

Analysis of Genetic Diversity on 15 Sheep Breeds in Xinjiang of China

¹X. Yu, ¹X.X. Huang, ¹W.J. Liu, ²Y. Fang, ¹L. Shi, ¹W.T. Xing, ¹C.W. Tang and ³M.A. Brown

¹College of Animal Science, Xinjiang Agricultural University, 830052 Urumqi, P.R. China

²Xinjiang TYCOON Co. Ltd., 831000 Changji, P.R. China

³USDA-ARS, Grazinglands Research Laboratory, OK 73036, El Reno, China

Abstract: The genetic variability and genetic relationship of the fifteen sheep breeds were studied. The genotypes of 14 indigenous Chinese sheep breeds and one crossbreed of wild sheep were investigated using 13 microsatellite DNA markers recommended by the Food and Agriculture Organization of the United Nations (FAO) and the International Society of Animal Genetics (ISAG) through PCR. The allele frequency, heterozygosity and Genetic Differentiation Index (F_{st}) were computed to estimate the genetic variation of each population. To determine the genetic relationships among the breeds, phylogenetic trees were constructed based on Nei's genetic distance using the unweighted pair-group method with arithmetic mean and neighbor-joining method. A total of 15 breeds were clustered into three groups. The first group included Altay sheep and Duolang sheep, the second group was very large and complicated, the third group was a Kazark sheep. The thirteen microsatellite loci were effective markers for the analysis of genetic relationship among sheep breeds. In addition, genetic distance among groups is not according with their geographic distribution and groups or breeds with low production performance can easily impacted by breeds with better production performance. As for the results, it may be result from applying on artificial insemination and extension of commercial cross breeding technique in sheep production system recent years in China.

Key words: Sheep breed, microsatellite, genetic diversity, genetic distance, genetic variation, China

INTRODUCTION

There is long history of sheep breeding in China and abundant sheep breeds resources. They have many kinds of products such as wool, mutton, cashmere and hide. All these indigent groups have good performances such as good adaptability, resistance against harsh environment and disease. Due to lack of overall and comprehensive understanding on local sheep breeds and in sheep production practice, they were often used as maternal part while introduced sheep breeds were used as father part. As a result, it lead to decline of biodiversity of some of them to some extent, some resources even endanger to extinction. So identify and study genetic diversity of local sheep breeds play a important role in making best use of current local sheep breeds resources.

Microsatellite DNA has been widely used in sheep genetic biodiversity (Du and Cao, 2003), lambing trait (Chu *et al.*, 2001) growth performances (Sun *et al.*, 2006), affinity (Yuan *et al.*, 2006) and wool traits (Zhao *et al.*, 2006) and research results has been achieved. Meantime, it has been used in genetic diversity and research of production performances in other spechies (Ouyang *et al.*, 2006; Chu *et al.*, 2006; Shao *et al.*, 2006). Microsatellite DNA has some characters such as wide

distribution, abundant polymorphism easily identifying and showed Mendelian codominant (Chu *et al.*, 2002; Fan *et al.*, 1999), it is recognized as the most valuable mean among genetic markers used in research on genetic biodiversity. This research was designed to study on genetic diversity of 15 groups and breeds of Xinjiang indigent sheep breeds in China with 13 microsatellite locus, aimed at providing references and data for sheep breeds conservation and development by studing genetic background of these sheep breeds (or groups) through allelic variation, heterozygosity, genetic distance eatimation, phylogenetic tree consrtruction and analysis of population genetic structure derivation.

MATERIALS AND METHODS

Animal sample: About 508 individuals were randomly selcted in China: 34 Ka (Kazark sheep) sheep, 40 Al (Altay sheep) sheep, 45 B (Bashibai sheep) sheep, 36 Me (China Merino) sheep, 35 X (Xinjiang Finewool sheep) sheep, 8 F1 (Pan and Bashibai's hybrid generation sheep) sheep, 27 F2 (Pan and Bashibai's hybrid generation sheep) sheep, 19 F3 (Pan and Bashibai's hybrid generation sheep) sheep, 38 BY (Bayinbluk sheep) sheep, 39 KZ (Keerkezi sheep) sheep, 30 SH (Shanqu Hetian sheep) sheep, 40 NH (Nongqu Hetian sheep) sheep, 38 DL

(Duolang sheep) sheep, 39 CL (Cele sheep) sheep, 39 TS (Tashikuergan sheep) sheep from XinJiang 15 individuals farm of China.

