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Comparative Research on Genetic Diversity of Main Sheep Breeds of Mongolia Sheep Group in China Using Structure Loci and Microsatellite Makers

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Abstract: It was studied with Tan sheep, small-tailed Han sheep, Hu sheep, Tong sheep and Wadi sheep and their close related species goat was served as a referenced population. The distribution of gene frequency of 14 polymorphic structure loci and 7 microsatellite sites was detected, the result showed that: The variance analysis of the Heterozygosity (H), Polymorphic Information Content (PIC), effective allele Number (Ne) of alleles at each structural locus showed there were not significant differences either between species or among breeds ($p>0.05$). The variance analysis of the same 3 genetic indexes at microsatellite makers showed that there were not significant differences among populations in heterozygosity and PIC ($p>0.05$) as for effective allele number there were not significant differences both among Tan sheep, Hu sheep, Tong sheep and Wadi sheep, and between Wadi sheep and small-tailed Han sheep ($p>0.05$) but between the pre-four populations and the post-two populations there were significant difference ($0.01>p>0.05$). The variation levels of Tan sheep was the highest in 5 sheep populations followed by Tong sheep, Hu sheep, small-tailed Han sheep and then Wadi sheep. The variation level of small-tailed Han sheep was the highest in the 5 populations based on microsatellite makers followed by Wadi sheep, Tong sheep, Tan sheep and then Hu sheep. The results of population genetic equilibrium status based on the 2 level makers were different as to structure loci, Hu sheep, Tong sheep and Tan sheep were in the status of Hardy-Weinberg extreme disequilibrium ($p<0.01$) and Yangtse River Delta White goat, small-tailed sheep and Wadi sheep were in Hardy-Weinberg disequilibrium as to microsatellite makers, Hu sheep and goat were in the status of Hardy-Weinberg extreme disequilibrium ($p<0.01$) and other populations including Tong sheep, small-tailed Han sheep, Tan sheep and Wadi sheep were in Hardy-Weinberg disequilibrium ($p<0.05$). The average coefficient of gene differentiation (Gst) at each structural locus in 5 sheep and one goat populations was all higher than that in 5 sheep populations and the average coefficient of gene differentiation (Gst) at structural loci all was higher than that at microsatellite loci.

Key words: Mongolia sheep group, structure loci, microsatellite makers, genetic diversity, gene differentiation, average coefficient

INTRODUCTION

According to the classic zoological taxonomy, Zhang divided the indigenous sheep breeds of the country into three groups, Mongolia sheep, Tibetan sheep and Kazakhstan sheep (Editorial Section of Records of Sheep and Goat Breeds in China, 1989). As a fact, each breed in the Mongolia sheep group is an important element in Chinese local sheep gene pool for the selection, breeding and genetic improvement of other sheep breeds and a major genetic resource to further development of unique local sheep breeds with high international competitively.

However, because of the great difference in the ecological conditions these breeds inhabited and the wide areas they distributed, many hypotheses were proposed with many pending questions like the relations between the breeds in the Mongolia sheep group and genetic diversity level of different breeds under different ecological type. The solutions to these problems are great reference for genetic evaluation and kinship determination within the Mongolia sheep group and further rational utilization and protection of these breeds.

MATERIALS AND METHODS

About 63 Hu sheep (Hu), 65 Tong sheep (Tong), 70 Small-tailed Han sheep (Han), 60 Tan sheep (Tan) and 76 Wadi sheep (WD) were sampled in China from Huzhou of Jiangsu, Baishui of Shaanxi, Liangshan of Shandong, Yinchuan of Ningxia and Dongying of Shandong by

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Random sampling in typical colonies of central area and 40 Yangtse River Delta White goats (Goat) with independent blood lineage were from the suburb of Yangzhou city of Jiangsu Province of China and any two individual samples with traceable phylogenic relationship were avoided. About 8 mL blood samples were collected through jugular vein and treated with heparin and SDS-EDTA and genome DNA were extracted according to the literature with some changes (Liu *et al*., 1997).

