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

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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.

Microsattelite 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). Microsattelite 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 induviduals were randomly seleted 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

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(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 addedto 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 microsattellites 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 (version16.0).

Allele frequencies:

$$Pi = (2(ii)+(ij_1)+(ij_2)+\ldots+(ij_n))/2N$$

Where:

P_i = The frequency of the ith allele
I = The frequency of the ith allele
n = The total number of the alleles
$j_1, j_2, \ldots j_n$ = The co-dominant alleles to i

Gene heterozygosity and effective allele numbers:

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

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

Where:

m = The number of the allele
P_i and P_j = The frequencies of the ith and the jth allele

Table 1: Microsatellite primer sequences and annealing temperature

Microsatellite name	Primer sequence (5′-3′)	Annealing temp (°C)
OarFcb48	GAGTTAGTACAAGGATGACAAGAGGCAC	58
	GACTCTAGAGGATCGCAAAGAACCAG	
OarFcb128	CAGCTGAGCAACTAAGACATACATGCG	55
	ATTAAAGCATCTTCTCTTTATTTCCTCGC	
OarHH35	AATTGCATTCAGTATCTTTAACATCTGGC	55
	ATGAAAATATAAAGAGAATGAACCACACGG	
OarHH41	TCCACAGGCTTAAATCTATATAGCAACC	56
	CCAGCTAAAGATAAAAGATGATGTGGGAG	
BM827	GGGCTGGTCGTATGCTGAG	58
	GTTGGACTTGCTGAAGTGACC	
CSSM47	TCTCTGTCTCTATCACTATATGGC	58
	CTGGGCACCTGAAACTATCATCAT	
HUJ616	TTCAAACTACACATTGACAGGG	54
	GGACCTTTGGCAATGGAAGG	
OarJMP8	CGGGATGATCTTCTGTCCAAATATGC	58
	CATTTGCTTTGGCTTCAGAACCAGAG	
SRCRSP5	GGACTCTACCAACTGAGCTAC AAG	56
	GTT TCTTTGAAATGAAGCTAAAGCAATGC	
RM4	CAGCAAAATATCAGCAAACCT	58
	CCACCTGGGAAGGCCTTTA	
OMHC1	ATCTGGTGGGCTACAGTCCATG	55
	GCAATGCTTTCTAAATTCTGAGGAA	
MAF33	GATCTT TGTTTCAATCTATTCCAATTTC	60
	GAT CATCTGAGTGTGAGTATATACAG	
OarFcb226	CTATATGTTGCCTTTCCCTTCCTGC	60
	GTGAGTCCCATAGAGCATAAGCTC	

The F-statistic:

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

Where:

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

Reynolds genetic distance (Dr):

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

The value of gene flow (Nm):