DNA preparation and microsatellite primer: About 5 mL blood was collected from each individual from the central artery vein of the ear into tubes containing 1 mL ACD (Citric acid, Sodium citrate, Dextrose) as anticoagulant and was then preserved in a -20°C freezer. Genomic DNA were extracted according to the Molecular Cloning-A Laboratory Manual. Thirteen microsatellite primers were designed based on the Genebank and were synthesized by the Shenggong biological engineering technology company, the information of primers are shown in Table 1.

PCR condition: PCR amplification was carried out in 20 µL of a mixture containing 1 µL DNA template (100 ng µL⁻¹) 2 µL 10×PCR Buffer, 1.5 µL dNTP (10 m mol L⁻¹) 1 µL of each primer (10 p mol µL⁻¹) and 0.2 µL Taq DNA polymerase (5 U µL⁻¹). Double-distilled water was added to a final volume of 20 µL. After a denaturing step of 5 min at 94°C, samples were processed through 35 cycles of 40 sec at 94°C, 40 sec at an optimal annealing temperature and 40 sec at 72°C. Then the last elongation step was at 72°C for 10 min.

Statistical methods and analysis: In these sheep breeds base on the microsatellites DNA genotypes of 13 locus in 15 sheep breeds, the genotypic frequencies, the allelic frequencies and Hardy-Weinberg equilibriums were directly. Differences genotypic frequencies at these locus among 15 indigenous sheep breeds in China were analyzed which were performed by SPSS software (version 16.0).

Allele frequencies:

$$P_i = (2(i_i) + (j_1) + (j_2) + \dots + (j_n)) / 2N$$

Where:

- P_i = The frequency of the *i*th allele
- I = The frequency of the *i*th allele
- n = The total number of the alleles
- j_1, j_2, \dots, j_n = The co-dominant alleles to *i*

Gene heterozygosity and effective allele numbers:

$$H_e = 1 - \sum_{i=1}^m P_i^2$$

$$N_e = \sum_{i=1}^m P_i^2$$

Where:

- m = The number of the allele
- P_i and P_j = The frequencies of the *i*th and the *j*th allele

Table 1: Microsatellite primer sequences and annealing temperature

Microsatellite name	Primer sequence (5'-3')	Annealing temp (°C)
OarFcb48	GAGTTAGTACAAGGATGACAAGAGGCAC GACTCTAGAGGATCGCAAAGAACCAG	58
OarFcb128	CAGCTGAGCAACTAAGACATACATGCG ATTAAGCATCTTCTTTATTTCCTCGC	55
OarHH35	AATTGCATTCAGTATCTTTAAACATCTGGC ATGAAAATATAAAGAGAAATGAACCACACGG	55
OarHH41	TCCACAGGCTTAAATCTATATAGCAACC CCAGCTAAAGATAAAAGATGATGTGGGAG	56
BM827	GGGCTGGTCGTATGCTGAG GTTGGACTTGCTGAAATGACC	58
CSSM47	TCTCTGTCTCTATCACTATATGGC CTGGCACCTGAAACTATCATCAT	58
HUJ616	TTCAAACTACACATTGACAGGG GGACCTTTGGCAATGGAAAGG	54
OarJMP8	CGGGATGATCTTCTGFCCAAATATGC CATTTGCTTTGGCTTCAGAACCCAGAG	58
SRCSRSP5	GGACTTACCAACTGAGCTAC AAG GTT TCTTTGAAATGAAGCTAAAGCAATGC	56
RM4	CAGCAAATATCAGCAAACCT CCACCTGGGAAGGCCITTA	58
OMHC1	ATCTGGTGGGTACAGTCCATG GCAATGCTTTCTAAATTCCTGAGGAA	55
MAF33	GATCTT TGTTC AATCTATTCCAATTTT GAT CATCTGAGTGTGATATATACAG	60
OarFcb226	CTATATGTTGCCCTTTCCCTTCTCTGC GTGAGTCCCATAGAGCATAAGCTC	60

The F-statistic:

$$1 - F_{ST} = (1 - F_{IS}) \times (1 - F_{IT})$$

Where:

- F_{IT} = The fixation indices of individuals related to its subpopulations
- F_{IS} = The fixation indices of individuals related to the total population
- F_{ST} = The fixation indices of subpopulation related to the total population

Reynolds genetic distance (Dr):

$$D_r = -\ln(1 - F_{ST})$$

The value of gene flow (Nm):

$$N_m = (1 - F_{ST}) / (4 \times F_{ST})$$

The dendrogram between different populations based on the Nei and Roychoudry (1974) genetic distance and Reynolds genetic distance were estimated by Unweighed Pair-Group Method with Arithmetic Averaging (UPGMA) by PHYLIP 3.0 software. Gene heterozygosity, gene homozygosity, effective allele numbers and polymorphism information content were calculated according to Nei and Roychoudhury (1974).

RESULTS AND DISCUSSION

In this study, Fig. 1 shows the agarose gel (0.6%) of genomic DNA, Fig. 2-6 shows some of the polyacmlamide

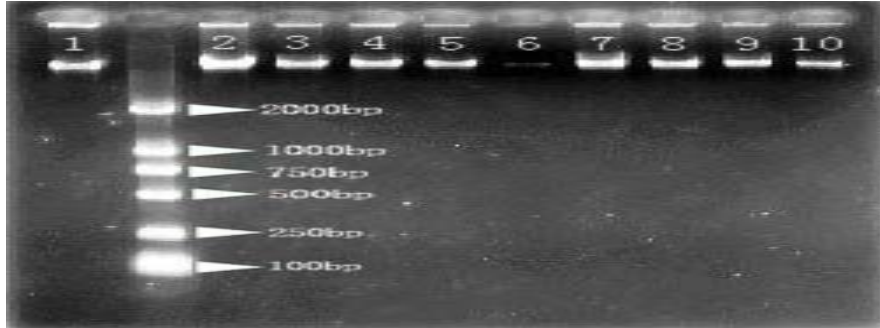


Fig. 1: Genomic DNA agarose gel electrophoresis (0.6%) results



Fig. 2: A portion of 1% agarose gel electrophoresis results of oarfcb48

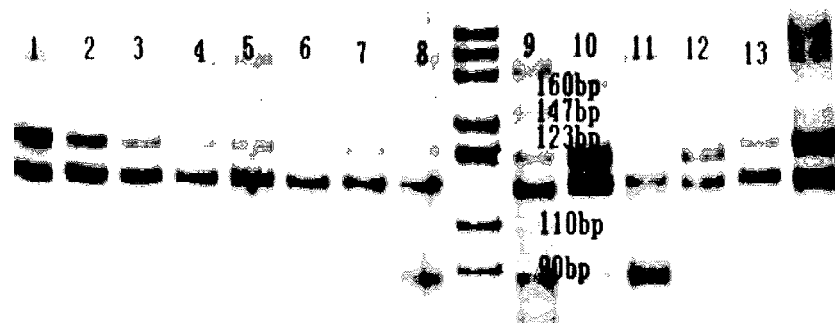


Fig. 3: A portion of 8% PAGE results of CSSM47

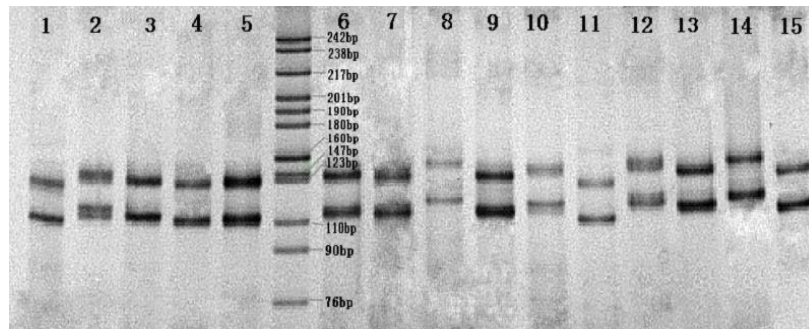


Fig. 4: A portion of 8% PAGE results of OarHH35

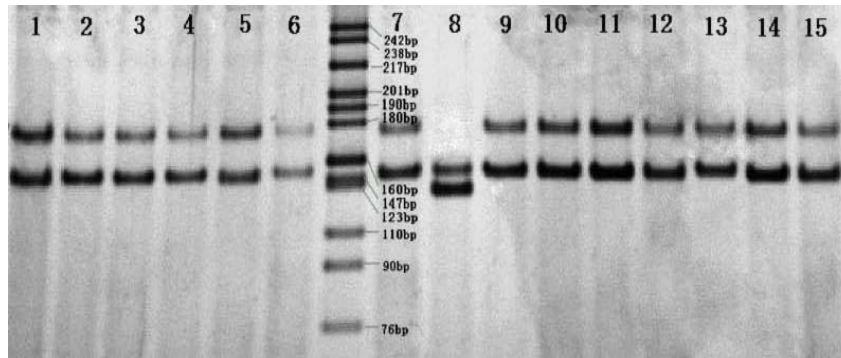


Fig. 5: A portion of 8% PAGE results of OarAE129



Fig. 6: A portion of 8% PAGE results of MAF33

3Gels (PAGE) of PCR products. The total number of alleles in 13 microsatellite loci of 15 populations was 507.

The effective Number of alleles (Ne) and the alleles frequencies (Na): The effective number of alleles is also an index used to reveal the genetic diversity of the populations. The results for the effective number of alleles were showed in Table 2. The value of 13 microsatellite loci varied from 2.459 (CSSM47)-9.554 (OarJMP8) and the mean was 5.362 ± 1.334 , the average value of alleles frequencies was 7.436 ± 1.129 .