Structure loci detection: Starch gel electrophoresis was used to determine the variations of 12 loci: Albumin (Alb), binding protein (Gc), Transferrin (Tf), Alkaline Phosphatase (Alp), Leucine Aminopeptidase (Lap), Arylesterase (Ary-Es), Hemoglobin-β (Hb-β), X-protein(X-p), Carbonic Anhydrase (CA), Catalase (Cat), Malate Dehydrogenase (MDH) and Acerbity Enzyme-D (Es-D-); Lysine (Ly) was tested with cellulose acetate film electrophoresis. The types of the variations were determined according to the standards universally accepted in the neighboring countries of China (Tsunoda *et al*., 1988, 1992).

Microsatellite marker detection: About 7 sheep microsatellite markers: OarFCB48, Oar-AE101, MAF33, OarFCB11, MAF70, OarFCB30 and OarFCB128 co-recommended by ISAG and FAO (Project MoDAD, http://www.fao.org/dad_is) at different chromosome were adopted to detect the genetic background (Crawford *et al*., 1995). The Primer sequences were shown in Table 1. The primers were procured from Shanghai Sangon Biological Engineering Company. The PCR system and protocols referred to literature with some changes (Buchanan and Crawford, 1992a, b, 1993; Crawford *et al*., 1990). A final volume of 25 μL containing: 10×buffer 2.5 μL, 25 mmol L^{-1} $MgCl_2$ 1.3-3.0 μL, 10 mmol l day NTP 0.5 μL, 8 pmol $μL^{-1}$ GT and CA primer 1-2 μL each, 5 U $μL^{-1}$ Taq DNA polymerase 0.4 μL, 50 ng $μL^{-1}$ DNA 2 μL. The primer sequences, volume of $MgCl_2$ and annealing temperature are shown in Table 1. All reagents were procured from Shanghai Sangon Biological Engineering Company.

The cycling conditions were as follows: an initial denaturation step of 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 53-66°C and 1 min at 72°C. The last polymerization step was extended for 5 min at 72°C. The products were stored at 4°C. About 5-6 μL valid product was detected first in the 1.5-3.0% agarose gel electrophoresis then in 12-14% non-denaturated PAGE and was dyed with EB. Fragment sizes were calculated by Kadak Digitial Sience ID Image Analysis Software according to pBR322/Msp-Marker.

Statistical analysis: Gene frequencies were computed by the counting method for the Al, Po, Tf, Hb-β, CA, Cat and MDH loci and by the square root method for the Alp, Lap, Ary-Es, X-p and Ly loci (Tsunoda *et al*., 1990) microsatellite sites were also determined by counting method.

Heterozygosity (H) (Nei, 1978), Polymorphic Information Content (PIC) (Botstein *et al*., 1980), effective allele Number (Ne) (Kimura and Crow, 1964) of each locus and significance test of difference between populations computed by the SAS software version 8.1 (SAS Institute Inc., NC. USA). Deviations from Hardy-Weinberg (HW) equilibrium based on hypothesis of heterozygote deficiency were estimated by GENEPOP software (Raymond and Rousset, 1995). The coefficient of gene differentiation (Nei, 1973) were calculated with GENEPOP (Raymond and Rousset, 1995) and Popgene (Yeh *et al*., 1999) software.

Table 1: Heterozygosity (h), Polymorphism Information Content (PIC), effective Number of alleles (Ne) at each structural locus and their mean

