A Comparison of Two Kinds of Markers Applied in Analysis of Genetic Diversity in Sheep and Goat Populations

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ABSTRACT : A genetic examination using 14 structural loci and 7 microsatellite markers was carried out among random samples of Hu sheep (Hu), Tong sheep (Tong) and Yantse River Delta White goat (YRD); The mean heterozygosity (H), mean polymorphism information contents (PIC) and mean effective numbers of alleles (Ne) calculated based on the data from the above two types of genetic markers were compared. The standard genetic distances among the three populations based on two types of gene frequencies were calculated and compared. The results show that the mean heterozygosity (H), mean polymorphism information contents (PIC) and mean effective numbers of alleles (Ne) based on 7 microsatellite markers are greater than those based on the structural loci. The standard genetic distances based on structural loci among the three populations are: 0.0268-0.2487, the standard genetic distances based on microsatellite markers are: 0.2321-1.2313. The study indicates that structural and microsatellite markers reflect the genetic variation of the three populations consistently: Tong>Hu>YRD. The differentiation between related species or interpopulations can be expressed more effectively by microsatellite markers than structural markers. Oar FCB11, MAF33, Oar AE101, Oar FCB128 and OarFCB304 can be used as representative loci for research on genetic differentiation between sheep and goat. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 892-896)***

Key Words : Structural Loci, Microsatellite Marker, Mean Heterozygosity, Standard Genetic Distance, Genetic Differentiation

INTRODUCTION

Since the development of enzyme electrophoresis (Hunter and Markert, 1957), numerous natural animal populations have been investigated for genetic variation by using a range of protein loci (Nevo et al., 1984). More recently, many new DNA based methods, usually offering much greater resolution of differences between individuals and populations, have become available. These include mitochondria DNA variation (Cann et al., 1987), restriction fragment length polymorphism (RFLP) (Quinn et al., 1987), random amplified polymorphic DNA (RAPD) (Williams et al., 1990) and microsatellite DNA variation (Tautz, 1989). This paper attempts to compare the results from the application of structure loci and microsatellite markers in the analysis of genetic differentiation in sheep and goat populations.

With regards to sheep and goat, there are rich resources in China. Hu sheep distributed along the Taihu valley, and it is famous for its high fertility and beautiful lambskin. Tong sheep distributed in Baishui county Shanxi province. Tang Dynasty ago, the area had been empire pasture (Lei, 1999). Now the total number is less than 1 thousand. Yantse River Delta white goats (YRD) distributed along lower Yantse River valley of Jangsu province with total number of about 10 millions. This study also wants to describe the genetic constitution diversity of the three populations, so as to provide a basis for sheep and goat husbandry and the genetic resources protection.

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MATERIAL AND METHOD

Sampling methods and experiment materials

The 63 Hu and 65 Tong sheep were from Lianshi Town of Huzhou city in Zhejiang province and Baishui countryside of Shanxi province of China respectively. The 49 Yantse River Delta white goats (YRD) were from the suburb of Yang Zhou city of Jiangsu province of China. The method of "Random sampling in typical colonies of central area" was performed and we tried to avoid sampling two (or more) individuals that have traceable genetic relationship.

Collecting and treatment of blood samples

16 ml of blood was collected from the cervical vein of each animal.8 ml of the total was put into a centrifuge tube using heparin as an anticoagulant for separating blood cells and serum (Sun et al., 2002). Another 8ml mixed with SDS-EDTA was used to extract DNA (Xiong et al., 1999; Li et al., 2000).