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

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

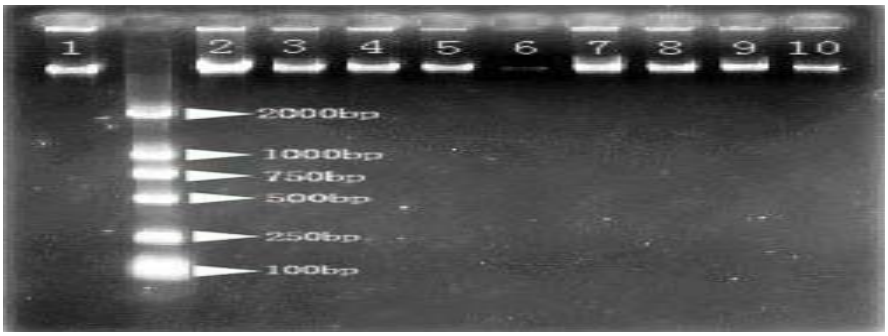

Fig. 1: Genomic DNA agarose gel electrophoresis (0.6%) results

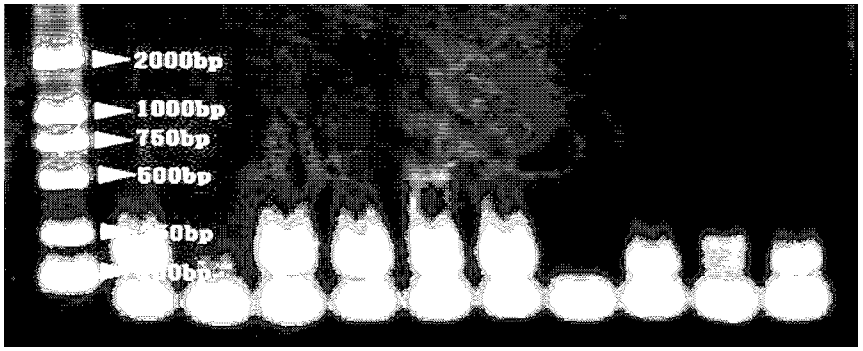

Fig. 2: A portion of 1% agarose gel electrophoresis results of oarfcb48

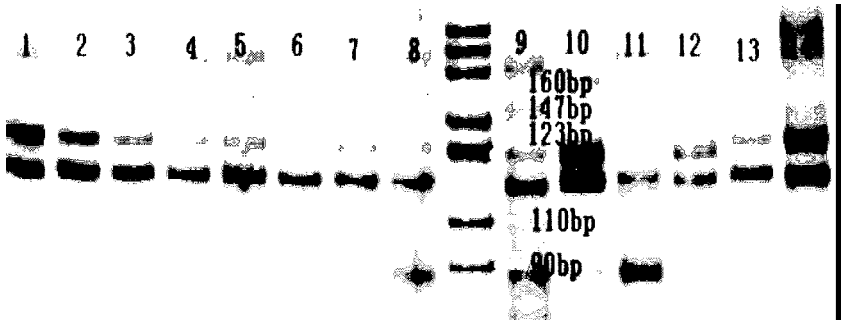

Fig. 3: A portion of 8% PAGE results of CSSM47

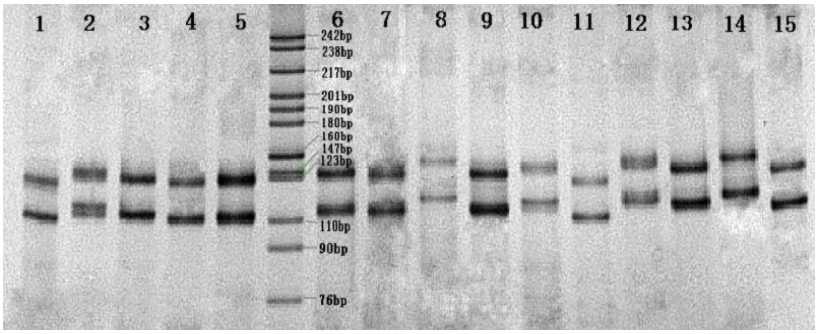

Fig. 4: A portion of 8% PAGE results of OarHH35

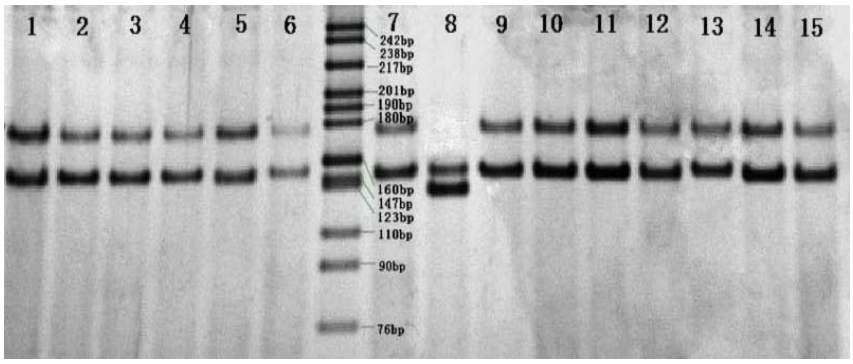

Fig. 5: A portion of 8% PAGE results of OarAE129

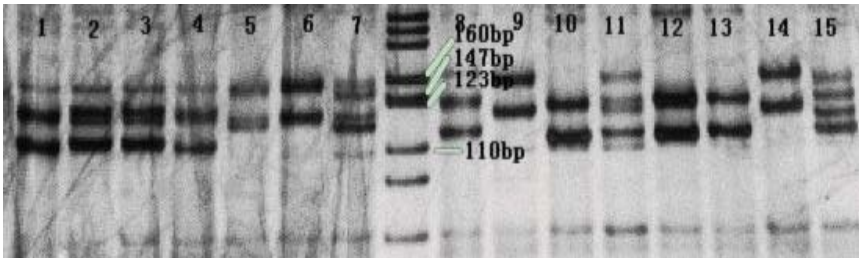

Fig. 6: A portion of 8% PAGE results of MAF33

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 heterozygosty (He): As shown in Table 3 and 4, the microsatellite OarJMP8 in the Me sheep population showed the highest expected gene heterozygosty (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 (Dr) and Nm 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 (Nm) 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 Cninese 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, NHNongqu Hetian sheep, KZ: Keerkezi sheep, DL: Duolang sheep, CL: Cele sheep,TS: Tashikuergan sheep

Table 3: The Gene Heterozygosty (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															Ka
0.080	0.000														Al
0.096	0.088	0.000													B
0.182	0.150	0.141	0.000												Me
0.141	0.191	0.159	0.171	0.000											X
0.357	0.386	0.417	0.481	0.418	0.000										F1
0.219	0.209	0.186	0.266	0.230	0.178	0.000									F2
0.232	0.225	0.190	0.291	0.271	0.223	0.098	0.000								F3
0.084	0.122	0.093	0.149	0.107	0.421	0.251	0.252	0.000							BY
0.124	0.112	0.103	0.154	0.128	0.349	0.180	0.225	0.124	0.000						KZ
0.127	0.111	0.085	0.145	0.140	0.375	0.170	0.237	0.135	0.077	0.000					SH