	Hu			Tong			Han			Tan			WD			Goat		
Loci	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne
Al	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000
Gc	0.0328	0.0323	1.0340	0.0000	0.0000	1.0000	0.0651	0.0641	1.0697	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000
Tf	0.8058	0.7784	5.1492	0.4635	0.2867	1.8640	0.7464	0.7042	3.9434	0.7107	0.6611	3.4564	0.7418	0.7027	3.8732	0.0455	0.0445	1.0477
Alp	0.5403	0.4440	2.1754	0.6208	0.5442	2.6372	0.0677	0.0654	1.0727	0.6135	0.5350	2.5874	0.6535	0.5788	2.8858	0.2617	0.2274	1.3544
Ary-Es	0.1789	0.1629	1.2179	0.2003	0.1803	1.2505	0.2952	0.2516	1.4188	0.1546	0.1426	1.1828	0.0852	0.0799	1.0932	0.0000	0.0000	1.0000
Lap	0.4921	0.3710	1.9690	0.4997	0.3749	1.9990	0.4364	0.3412	1.7741	0.4855	0.3676	1.9436	0.4041	0.3224	1.6781	0.2103	0.1882	1.2663
Hb-B	0.5403	0.4440	2.1754	0.6208	0.5442	2.6372	0.6640	0.5899	2.9765	0.6135	0.5350	2.5874	0.6535	0.5788	2.8858	0.2729	0.2356	1.3753
Xp	0.1789	0.1629	1.2179	0.2003	0.1803	1.2505	0.2952	0.2516	1.4188	0.1546	0.1426	1.1828	0.0852	0.0799	1.0932	0.1374	0.1280	1.1593
MDH	0.5000	0.3750	0.2000	0.4616	0.3551	1.8575	0.4950	0.3725	1.9802	0.4985	0.3743	1.9940	0.4997	0.3748	1.9986	0.5000	0.3750	2.0000
Cat	0.3350	0.2789	1.5038	0.4072	0.3243	1.6869	0.4861	0.3680	1.9460	0.4887	0.3693	1.9556	0.2596	0.2259	1.3506	0.0000	0.0000	1.0000
Ly	0.4047	0.3228	1.6798	0.3731	0.3035	1.5951	0.0000	0.0000	1.0000	0.4276	0.3362	1.7469	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000
EsD	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.4992	0.3746	1.9967
CA	0.0644	0.0623	1.0688	0.0881	0.0842	1.0967	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000
Ke	0.1519	0.1404	1.1791	0.2917	0.2492	1.4119	0.1889	0.1711	1.2329	0.1109	0.1047	1.1247	0.0899	0.0859	1.0988	0.1184	0.1114	1.1343
Mean	0.3018	0.2554	1.6122	0.3019	0.2448	1.5918	0.2671	0.2271	1.6309	0.3042	0.2549	1.6973	0.2480	0.2164	1.6398	0.1461	0.1203	1.2381

RESULTS AND DISCUSSION

Genetic variation in population: The average Heterozygosity (H), Information Content of Polymorphism (PIC), Number of effective allele (Ne) were calculated for each locus and the result showed that the H, PIC and Ne in different populations all obeyed normal distribution. The variance analysis of the heterozygosity, polymorphic information content, effective number of alleles at each structural locus showed that there were not significant differences either between species or among breeds ($p>0.05$); variance analysis of the 3 genetic variables at microsatellite makers showed that there were not significant differences among populations in heterozygosity and PIC ($p>0.05$)as to effective number of alleles there were not significant differences both among Tan sheep, Hu sheep, Tong sheep and Wadi sheep and between Wadi sheep and small-tailed sheep, ($p>0.05$) but between the pre-four populations and the post-two populations, there were significant differences ($0.01<p<0.05$). The results indicated that the three indices were in a broad sense a quantitative explanation of genetic variation within species, exported no obvious borderline among species or breeds. Meanwhile, comparing Table 1 with Table 2, the value of H, PIC and Ne at microsatellite loci and the average value of each population were all higher than the indexes at structural loci which showed microsatellite marker revealed higher level of genetic variance than structural loci. The indexes of genetic variance based on structural loci in Table 1 showed that the variation level of Yangtse River Delta White goats was lowest and the variation levels of 5 sheep populations were adjacent among which Tan sheep was the highest followed by Tong sheep, Hu sheep, small-tailed Han sheep and then Wadi sheep. The variation level of small-tailed Han sheep was the highest in the 5 populations based on microsatellite makers data, followed by Wadi sheep, Tong sheep, Tan sheep and then Hu sheep.

