of Xinjiang and one western breed, Texel were chosen to sequencing. The entire base pairs of Cytochrome b gene (1140bp) were analyzed, the results were compared with the data in previous studies obtained from Genbank database. The available sheep sequences of cytochrome b including the wild sheep such as Ovis vignei (Genbank access number AF034729), Ovis orientalis (Genbank access number AJ867261), Ovis ammon (Genbank access number AJ867272) and Chinese breeds as Lanzhou big tail sheep，Ganjia, Oula，Heiqiupi, Hanzhong，Henan big tail sheep(Genbank access number DQ903211~903224) and Turkish breeds as Akkaraman, Tuj, Karakaya, Hemsin, Morkaraman, Awassi from Israel (Genbank access number DQ097407~097430 and DQ851912, DQ852045, DQ852072, DQ852077~852082) .

All sequences were manually edited using the BioEdit package (Hall, 1999) and aligned using the Clustal W program (Thompson *et al*., 1994). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura, Dudley, Nei and Kumar, 2007), with a Kimura 2-parameter (with transitions and transversions) model and a bootstrap (1000 replications) test. A pairwise distance matrix between Cytochrome b haplotypes was calculated and a neighbor joining (NJ) tree was constructed based on the nucleotide *p*-distances and other parameters were set at the default values.

The median joining (MJ) networks (Bandelt *et al*., 1999) were plotted using the Network 4.1.0.9 program (http://www.fluxus-engineering.com) to reveal the possible relationships among haplotypes. Nucleotide and haplotypes diversities were calculated using DnaSP 5.10.00 (LIBRADO, P. and ROZAS, J. 2009). Fu's Fs statistics (Fu 1997) and mismatch distribution were computed using Arlequin3.11 (Excoffier *et al*, 2005).

RESULTS

The results of 167 Cytochrome b sequences analysis from the nineteen local breeds in Xinjiang and one Western breed revealed that the length of the sequences were considerably variable between 1082 and 1149bp. The difference of length of the sequences might be caused by tandem repeated sequence and also may be with transitions and transversions of base pairs. The sample information and number of haplotypes, haplotypes diversity, observed lineage and nucleotide diversity of each population are given in Table 1.

A phylogenetic analysis of the genetic diversity was applied, based on constructing a Neighbor-joining phylogenetic tree (Saitou and Nei, 1987) to determine maternal ancestors of the nineteen Xinjiang local sheep breeds. The results of sequence alignments with Ovis ammon (argali), *Ovis vignei* (Urial), Asian mouflon (*Ovis orientalis*) and other domestic sheep breeds of China, Turkey and Israel retrieved from Genbank showed that the same three clades A, B and C were detected, which were reconciled with haplotypes A, B and C (Figure 1

and Figure 2) where as the NJ and UPGMA tree constructed by Mega 4.0 (Figure 3 and Figure 4). All of the sheep breeds in Xinjiang were clustered with other sheep breeds of China, as Lanzhou, Ganjia, Oula, Heiqiupi, Hanzhong, Henan, and also the breeds in other countries mainly around Silk Road, as Awassi, Akkaraman, Karakaya, Hemsin, Tuj and Morkaraman, which showed obvious genetic relationship with their geographical distribution. But the Baqiangzi breed was not clustered with Xinjiang sheep breeds but with Oula, Texel and Tuj； The Emil breed was clustered with Karakaya at first and then clustered with Hemsin despite of long geographical distances between them. The results for Fu's Fs statistics (Fu 1997) gave a very significant negative value when a population expansion occurs. Based on the results of the Fu's Fs statistic and mismatch distributions（data not shown）from Arlequin, the probability of observing a random neutral sample with a number of alleles is similar than the observed value. The Fs values for haplotypes A, B and C were -25.492 ($P<0.000$), -5.119 ($P < 0.000$) and - 4.147 ($P<0.00$), respectively, which suggested that lineages A, B and C depart significantly from the neutral model and have a quite different demographical history. The results for network analysis showed that the three clusters correspond with the phylogenetic clades (Figure. 2). The phylogenetic clades were essential to reveal the genetic structure between the lineages. Each phylogenetic clade was clearly isolated, but there was some deviation in clade A and C. The results shows that there were more than one common maternal ancestor in this two clades. Parameters of Cyt b variability within each cluster were given in Table 2.