Gene heterozygosity (He): As shown in Table 3 and 4, the microsatellite OarJMP8 in the Me sheep population showed the highest expected gene heterozygosity ($He = 0.895$) while the locus CSSM47 in CL sheep breed had the lowest He ($He = 0.593$). The average He of all loci and populations was range from 0.751 ± 0.070 in F1 sheep breed to 0.823 ± 0.032 in X sheep breed.

F-statistic analysis, Dr genetic distances and Nm among the 13 sheep breeds: The F-statistic was used to test the genetic differentiation among subpopulations. The F-statistics were calculated for 13 microsatellite locus in this study, the results are shown in Table 4. The range of F_{ST} was from 0.024 (OarHH41)-0.057 (OarJMP8). The means of F_{ST} , F_{IT} and F_{IS} were 0.037 ± 0.010 , -0.204 ± 0.053

and -0.251 ± 0.053 , respectively. The Reynolds genetic distance (D_r) and N_m was calculated by F_{ST} and the results are shown in Table 4 and 5. Genetic distance between TS and B seep was the nearest (0.064) while the genetic distance between F1 and Me sheep breeds was the farthest (0.481). The highest value of gene flow (N_m) was 10.358 and the lowest was 4.145 between all breeds (Fig. 7).

Sampling and designing: Sampling plays a important role in overall experiment process. Barker *et al.* (1993) suggest that at least 25 samples should be selected for each breed, if 50 samples are selected as experiment subjects, it can make up accounting errors (Barker *et al.*, 1993). This research was deigned to take random sampling method in typical groups, typical groups were selected as samples, random sampling within typical groups, number of samples is controlled over 40.

The results can reflect whole genetic information of groups if it can meet the above criteria. As for the sheep breeds (or groups) selected as samples compared with similar researches before it, samples distributed in the widest area of Xinjiang and the number of the breeds (or groups) is the most, the majority of all sheep breeds within Xinjiang Autonomous region included in Chinese sheep breeds are selected as breeds samples.

Table 2: The effective Number of alleles (Ne) and the alleles frequencies (Na) of 13 microsatellite loci in 15 sheep breeds