Structural loci analysis

Starch gel electrophoresis was used to determine the variations of 12 loci: albumin (Al), post-albumin (Po), transferring (Tf), hemoglobin-β (Hb-β), alkaline phosphatase (Alp), leucine aminopeptidase (Lap), arylesterase (Ary-Es), X-protein (X-p), carbonic anhydrase (CA), catalase (Cat), Esterase-D (Es-D) and malate

Table 1. The primer sequence, chromosome assignment, annealing temperature and $MgCl_2$ volume

Marker	Chromosome assignment	Primer sequence	Annealing temperature	$MgCl_2$ (25 m mol·L^{-1}, μl)
OarFCB 11	2	(CA strand): GGCCTGAACTCACAAGTTGATATATCTATCAC	63	1.8
		(GT strand): GCAAGCAGGTTCTTTACCACTAGCACC		
OarFCB 128	2	(CA strand): CAGCTGAGCAACTAAGACATACATGGCG	60	1.0
		(GT strand): ATTAAAGCATCTTCTCTTTATTTCCTCGC		
OarFCB 304	19	(CA strand): CCCTAGGAGCTTTCAATAAAGAATCGG	61	1.5
		(GT strand): CGCTGCTGTCAACTGGGTCAGGG		
OarFCB 48	17	(CA strand): GAGTTATGTACAAGGATGACAAGAGGCAC	53	1.6
		(GT strand): GACTCTAGAGGATCGCAAAGAACCAG		
MAF 70	4	(CA strand): GCAGGACTCTACGGGGCCTTTGC	63.5	1.0
		(GT strand): CACGGAGTCACAAAGAGTCAGACC		
MAF 33	9	(CA strand): GATCATCTGAGTGTGAGTATATACAG	58	1.5
		(GT strand): GACTTTGTTTCAATCTATTCCAATTTC		
OarAE 101	6	(CA strand): TAAGAAATATATTTGAAAAAACTGTATCTCCC	57	1.0
		(GT strand): TCCTTATAGATGCACTCAAGCTAGG		

Table 2 (i). Alleles frequencies of 7 microsatellite and 14 structural loci in 3 sheep and goat populations

Locus/allele	Hu	Tong	YRD	Locus/allele	Hu	Tong	YRD	Locus/allele	Hu	Tong	YRD	Locus/allele	Hu	Tong	YRD
OarFCBll				OarFCB48				MAF33				OarAE101			
120	0.0405	0.0357	0	127	0	0.0122	0	110	0	0	0.1200	75	0	0	0.0526
124	0.0135	0.0238	0	141	0.0125	0.0122	0	112	0	0	0.4800	77	0	0	0.0263
126	0	0.0715	0	145	0.0125	0.0122	0	114	0	0	0.0800	79	0	0	0.0263
128	0.0405	0.1548	0	147	0.0250	0.0366	0	116	0.0250	0	0.1600	85	0	0.0172	0
130	0.0270	0.0833	0	149	0.1125	0.0975	0	120	0.0125	0.0125	0	87	0	0	0.0263
132	0	0.0833	0	151	0.0750	0.0610	0	122	0.0125	0	0.0600	93	0.0589	0.0517	0.0263
134	0.0541	0.0715	0	153	0.0375	0.0488	0.0227	124	0.0250	0.0750	0.0600	95	0.1176	0.0690	0.0263
136	0.0541	0.0357	0.0477	155	0.0375	0.0975	0.0683	126	0.1500	0.1500	0.0400	97	0.2059	0.1724	0.0263
138	0.0455	0.0715	0	157	0.0500	0.0610	0.0909	128	0.0500	0.0875	0	99	0.1765	0.1034	0
140	0.0812	0.0357	0.0238	159	0.0375	0.0366	0.1136	130	0.0125	0.0250	0	101	0.0587	0.0690	0
142	0.0270	0.0357	0.0238	161	0.0250	0.0366	0.0227	132	0.0500	0.0500	0	103	0.1176	0.0862	0.0789
144	0.0541	0.0952	0	163	0.0500	0.0244	0.0909	134	0.0250	0.0500	0	105	0.1029	0.0862	0.0586
146	0.0946	0.0595	0	165	0.0125	0.1097	0.0227	136	0.1750	0.0750	0	107	0.1176	0.1208	0.1055
148	0.0405	0.0357	0.2143	167	0.0875	0.0122	0.0445	138	0.1250	0.1625	0	109	0	0.0517	0.1055
150	0.0405	0.0238	0.1904	169	0.1375	0.0732	0.0445	140	0.0750	0.1125	0	111	0.0294	0.0517	0.0526
152	0.0315	0.0119	0.0952	171	0.0500	0.0610	0.1136	142	0.0750	0.0500	0	113	0.0147	0.0690	0.0789
154	0.0541	0.0119	0.0477	173	0.1000	0.0853	0	144	0	0.0125	0	115	0	0.0345	0.0263
156	0.0541	0	0.0238	175	0.0125	0.0732	0.1136	146	0.0375	0	0	117	0	0	0.0526
158	0.0405	0.357	0	177	0.0375	0.0122	0.0227	148	0	0.0250	0	119	0	0.0172	0.0789
160	0.0270	0.0238	0	179	0.0125	0.0244	0.0909	150	0.0500	0.0500	0	121	0	0	0.0263
162	0.0405	0	0	181	0.0250	0.0122	0.0227	152	0.0500	0.0375	0	123	0	0	0.0526
164	0.0946	0	0.1190	185	0	0	0.0227	154	0.0125	0.0250	0	125	0	0	0.0263
166	0.0541	0	0.0714	187	0.0125	0	0.0683	156	0.0125	0	0	127	0	0	0.0263
168	0	0	0.0714	189	0.0125	0	0.0227	158	0.0125	0	0	131	0	0	0.0263
170	0.0135	0	0.0477	197	0.0250	0	0	160	0.0125	0	0				
174	0	0	0.0238												