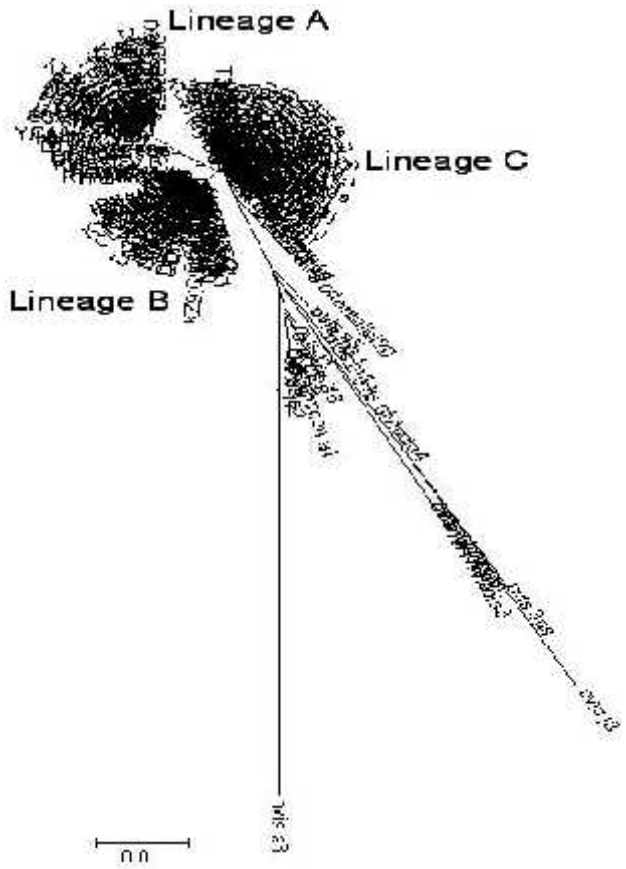

Figure1 the Neighbor joining phylogenetic tree constructed based on Cyt b sequences.

The A, B and C indicate sheep maternal lineages. Wild sheep serves an out group.

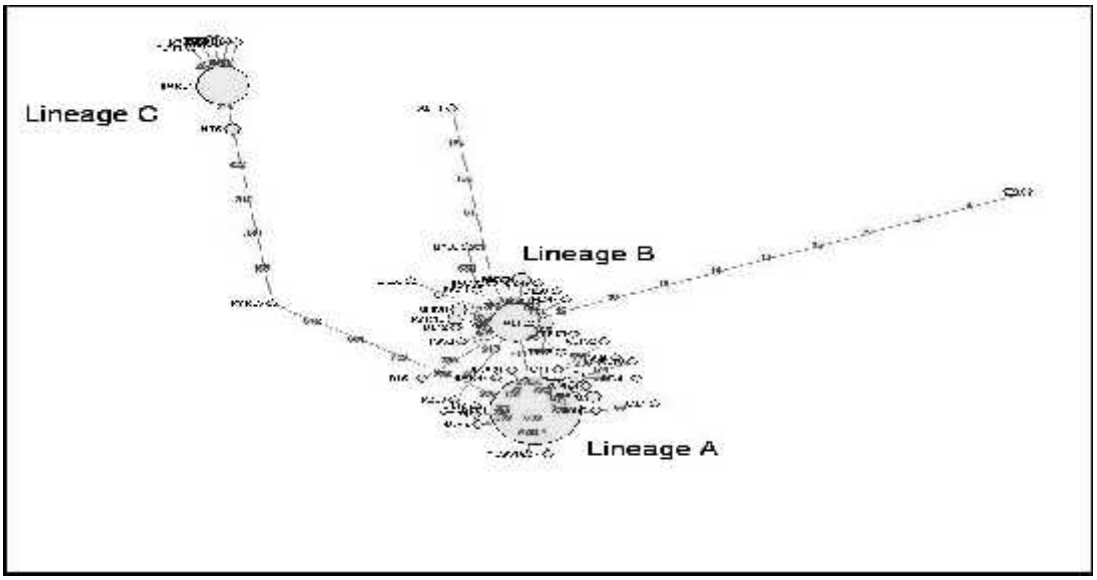

Figure 2 Network of three lineages in nineteen sheep breeds in Xinjiang based on Cyt b sequences. The hollow circles are 163 samples and the solid ones indicate median vectors.