Loci	Ka	AL	B	Me	X	F1	F2	F3	BY	KZ	SH	NH	DL	CL	Ts	Na
OarFcb48	5.415	4.741	5.878	7.902	7.704	5.333	4.486	4.272	6.237	7.191	4.296	4.595	4.085	2.624	3.233	7.733
OarFcb128	5.514	4.503	3.868	5.279	5.457	3.765	4.646	3.557	4.265	5.967	5.702	7.091	6.496	5.586	4.827	7.667
OarHH35	5.943	7.501	6.519	4.597	6.712	5.765	8.428	5.121	4.940	5.729	5.455	5.614	6.017	6.528	8.817	8.600
OarHH41	4.519	5.986	5.610	6.331	5.898	3.200	6.863	5.511	5.389	4.940	6.316	6.004	6.251	5.121	6.324	7.467
BM827	5.352	5.200	5.195	7.863	5.658	4.267	4.864	6.067	5.894	4.846	4.876	4.895	5.429	5.379	5.530	6.333
CSSM47	4.151	4.015	3.791	3.090	3.982	3.765	4.265	3.828	4.050	3.669	3.216	3.631	3.636	2.459	4.263	4.600
HUJ616	3.974	4.434	7.124	6.933	6.302	3.879	6.696	6.416	5.487	5.408	5.348	5.093	5.490	4.327	4.737	8.600
OarJMP8	5.689	9.246	6.084	9.554	5.371	3.657	4.876	3.560	5.098	7.405	8.881	8.658	8.181	7.380	4.098	8.467
SRCRSP5	6.380	5.024	3.707	4.923	5.048	6.400	4.899	4.629	4.531	5.678	4.976	7.009	5.286	5.309	3.968	7.667
RM4	5.496	6.695	4.863	3.787	6.059	5.818	6.377	4.173	6.531	6.048	5.930	6.343	6.265	5.965	5.902	7.533
OMHC1	3.959	5.503	5.200	5.855	6.980	4.267	5.452	5.294	4.854	6.629	6.444	6.993	6.811	4.281	4.658	8.400
MAF33	6.840	6.695	5.608	4.529	5.338	2.560	7.327	5.311	6.145	5.501	6.767	7.175	5.251	6.656	6.542	7.200
OarFcb226	4.853	4.315	3.682	4.992	4.762	3.200	2.509	2.788	4.353	4.067	3.379	3.980	5.799	3.675	4.757	6.400

Ka: Kazark sheep, X: Xinjiang Finewool sheep, B: Bashibai sheep, Me: China Merino, Al: Altay sheep, BY: Bayinbluk sheep, SH: Shanqu Hetian sheep, NH: Nongqu Hetian sheep, KZ: Keerkezi sheep, DL: Duolang sheep, CL: Cele sheep, TS: Tashikuergan sheep

Table 3: The Gene Heterozygosity (He) of 13 microsatellite loci in 15 sheep breeds

Loci	Ka	AL	B	Me	X	F1	F2	F3	BY	KZ	SH	NH	DL	CL	Ts
OarFcb48	0.815	0.789	0.830	0.873	0.870	0.813	0.777	0.766	0.840	0.861	0.767	0.782	0.755	0.619	0.691
OarFcb128	0.819	0.778	0.741	0.811	0.817	0.734	0.785	0.719	0.766	0.832	0.825	0.859	0.846	0.821	0.793
OarHH35	0.832	0.867	0.847	0.782	0.851	0.827	0.881	0.805	0.798	0.825	0.817	0.822	0.834	0.847	0.887
OarHH41	0.779	0.833	0.822	0.842	0.830	0.688	0.854	0.819	0.814	0.798	0.842	0.833	0.840	0.805	0.842
BM827	0.813	0.808	0.808	0.873	0.823	0.766	0.794	0.835	0.830	0.794	0.795	0.796	0.816	0.814	0.819
CSSM47	0.759	0.751	0.736	0.676	0.749	0.734	0.766	0.739	0.753	0.727	0.689	0.725	0.725	0.593	0.765
HUJ616	0.748	0.774	0.860	0.856	0.841	0.742	0.851	0.844	0.818	0.815	0.813	0.804	0.818	0.769	0.789
OarJMP8	0.824	0.892	0.836	0.895	0.814	0.727	0.795	0.719	0.804	0.865	0.887	0.884	0.878	0.864	0.756
SRCRSP5	0.843	0.801	0.730	0.797	0.802	0.844	0.796	0.784	0.779	0.824	0.799	0.857	0.811	0.812	0.748
RM4	0.818	0.851	0.794	0.736	0.835	0.828	0.843	0.760	0.847	0.835	0.831	0.842	0.840	0.832	0.831
OMHC1	0.747	0.818	0.808	0.829	0.857	0.766	0.817	0.811	0.794	0.849	0.845	0.857	0.853	0.766	0.785
MAF33	0.854	0.851	0.822	0.779	0.813	0.609	0.864	0.812	0.837	0.818	0.852	0.861	0.810	0.850	0.847
OarFcb226	0.794	0.768	0.728	0.800	0.790	0.688	0.602	0.641	0.770	0.754	0.704	0.749	0.828	0.728	0.790