The different order of genetic variation level of 5 sheep populations based on the two level makers, i.e., structural loci and microsatellite makers were different which showed that genetic variance of 5 sheep populations at structural loci (in coding region) and at microsatellite loci (in noncoding region) were different. There wasn't necessarily association between the two level markers and the delineation of population variation was concerned with the marker.

Detection for Hardy-Weinberg (HW) equilibrium: From Table 3 based on structural loci, Hu sheep, Tong sheep and Tan sheep were in the status of Hardy-Weinberg extreme disequilibrium ($p<0.01$) and Yangtse River Delta White goat, small-tailed sheep and Wadi sheep were in Hardy-Weinberg disequilibrium. But from Table 4 based on microsatellite makers, the status of

Table 2: Heterozygosity (h) Polymorphism Information Content (PIC), effective number of alleles (Ne) per microsatellite locus and their mean

	Han			Tan			Hu			Tong			WD			Goat		
Locus	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne	H	PIC	Ne
OarAE101	0.8914	0.8816	9.2081	0.8945	0.8855	9.4831	0.8664	0.8520	7.4839	0.9061	0.8987	10.6474	0.8597	0.8439	7.1298	0.9378	0.9343	16.0637
OarFCB48	0.9173	0.9114	12.0948	0.8983	0.8898	9.8370	0.9284	0.9240	13.9738	0.9296	0.9252	14.2043	0.9348	0.9310	15.3332	0.9195	0.9138	12.4183
MAF33	0.9528	0.9507	21.1864	0.8953	0.8866	9.5482	0.9056	0.8985	10.5960	0.9059	0.8986	10.6312	0.9491	0.9461	19.6549	0.7144	0.6869	3.5014
OarFCB11	0.9392	0.9359	16.4584	0.9230	0.9178	12.9903	0.9438	0.9409	17.7772	0.9246	0.9198	13.2683	0.9319	0.9278	14.6936	0.8753	0.8636	8.0218
MAF70	0.9382	0.9347	16.1812	0.9096	0.9028	11.0561	0.9000	0.8926	10.0796	0.9008	0.8856	9.4382	0.9373	0.9337	15.9497	0.9063	0.8992	10.6676
OarFCB304	0.9580	0.9563	23.8095	0.9233	0.9182	13.0389	0.9101	0.9036	11.1285	0.9331	0.9291	14.9520	0.9267	0.9219	13.6430	0.9406	0.9374	16.8237
OarFCB128	0.9384	0.9351	16.2338	0.9370	0.9334	15.8653	0.9120	0.9055	11.3673	0.9288	0.9242	14.0351	0.9360	0.9324	15.6230	0.9133	0.9068	11.5385
Mean	0.9336	0.9294	16.4532	0.9116	0.9049	11.6884	0.9095	0.9024	11.7723	0.9184	0.9116	12.4538	0.9251	0.9195	14.5753	0.8906	0.8821	11.6104

Table 3: Hardy Weinberg equilibrium tests based on hypothesis of heterozygote deficiency

	Hu		Tong		Goat		Han		Tan		WD		All populations	
Locus	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	fis (W and C)	P
Al	-----No diversity----		----No diversity-------		---No diversity-----		---No diversity-----		----No diversity----		----No diversity----		----No diversity----	
Gc	-0.009	1	----No diversity------		---No diversity-----		-0.018	1	-0.021	1	----No diversity----		-0.0173	1
Tf	0.211	0.000	-0.030	0.1075	-0.012	1	-0.100	0.8586	0.204	0.000	0.018	0.6922	0.0677	0.000
Hb-β	0.551	0.0001	0.469	0.000	-0.048	0.7939	-0.046	0.6827	-0.087	0.9141	-0.020	0.6553	0.1335	0.0023
MDH	0.024	0.5250	0.241	0.0466	-1	1	0.334	0.0092	0.155	0.1371	-0.389	0.9999	-0.0812	0.9349
Cat	0.374	0.0192	0.214	0.0777	-----No diversity------		0.254	0.0435	0.110	0.2414	-0.175	1	0.1544	0.0092
EsD	----No diversity-----		----No diversity------		-0.882	1	----No diversity-----		----No diversity-----		---No diversity------		-0.8829	1
CA	-0.078	1	-0.041	1	-----No diversity------		---No diversity-----		----No diversity-----		---No diversity-----		-0.0639	1
All loci		0.0000		0.0007		1		0.2579		0.0003		0.9937	0.0332	0.0001