The A, B and C represent the sheep maternal lineages.

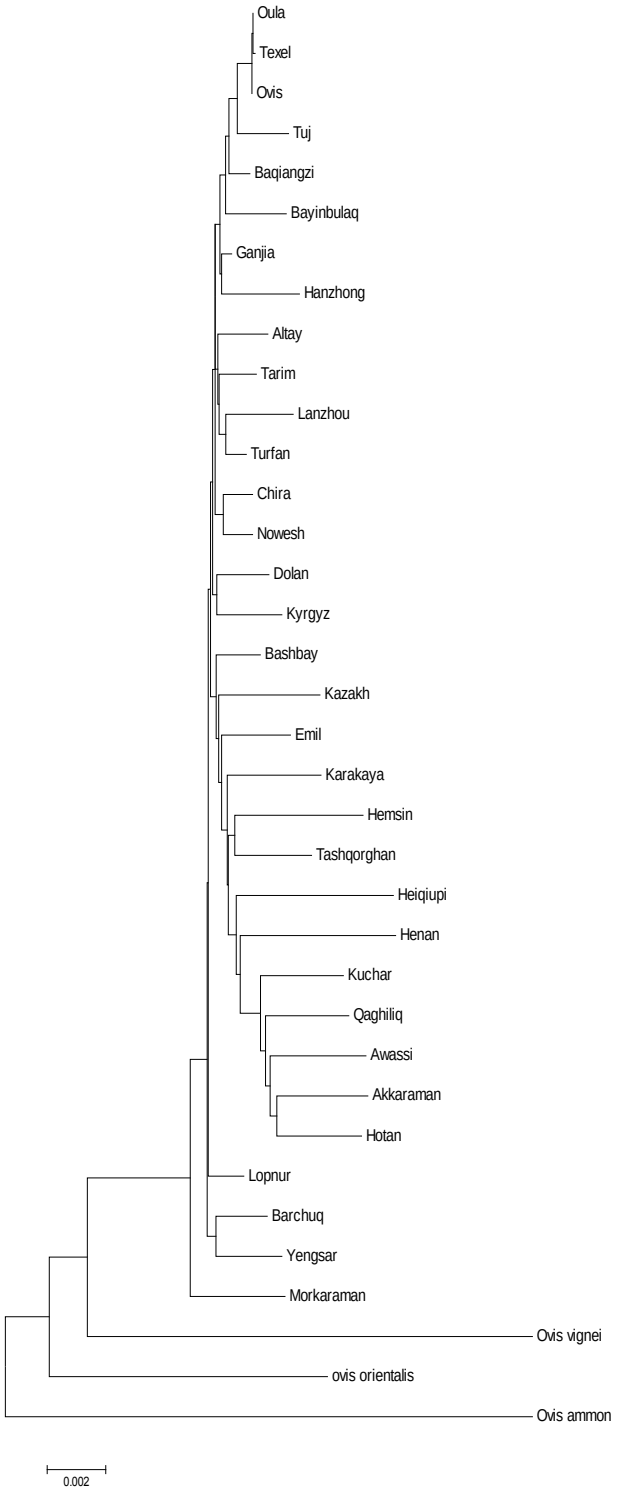

Figure 3. The Neighbor joining constructed phylogenetic relationships of Xinjiang local sheep breeds. Sequences of Lanzhou, Ganjia, Oula, Heiqiupi, Hanzhong, Henan, Awassi, Akkaraman, Karakaya, Hemsin, Tuj and Morkaraman were retrieved from Genbank.