Ka: Kazark Sheep, X: Xinjiang Finewool sheep, B: Bashibai sheep, Me: China Merino, Al: Altay sheep, BY: Bayinbluk sheep, SH: Shanqu Hetian sheep, NH: Nongqu Hetian sheep, KZ: Keerkezi sheep, DL: Duolang sheep, CL: Cele sheep, TS: Tashikuergan sheep

Table 4: F-value for all locus

All pops.	OarFcb48	OarFcb128	OarHH35	OarHH41	BM 827	CSSM47	HUJ616	OarJMP8	SRCRSP5	RM4	OMHC1	MAF33	OarFcb226	Mean
Fis	-0.266	-0.256	-0.198	-0.225	-0.231	-0.378	-0.235	-0.206	-0.247	-0.217	-0.229	-0.222	-0.347	-0.251
Fit	-0.199	-0.214	-0.166	-0.197	-0.186	-0.336	-0.191	-0.137	-0.195	-0.167	-0.196	-0.178	-0.292	-0.204
Fst	0.053	0.034	0.026	0.024	0.037	0.030	0.036	0.057	0.042	0.041	0.027	0.036	0.041	0.037
Nm	4.458	7.194	9.188	10.358	6.507	8.120	6.715	4.145	5.725	5.830	9.120	6.733	5.876	6.484

Table 5: Dr genetic distances among the 15 sheep breeds, pairwise population matrix of Nei genetic distance

Genetic distancing for Xinjiang							Pairwise population matrix of Nei genetic distance									
Ka	Al	B	Me	X	F1	F2	F3	BY	KZ	SH	NH	DL	CL	TS		
0.000																
0.080	0.000															
0.096	0.088	0.000														
0.182	0.150	0.141	0.000													
0.141	0.191	0.159	0.171	0.000												
0.357	0.386	0.417	0.481	0.418	0.000											
0.219	0.209	0.186	0.266	0.230	0.178	0.000										
0.232	0.225	0.190	0.291	0.271	0.223	0.098	0.000									
0.084	0.122	0.093	0.149	0.107	0.421	0.251	0.252	0.000								
0.124	0.112	0.103	0.154	0.128	0.349	0.180	0.225	0.124	0.000							
0.127	0.111	0.085	0.145	0.140	0.375	0.170	0.237	0.135	0.077	0.000						
0.096	0.077	0.108	0.144	0.092	0.359	0.179	0.244	0.104	0.082	0.090	0.000					
0.090	0.084	0.117	0.168	0.153	0.434	0.227	0.241	0.105	0.119	0.136	0.101	0.000				
0.100	0.105	0.104	0.203	0.235	0.518	0.306	0.295	0.106	0.120	0.129	0.158	0.108	0.000			
0.074	0.091	0.064	0.161	0.164	0.448	0.213	0.226	0.099	0.127	0.114	0.129	0.101	0.095	0.000		

Ka: Kazark sheep, X: Xinjiang Finewool sheep, B: Bashibai sheep, Me: China Merino, Al: Altay sheep, BY: Bayinbluk sheep, SH: Shanqu Hetian sheep, NH: Nongqu Hetian sheep, KZ: Keerkezi sheep, DL: Duolang sheep, CL: Cele sheep, TS: Tashikuergan sheep

Variations with sheep groups or breeds: Heterozygosity, also known as gene diversity, reflected in the locus to be detected population genetic variation. Groups with low heterozygosity, indicating the genetic uniformity of the

high genetic iversity of difference. The arerage expected heterozygosity of 14 sheep breeds included in this experiment is 0.801, bigger than that of (Jia *et al.*, 2003) ($H = 0.5721$) (Peter *et al.*, 2007) ($He = 0.6$) (Arranz *et al.*,