Table 4: Hardy Weinberg equilibrium tests based on hypothesis of heterozygote deficiency

	Hu		Tong		Han		Tan		WD		Goat		All population	
Site	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P	Fis (W and C)	P
OarAE101	0.134	0.0436	0.142	0.0057	-0.089	0.9713	-0.079	0.8464	-0.138	1.0000	0.017	0.2437	-0.0387	0.7505
OarFCB48	0.016	0.1517	-0.009	0.6933	-0.011	0.7612	-0.089	0.9973	-0.030	0.8228	-0.065	1.0000	-0.0332	0.9878
MAF33	0.074	0.0939	-0.065	0.7192	0.003	0.7069	-0.107	1.0000	-0.046	1.0000	0.566	0.0008	0.0038	0.8526
OarFCB128	0.209	0.0019	-0.051	1.0000	-0.012	0.2605	0.021	0.0593	-0.041	0.7953	0.585	0.0000	0.0423	0.0018
OarFCB11	-0.017	0.6314	0.059	0.0573	-0.029	0.8814	-0.046	0.4180	-0.050	0.9624	0.045	0.4051	-0.0178	0.6969
MAF70	-0.098	1.0000	-0.103	1.0000	-0.056	1.0000	-0.076	0.6359	-0.027	0.8135	0.109	0.0081	0.0509	0.9810
OarFCB304	0.225	0.0023	-0.014	0.5305	-0.035	1.0000	-0.043	0.2601	-0.072	1.0000	0.101	0.0244	-0.0000	0.2045
All sites	-	0.0006	0.000	0.2596	0.000	0.9991	0.000	0.8834	0.000	1.0000	0.000	0.0000	0.0000	0.8376

Table 5: Gene differentiation coefficients at each microsatellite sites s in 5 sheep and goat populations

5 sheep and one goat populations				5 sheep populations			
Site	Gst	Ht	Hs	Site	Gst	Ht	Hs
OarAE101	0.043702	0.933415	0.892623	OarAE101	0.046742	0.926933	0.883606
OarFCB48	0.023781	0.944045	0.921594	OarFCB48	0.017764	0.938699	0.922024
MAF33	0.071255	0.955259	0.887192	MAF33	0.029177	0.949453	0.921750
OarFCB128	0.031599	0.957846	0.927579	OarFCB128	0.019368	0.948805	0.930428
OarFCB11	0.029797	0.951334	0.922987	OarFCB11	0.017639	0.949261	0.932517
MAF70	0.026098	0.938859	0.914357	MAF70	0.025232	0.939693	0.915982
OarFCB304	0.033347	0.964122	0.931971	OarFCB304	0.027612	0.939693	0.915982
All sites	0.037108	0.949269	0.914043	All sites	0.026167	0.944216	0.919509

Table 6: Gene differentiation coefficients at each structural locus in 5 sheep and goat populations