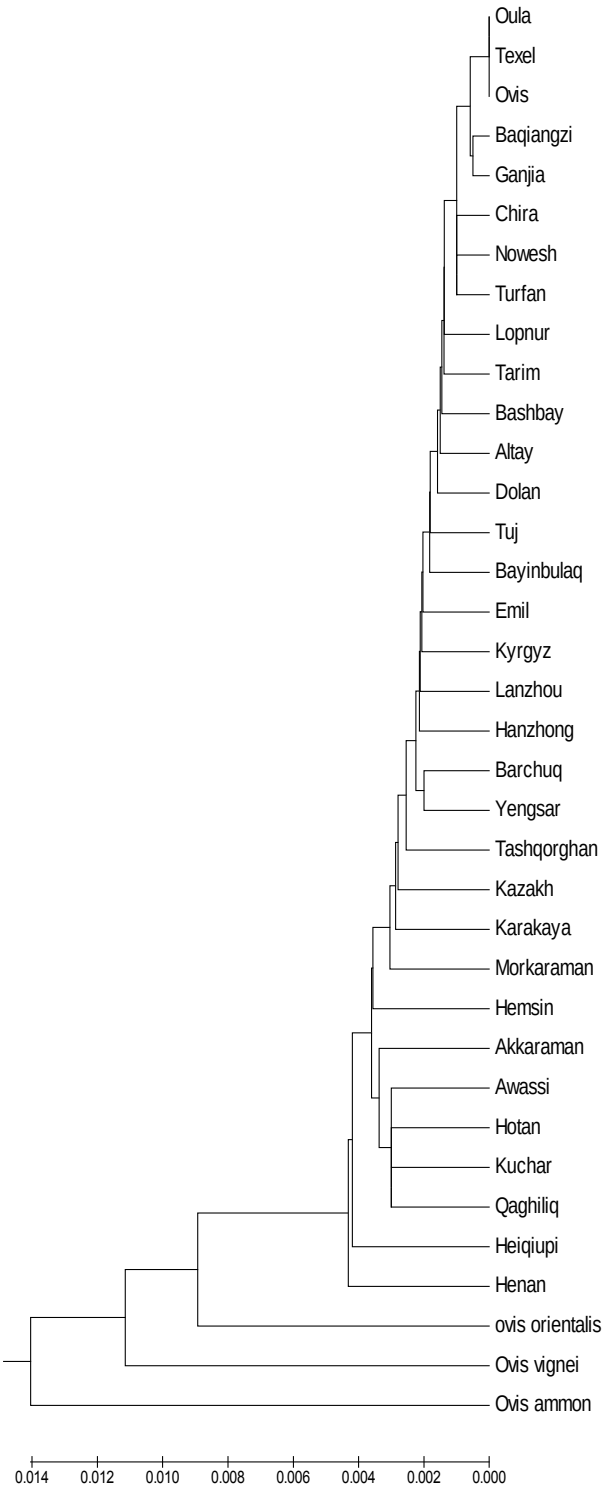

Figure4. The UPGMA phylogenetic relationships of Xinjiang local sheep breeds. Sequences of Lanzhou, Ganjia, Oula, Heiqiupi, Hanzhong, Henan, Awassi, Akkaraman, Karakaya, Hemsin, Tuj and Morkaraman were retrieved from Genbank.

Table 1. The sample information and some diversity indices of the studied population.

Breeds	Code	Sample size	Number of haplotypes	Lineage observed	Haplotype diversity (SE)	Nucleotide diversity (SE)
Altay	ALT	9	6	A	0.889±0.091	0.00345±0.00224
Barikol	Bac	6	4	A,B	0.800±0.172	0.00193±0.00091
Barchuq	Bar	10	5	A,B,C	0.756±0.130	0.00430±0.00132
Bashbay	BS	9	5	A,B,C	0.722±0.159	0.00379±0.00142
Bayinbulaq	BY	7	5	A,B,C	0.905±0.103	0.00502±0.00163
Chira	CR	9	5	A,B	0.806±0.120	0.00230±0.00101
Dolan	DL	10	6	A,B,C	0.778±0.137	0.00407±0.00151
Hotan	HT	12	4	A,B,C	0.742±0.084	0.00583±0.00119
Kuchar	KC	9	4	A,B,C	0.750±0.112	0.00666±0.00142
Kyrgyz	KR	8	6	A,B,C	0.893±0.111	0.00491±0.00149
Kazakh	KZ	10	6	A,B,C	0.844±0.103	0.00726±0.00179
Lopnor	LN	8	3	A,B,C	0.464±0.200	0.00310±0.00185
Nowesh	NW	5	3	A,B	0.800±0.164	0.00228±0.00099
Turpan	TP	7	4	A,B	0.714±0.181	0.00187±0.00094
Tarim	TR	10	4	A,B,C	0.533±0.180	0.00290±0.00137
Tashqurghan	TS	8	6	A,B,C	0.893±0.111	0.00665±0.00160
Texel	TX	4	2	A,B	0.500±0.265	0.00052±0.00056
Qaghiliq	YC	9	6	A,B,C	0.889±0.091	0.00666±0.00142
Yengsar	YS	8	4	A,B,C	0.750±0.139	0.00495±0.00138
Emil	YM	9	3	A,B,C	0.667±0.132	0.00517±0.00132
All		167	91			