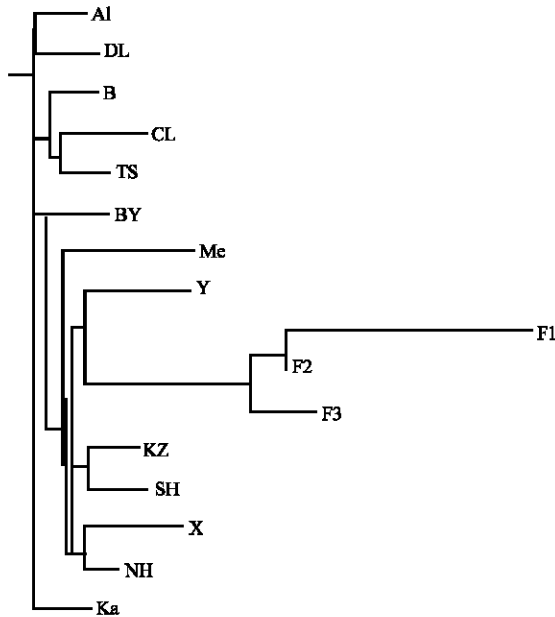


Fig. 7: The dendrogram based on the Reynolds genetic distance were estimated by UPGMA

2001) research results from 19 microsatellite locus on Spain (Gutiérrez-Espeleta *et al.*, 2000) ($H = 0.74$). The results show: Xinjiang 14 sheep species, 1 hybrid generation of variation within the sheep populations generally slightly lower than other varieties of foreign sheep. In sheep, genetic diversity is relatively high.

In terms of average expected heterozygosity among all sheep breeds included in the experiment, Xinjiang Finewool sheep is the highest, it is in accordance with its breed history. During its breeding process, the maternal is local sheep breeds like Kazark sheep, Altay sheep, Duolang sheep etc., its farther family is very large, many famous finewool sheep breeds were introduced in Xinjiang, so it results in high genetic diversity. Then after it, it is Duo Lang sheep and Altay sheep. Concerning these two breeds, they are dominant within Xinjiang, regarding to their distribution and populations, so it confirms high genetic diversity.

On the contrary, results from three groups including Cele sheep, Tashikuergan sheep and Bashibai sheep, they have lowest average expected Heterozygosity (H_e). Compared with other sheep breeds, they live in narrow geography area, Cele sheep lived in two counties within Hetian prefecture, Tashikuergan sheep only lives in one county area with high altitude (over 4000 m above sea level) and cold climate and Bashibai sheep only lives in few counties within Tacheng prefecture. This can indicate that a degree of choice and inbreeding among these groups exists, gene flow between other sheep breeds is the relatively poor.

Genetic distance among sheep breeds: In terms of the genetic distance among breeds, it is not fully consistent with the traditional classification and the geographical relationship. The reasons should be as specific as possible: different cluster methods may result in different results even contradiction results. In addition, artificial selection, migration, mutation and how familiar with biotechnology by technical staff which may influence the analysis. Kazark sheep, Duolang sheep and Altay sheep are ancient indigent species in Xinjiang with large populations, they are clustered into first group alone; genetic distance of some groups are consistent with geographical distribution such as those lives in Tacheng basin were clustered in one group which are Yemule sheep and crossbreeds of wild sheep and local sheep, Keerkezi sheep, Shanqu Hetian sheep, Cele sheep and TaShikuergan sheep are clustered in one group due to their close geographic distribution. One case on Microsatellite DNA analysis of 8 Chinese sheep breeds in Xinjiang by Yan Jingjuan (Jin-Juan, 2004) showed: Keerkezi sheep, Duolang sheep and Hetian sheep (include Shanqu Hetian sheep and Nongqu Hetian sheep) are close relatives, it is similar with this result. As the traditional classification: AL and B were the KA's generation. Analysis from the genetic distance was 0.08 (between AL and KA) and 0.096 (between Band KA), it has some challenge with the traditional classification. As for blood and origin of Bashibai sheep, there is not a certain conclusion from traditional classification. By now, there is not deep study on genetic relation of all Xinjiang sheep breeds. So it is necessary for us to study on the genetic diversity of Xinjiang indigent sheep breeds of China by making best use of biotechnology technique such as MtDNA, SNP, Csnp. Then verify reliability of the methods and results together with Microsatellite Marker. All above research can provide valuable scientific data and basis for us to conservation of genetic resources and its development.

CONCLUSION

Genetic variation within populations are higher than that of between breeds. The results suggested that genetic differentiation already occurred among the 15 populations although, the degree of differentiation was low. The invasion of introduced breeds in Xinjiang region was at the primary stage.