dehydrogenase (MDH). Lysine (Ly) was analyzed by cellulose acetate electrophoresis and potassium (Ke) in red cell was tested with a Na/K/Cl Analytical Instrument (MEDICA, USA). The methodologies used and the types of the variations present were determined according to the universally accepted methods (Tsunoda et al., 1998,1999).

The 7 sheep microsatellites studied and their characteristics are shown in Table 1. PCR amplification was performed on a HBPX200 (Hybird company). Each 20 μl PCR reaction contained 100 ng template DNA, 1-2 μl 5-10 pmol/μl each primer, 0.4 μl 20 mmol/μl dNTP, 1.0-1.8 μl 25 mmol/ml $MgCl_2$, 1 U Taq DNA polymerase, 2 μl 10×buffer. An initial denaturation at 94°C for 5 min was followed by 30 cycles of 60 s at 94°C denaturation, 60 s of 53-63.5°C annealing, 60 s of 72°C extension. The final cycle was followed by an extension at 72°C for 10 min.

The amplified fragments were electrophoresed on an 8% polyacrylamide gels in 1×TBE with 150-180 v of running voltage. Then the gels were detected by ethidium bromide staining. The fragment sizes were calculated by using the Kodak Digital Science ID Image Analysis Software.

Statistical analyses

The allele frequencies for the polymorphisms of blood proteins and non-protein systems were computed by the gene counting or square root methods. Microsatellite allele frequencies were determined by direct counting.

Genetic variability measures such as average heterozygosity (H) (Nei, 1978), Information content of

Table 2 (ii). Alleles frequencies of 7 microsatellite and 14 structural loci in 3 sheep and goat populations