SE: standard error

Table 2. Parameters of mtDNA variability within the three clusters in Xinjiang local sheep

Parameters	Clusters			
	A	B	C	All
Sequences	89	46	32	
Polymorphic sites	37	21	9	
Nucleotide diversity（Pi）	0.00196	0.00202	0.00070	
Haplotypes	28	13	7	
Haplotypes diversity	0.667±0.056	0.721±0.051	0.393±0.0109	
Average number of pair wise nucleotide differences（k）	1.891	1.950	0.675	
Fu's Fs statistic:	-25.492 （P < 0.00）	-5.119 （P < 0.00）	- 4.147 （P < 0.00）	
Tajima's D:				-1.7539（0.10 > P > 0.05）

The A, B and C represent the sheep maternal lineages.

DISCUSSION

In this study, we conducted a comprehensive phylogenetic analysis to reveal the genetic origins and evolutionary history of the nineteen Xinjiang local sheep breeds and compared with other sheep breeds of China and other countries.

In previous studies, analysis based on mtDNA control region (D-Loop) were revealed that three maternal lineages A（Asian type）, B（European type）and C（Central Asian and Middle East type）were identified in modern domestic sheep breeds sampled from different geographical regions of the world (Hiendleder *et al*., 1998, 2002; Meadows *et al*., 2005; Wood and Phua, 1996, Guo *et al*., 2005; Pedrosa *et al*., 2005, Tsunada *et al*., 2009, Chang Hong , 2009), and a small number of individuals of maternal lineage D（Caucasian type）also were reported .(Guo *et al*., 2005, Pedrosa *et al*., 2005).

The lineage A, B, and C were found in previous studies of Xinjiang local sheep breeds inferred from mtDNA control region (D-Loop) (Sulaiman *et al*., 2011). Based on genetic markers such as microsatellite analysis, mtDNA control region and phylogenetic analyses on the local sheep breeds of Xinjiang (Wang *et al*, 2007; Yang *et al*., 2002; Tang *et al*., 2009; Tsunada *et al*., 2009; Guo *et al*., 2005 reported previously, suggested that Xinjiang sheep breeds are mainly maternally originated from Kazakh and Mongolian sheep in China. However, there were few studies about the origins and phylogeny of the many unregistered Xinjiang sheep breeds in previous studies (Sulaiman *et al*, 2011). Previous studies based on the of archaeological, morphological, historical and available molecular genetic information suggested that sheep breeds in China are mainly categorized into four groups as Mongolian, Tibetan and Kazakh and Yunnan sheep groups (Chang Hong, 2009). Previous studies reveal that Xinjiang local sheep breeds share the same haplotypes with other sheep breeds of China and belong to Kazakh groups and also have close genetic relationship with Mongolian Sheep (Yu *et al*., 1992, Tu *et al*. 1989, Chen and Xu., 2004, Chang Hong, 2009), but in our study, there are some exceptional cases. The Lopnur, Barchuq and Yengsar breeds were not clustered with other sheep breeds of China, but with Morkaraman of Turkey. The Baqiangzi breed was not clustered with the sheep breeds of Xinjiang, but with Oula, Texel and Tuj. The Emil breed was clustered with Karakaya at first and then clustered with Hemsin, despite being the thousands kilometer distance between China and Turkey. Some individuals are deviated from phylogenetic clade A and C. Unlike sheep maternal lineage B (European type), lineage A and C have more complex phylogenetic branches and indicates that the two lineages might be derived from a number of maternal founders, instead of a single common ancestor of maternal origin. Xinjiang local sheep breeds may have more complicated phylogenetic history than the other Chinese sheep breeds. The breeds like Lopnur, Yengsar, Kuchar, Qaghiliq, Hotan and Barchuq were clustered with Turkey and Israel sheep breeds. This indicates that they had much genetic distance from other Chinese sheep breeds.