5 sheep and one goat populations				5 sheep populations			
Locus	Gst	Ht	Hs	Locus	Gst	Ht	Hs
Al	----------No diversity----------			Al	----------No diversity----------		
Gc	0.015875	0.016592	0.016328	Gc	0.014587	0.019884	0.019594
Tf	0.126660	0.712100	0.621906	Tf	0.030178	0.760123	0.737184
Alp	0.115954	0.276442	0.244388	Alp	0.137499	0.279339	0.240930
Ary-Es	0.185611	0.493399	0.401819	Ary-Es	0.031899	0.498071	0.482183
Lap	0.155404	0.498872	0.421345	Lap	0.064400	0.495465	0.463557
Hb-β	0.079896	0.609533	0.560834	Hb-β	0.023477	0.633296	0.618428
X-p	0.017611	0.181305	0.178112	X-p	0.018400	0.189749	0.186257
MDH	0.014467	0.499693	0.492464	MDH	0.017218	0.499558	0.490957
Cat	0.322093	0.485961	0.329436	Cat	0.209352	0.499999	0.395324
Ly	0.165313	0.240680	0.200892	Ly	0.137243	0.279419	0.241071
EsD	0.434389	0.147088	0.083195	ESD	No diversity	-	-
CA	0.047244	0.040990	0.039054	CA	0.043152	0.048978	0.046864
Ke	0.023455	0.162432	0.158622	Ke	0.024856	0.170912	0.166664
All loci	0.141278	0.311792	0.267743	All loci	0.065324	0.312485	0.292072

genetic equilibrium in each population was different from the results based on the structural loci: Hu sheep and goat were in the status of Hardy-Weinberg extreme disequilibrium ($p<0.01$) and other populations including Tong sheep, small-tailed Han sheep, Tan sheep and Wadi sheep were in Hardy-Weinberg disequilibrium ($0.01<p<0.05$). The results showed that genetic equilibrium detection based on the two level markers were different because the two level markers were distributed in different genome region, generally, structural loci were in coding region and microsatellite sites were in noncoding region. These also revealed that the detected result of genetic equilibrium was concerned with the marker.

Analysis of coefficient of gene differentiation: Form Table 5 and 6, it could find the average coefficient of gene differentiation (Gst) at each structural locus in 5 sheep and one goat populations was all higher than that in 5 sheep populations which revealed that the genetic variation level among 5 sheep and goat populations was higher than that among 5 sheep populations. It was also consistent with the information revealed from microsatellite markers which indicated the variation level among 5 sheep and one goat populations was higher than that among 5 sheep populations. From Table 5 and 6, it also found the average coefficient of gene differentiation (Gst) at structural loci all was higher than that at microsatellite loci.

Comparison of genetic variance among breeds or among species: The variance analysis of the 3 genetic variance index including H, PIC and Ne at structural loci indicated that there weren't significant differences either among species or among breeds ($p>0.05$); the variance analysis of the 3 genetic indexes at microsatellite makers showed that there were not significant differences among populations in heterozygosity and PIC ($p>0.05$) as to effective number of alleles even though there were significant differences among partial different populations, there were not significant differences among species or breeds.

The results indicated that the three indices were in a broad sense a quantitative explanation of genetic variation within species, exported no obvious borderline among species or breeds. Meanwhile, microsatellite markers revealed higher level of genetic variance than structural loci.

Comparison of structure loci and microsatellite sites approaching the status of genetic equilibrium and the level of genetic variance: The status of genetic equilibrium of 5 sheep populations was different based on structure loci and microsatellite sites. At the same time, 2 level markers of 5 sheep populations based on structural loci and microsatellite makers revealed that the extent of

genetic variance level was different among populations which showed that genetic variance of 5 sheep populations at structural loci in genome coding region and at microsatellite sites in non-coding region was different.

There wasn't necessarily association between the two markers and the detected result of genetic equilibrium was concerned with the marker used. The value of H, PIC and Ne at microsatellite loci in each population were all higher than the indexes at structural loci; all the coefficients of gene differentiation at microsatellite loci were higher than that at structural loci which showed microsatellite markers revealed higher level of genetic variance than structural loci and was more superior molecular marker to analyze the relationship of genetic differentiation among populations especially among consanguineous populations.

CONCLUSION

This study focus on the genetic diversity of five main sheep breeds under different ecological circumstance in China detected by using structure loci and microsatellite sites markers including Tan sheep, small-tailed Han sheep, Hu sheep, Tong sheep and Wadi sheep and compare the genetic variation, the extent of hereditary basis of each locus (site), so as to provide a basis for the genetic resources evaluation, protection and utilization of Mongolia sheep group in China.

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