0.096	0.077	0.108	0.144	0.092	0.359	0.179	0.244	0.104	0.082	0.090	0.000				NH
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			DL
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		CL
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	TS

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*.,

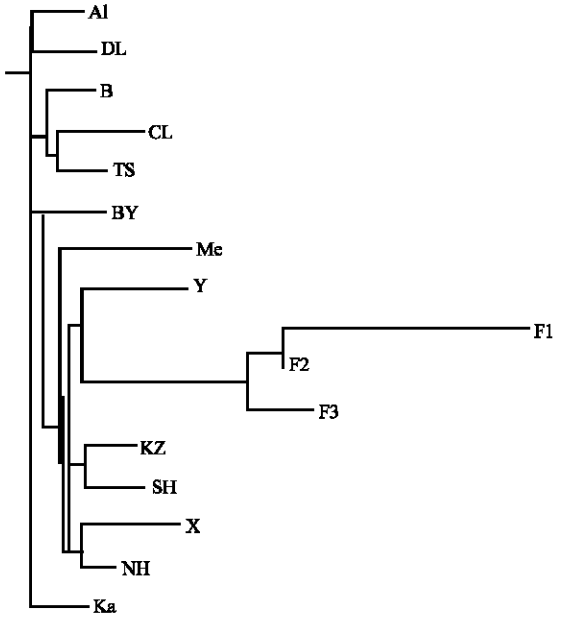

Fig. 7: The dendrogram based on the Reynolds genetic distance were estimated by UPGMA

2001) research results from 19 microsattelite locus on Spain (GutiErrez-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 arerage expected heterozygosity among all sheep breeds included in the experiment, Xinjiang Finewool sheep is the highest, it is in according with its breed history. During its breeding process, the marternal is local sheep breeds like Kazark sheep, Altay sheep, Duolang sheep etc., its farther familiy is very large, many famous finewool sheep breeds were introduced in Xinjiang, so it result 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 (He). 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, migriation, 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 clusterd into first group alone; genetic distance of some groups are consistant with geographical distribution such as those lives in Tacheng basin were clusterd in one group which are Yemule sheep and crossbreds of wild sheep and local sheep, Keerkezi sheep, Shanqu Hetian sheep, Cele sheep and TaShikuergan sheep are clusterd in to one groups due to their close geographic distribution. One case on Microsattelite 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 resul. 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 Microsattelite 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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