Most of Xinjiang sheep breeds were closed to the maternal origin of Asian types. However, some breeds of Xinjiang showed the genetic links with Turkish and Israel sheep breeds. The samples used in this study were collected by remote areas with the aim of preventing the influences of transportation and human communications. We supposed that they are hardly influenced by foreign sheep breeds depending geographic distribution, so represent the current genetic situations of domestic sheep breeds in Xinjiang. But the number of individuals that sequencing Cyt b on each breed were limited, and this may caused concealing some information on the potential genetic links with other sheep breeds. The available genetic information's in this study were not established the genetic relationships with Turkish sheep breeds. It needs more evidences to conduct further studies.

It was also found that some sheep breeds from the same region were clustered into different subgroups, like Tashqorghan, Kyrgyz and Tarim which were from South Xinjiang but results showed they belonged to different subgroups, respectively. Some breeds from different areas were also clustered to the same subgroups which revealed that there might be some gene flows between sheep breeds. Although this region was separated by mountains, large deserts, relatively isolated oasis and long geographical distances but gene flows probably existed between the different breeds in these regions along with Silk Road.

Historical and archaeological events indicated that Europoid people lived in Xinjiang from pre-historic times. In ancient time, Xinjiang territory was usually divided by many local oasis rulers like Huns, Turkic and Mongoloid or warring empires 'chasing grass and water' (Millward, 2007). Xinjiang were sweep away by imperators ruling over pastoralist and agrarian areas from Iran to China. Parts of Xinjiang were often ruled together with lands which now have been the Central Asian Republics. Later the dissolution of Turk empire because of wars, diseases and natural disaster led to one of history's great movement of Turkic-speaking tribes into Xinjiang and across Central Eurasia. As a result of the movement, there are now several Turkic language-spoken ethnic groups in Xinjiang including Turkish, Uyghur, Uzbek, Kazakh, Kyrgyz and others. The movement also led to migration and integrations of livestock breeds. For thousands of years, there were active commodities exchanges along the famous "Silk Road". Sheep, as main livestock animal in regions, might also be under the exchange. The above information and historical events may provide some clues to understand that why the two Xinjiang local sheep breeds were genetically closed to Turkish sheep breeds.

Our results show that genetic relationships of the three breeds with other Chinese sheep breeds are not close, and the origin and migration history of Loner, Bacchus, Yengsar, Kuchar, Qaghiliq and Hotan breeds in South Xinjiang were independent from the other breeds in the same areas. The high genetic diversity of Xinjiang local sheep breeds implies that they may have distinctive origins.

This study provided molecular evidences of genetic diversities in Xinjiang local sheep, which may have multiple maternal origins, which not only related to Kazak, Mongolian and Tibetan origin, but also to other two ancestors of Lopnur and Kuchar. The results would be also helpful to understand the diversity and origins of Central Asian and Middle East domestic sheep. Whether Lopnur and Kuchar are derived from two separate

maternal origins, it needs to enunciate with more evidences from the further studies on phylogeny of sheep breeds in Afghanistan, Pakistan, Iran and Central Asian Republics.

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REFERENCES

Academy of Chinese Science (ACA), (1964). Animal Husbandry in Xinjiang. Science Press of China, Beijing, in Chinese.

Bandelt H.J., P. Forster and A. Rohl (1999). Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16, 37–48.

Bunch, T.D., C. Wu, Y. P. Zhang and S. Wang (2006). Phylogenetic analysis of snow sheep (Ovis nivicola) and closely related taxa. J. Hered. 97 (1), 21-30

Chang Hong (2009). Animal Genetic Resources. Science Press of China, Beijing, in Chinese.

Chen Weisheng and Xu Guifang (2004). Color Atlas of Chinese Domestic Livestock's. Beijing, China Agricultural Press, in Chinese.

Excoffier, L. G. Laval and S. Schneider (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1**:47-50.

Fu Y.X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–25.