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REFERENCES

- Arranz, J.J., Y. Bayon and F. San Primitivo, 2001. Differentiation among Spanish sheep breeds using microsatellites. *Genet. Selection Evol.*, 33: 529-542.
- Barker, J.S., D.G. Bradley, R. Fries, W.G. Hill and M. Net *et al.*, 1993. An integrated global programme to establish the genetic relationships among the breeds of each domestic animal species. Rome: FAO Report. <http://www.genetics.org/cgi/content/full/162/1/473>.
- Chu, M.X., C. Wang, X.W. Li, S.C. Ye and L. Fang, 2006. Relationships between six microsatellite loci and milk composition traits in Beijing Holstein Cows. *J. Agric. Biotechnol.*, 14: 468-473.
- Chu, M.X., J.H. Cheng and W. Guo, 2001. Preliminary studies of microsatellite markers OarAE101 and BM1329 in five sheep breeds. *Acta. Genetica. Sinica.*, 28: 510-517.
- Chu, M.X., J.Z. Wang, A.G. Wang, N. Li and J.L. Fu, 2002. Genetic polymorphisms of five microsatellite loci in small tail Han sheep. *Acta. Genetic. Sinic.*, 29: 502-506.
- Du, L.X. and D.G. Cao, 2003. Studies on microsatellite and RAPD markers in little-tailed Han sheep. *Acta Genetica. Sinica.*, 30: 1041-1044.
- Fan, B., K. Li, Z.Z. Peng, Y. Chen, C. Moran, Y.Z. Gong and S.H. Zhao, 1999. Genetic variation of 27 microsatellite loci in three Hubei indigenous pig breeds. *Biodiversity Sci.*, 7: 91-96.
- Gutiérrez-Espeleta, G.A., S.T. Kalinowski, W.M. Boyce and P.W. Hedrick, 2000. Genetic variation and population structure in desert bighorn sheep: Implications for conservation. *Conserv. Genet.*, 1: 3-15.
- Jia, B., J. Chen, R.Q. Zhao, Q.J. Luo, G.Q. Yan and J. Chen, 2003. Microsatellite analysis of genetic diversity and phylogenetic relationship of eight sheep breeds in Xinjiang. *Acta. Genet. Sinica.*, 30: 847-854.
- Jin-Juan, Y., 2004. Study on microsatellite DNA genetic diversity of 8 sheep groups in Xinjiang China[D]. Inner Mongolian Agricultural University, Huhehaote, pp: 5.
- Nei, M. and A.K. Roychoudhury, 1974. Sampling variance of heterozygosity and genetic distance. *Genetics*, 76: 379-390.
- Ouyang, X.X., Q.S. Shi, S.Q. Huang, Z.F. Deng and H.X. Liu *et al.*, 2006. Studies of microsatellite markers OarHH35 and BMS2508 in four goat breeds. *J. Agric. Biotechnol.*, 14: 478-483.
- Peter, C., M. Bruford, T. Perez, S. Dalamitra, G. Hewitt, G. Erhardt and E. Consortium, 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Ani. Genet.*, 38: 37-44.
- Shao, G.B., C. Jia, H.L. Liu, R.Q. Zhao and H.M. Ding *et al.*, 2006. Effects of 5 microsatellite DNA markers linked with PPAR gene on meat quality traits in pig. *J. Agric. Biotechnol.*, 14: 162-165.
- Sun, Y.L., G.Q. Liu and G. Wang, 2006. Correlation analysis between microsatellite marks and body weight in meat sheep. *Scientia. Agric. Sinica.*, 39: 2095-2100.
- Yuan, C.Z., Y.H. Ma, J.M. Wang, Y.G. Shang, N.B. Zang and J.P. Li, 2006. Analysis of phenotype and genetic relationship between four indigenous sheep breeds in Shandong Province. *Acta. Veterinaria Zootechnica Sinica.*, 37: 858-863.
- Zhao, Z.S., G.L. Wang, Y.P. Ma, Y.J. Mu and D.Q. Li, 2006. Studies on relation between some wool traits and a few microsatellites on sheep. *Acta. Veterinaria Zootechnica Sinica.*, 37: 864-869.