Hiendleder, H. S. (1998). A low rate of replacement substitutions in two major Ovis Aries mitochondrial genomes. Animal Genetics, 1998, 29: 116~122.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

Hassanin, A., E. Pasquet and J. D. Vigne (1998). Molecular systematics of the subfamily Caprinae (Artiodactyla, Bovidae) as determined from Cytochrome b sequences. J. Mammal. Evol. 5 (3), 217-236.

Hiendleder S. (1998). A low rate of replacement substitutions in two

Hiendleder S., B. Kaupe, R. Wassmuth and A. Janke (2002). Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. Proceedings of the Royal Society of London Series B Biological Science 269, 893–904.

Hiendleder S., K. Mainz, Y. Plante and H. Lewalski (1998) Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep, J. Heredity 89, 113–20.

Guo, J., L. X. Du, Y.H. Ma, W.J. Guan, H.B. Li, Q.J. Zhao, X. Li and S.Q. Rao (2005). A novel maternal lineage revealed in sheep (Ovis Aries), Animal Genetics, 36, 331–336

James, A. Millward (2007). Eurasian Crossroads: A History of Xinjiang. Columbia University Press, NEW YORK.

Kenji Tsunada, Hong Chang, Guobin Chang,Wei Sun, Tashi Dorji, Gyem Tshering, Yoshio Yamamoto, Takao Namikawa (2009). Phylogeny of Local Sheep Breeds in East Asia, Focusing on the Bayanbulak Sheep in China and the Sipsu Sheep in Bhutan. Biochemical Genetics, 48 (1-2), pp. 1-12

Librado, P. and J. Rozas (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452.

Major Ovis aries mitochondrial genomes, Animal Genetics 29, 116–22.

Meadows, J.R., I. Cemal, O. Karaca, E. Gootwine and J. W. Kijas (2007). Five ovine mitochondrial lineages identified from sheep breeds of the near East. Genetics 175 (3), 1371-1379.

Pedrosa, S., M. Uzun, J.J. Arranz, B. Gutierrez-Gil, F. San Primitivo and Y. Bayon (2005). Evidence of three maternal lineages in Near Eastern sheep supporting multiple domestication events.Proc. Biol. Sci. 272 (1577), 2211-2217.

Saitou N and M. Nei (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Sambrook J., E.F. Fritsch and T. Maniatis (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York, NY.

Tamura K, J. Dudley, M. Nei and S. Kumar (2007). *MEGA4*: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

Tang Cunwei, Xing Weiting, Shi Liang, Yu Xiong, Liu Wujun, Huang Xixia, Jue Ken, Yan G Xiaozhong (2009). Microsatellite Analysis of the Genetic Polymorphism in 13 Sheep Breeds

and 1 Hybrid Group in Xinjiang. Chinese Veterinary J., 2009(36)6:73-78.in Chinese with English Abstract.

Thompson J.D., D.G. Higgins and T.J. Gibson (1994) .CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–80.

Tu, Y., (1989). Sheep and Goat Breeds in China. Shanghai Scientific & Technical Publishers, Shanghai, in Chinese.

Wang, X., Y.H. Ma, and H. Chen, (2006).Analysis of the genetic diversity and the phylogenetic evolution of Chinese sheep based on Cyt b gene sequences. Yi Chuan Xue Bao 33 (12), 1081-1086.

Wood N J, S H. Phua (1996). Variation in the control region sequence of the sheep mitochondrial genome. Animal Genetics, 27: 25~33.

Wang, X., Y.H. Ma, H. Chen, W.J. Guan (2007).Genetic and phylogenetic studies of Chinese native sheep breeds (Ovis Aries) based on mtDNA D-loop sequences. Small Ruminant Research 72 (2007) 232–236.

Yiming Sulaiman, Changxin Wu, Chunjiang Zhao (2011). Phylogeny of 19 Indigenous Sheep Populations in Northwestern China inferred from Mitochondrial DNA Control Region. Asian J. Anim. and Vet. Advances. 2011, 6(1):71-79.

Yu Daxin, Alimjan Sultan, Zhang Qikun, Li Yishan, Du Shikong, Dao Liangzuo, Li Zhaosheng, Kerem Seley, (1992). Livestock Breeds in Xinjiang. Xinjiang People's Publishers, Ürümchi, in Uyghur.