Locus/allele	Hu	Tong	YRD	Locus/allele	Hu	Tong	YRD	Locus/allele	Hu	Tong	YRD	Locus/allele	Hu	Tong	YRD
OarFCB128				MAF70				152	0	0	0.0690	F	0.0617	0	0
91	0	0	0.0667	133	0.0119	0	0	156	0.0156	0	0.0172	Alp			
93	0	0.0250	0.1333	135	0.0119	0.0469	0	158	0	0.0761	0	B^+	0.3945	0.1497	0.1548
95	0	0	0.0667	137	0.0595	0.0625	0.0625	160	0.0156	0.0761	0	B^-	0.6055	0.8503	0.8452
97	0	0.0250	0.1333	139	0.1667	0.2032	0.1042	162	0.0938	0.0362	0.0517	Ary-Es			
99	0.0313	0.1000	0.1000	141	0.0952	0.0625	0.0208	164	0.1407	0.0761	0.0690	Ary-Es^+	0.3292	0.5196	1.0000
101	0.0313	0.0500	0	143	0.0952	0.0312	0.0625	166	0.1719	0.0978	0.0517	Ary-Es^-	0.6708	0.4804	0
103	0	0.0500	0	145	0.0119	0.0625	0.1042	168	0.0938	0.0326	0	Lap			
105	0	0.0750	0	147	0.0238	0.0156	0.0208	170	0.0469	0.0326	0	A	0.4373	0.4886	0.1194
107	0.0937	0.0750	0	149	0.0447	0.0312	0.0833	172	0.0156	0.0435	0	B	0.5627	0.5114	0.8806
109	0.0937	0.1000	0.0333	151	0.	0.0156	0.0417	174	0	0.0326	0	Hb-β			
111	0.0313	0	0	153	0.0595	0.1719	0.1875	176	0.0625	0.0217	0.0345	A	0.0238	0.3538	0
113	0.1526	0.0750	0	155	0.0952	0.0782	0.0625	178	0.0469	0.0109	0.0345	B	0.9762	0.6462	1.0000
115	0.0937	0.0500	0	157	0.1548	0.0938	0.0208	180	0.0625	0.0435	0.0172	X-p			
117	0.0313	0.1000	0	159	0.0833	0.0469	0.1042	182	0.0781	0.0869	0.0172	X	0.2546	0.2768	0.0742
119	0	0.0750	0	161	0.0238	0.0156	0.0417	184	0.0156	0.0761	0	x	0.7454	0.7232	0.9258
121	0.1250	0.0750	0	163	0.0477	0.0156	0.0417	186	0.0156	0	0	CA			
123	0.0937	0	0	165	0.0119	0	0.0208	188	0.0156	0.1087	0	F	0.0794	0.0462	0
125	0.0313	0.0500	0	167	0	0.0156	0.0208	190	0.0156	0.0543	0	S	0.9206	0.9538	1.0000
127	0.0313	0	0	169	0	0.0312	0	192	0.0469	0.0435	0	Cat			
129	0.0313	0.0250	0.0333	OarFCB304				194	0.0156	0.0326	0	B	0.4440	0.4620	0
131	0.0623	0.0	0.0667	126	0	0	0.0172	196	0	0.0109	0	C	0.5560	0.5380	0
133	0.0313	0.0250	0	128	0	0	0.0172	198	0	0.0109	0	D	0	0	1.0000
135	0	0	0.1000	130	0	0	0.0345	Al	0			MDH			
137	0	0.0250	0.0667	132	0	0	0.0517	C	1.0000	1.0000	1.0000	F	0.5000	0.3615	0.5000
139	0	0	0.0333	134	0	0	0.0517	Po				S	0.5000	0.6385	0.5000
141	0.0313	0	0	136	0	0	0.0517	F	0.0167	0	0	EsD			
143	0	0	0.0333	138	0	0	0.0863	S	0.9833	1.0000	1.0000	F	0	0	0.4796
153	0	0	0.0667	140	0.0156	0	0.1207	Tf				S	1.0000	1.0000	0.5204
159	0	0	0.0667	142	0	0	0.0517	A	0.0416	0.0923	0	Ly			
				144	0	0	0.0517	B	0.1500	0.2615	0	A^+	0.7183	0.7519	0
				146	0	0	0.0517	C	0.3000	0.3385	0	A^-	0.2817	0.2481	1.0000
				148	0.0156	0	0.0172	D	0.3417	0.2769	0.9796	Ke-L	0.0828	0.1772	0.0632
				150	0	0	0.0345	E	0.1500	0.0308	0.0204	Ke-H	0.9172	0.8228	0.9368

Table 3. Mean heterozgosity (H), mean polymorphism information content (PIC), mean effective number of alleles (Ne) in 3 populations based on structural (left) and microsatellite (right) loci

	Hu	Tong	YRD
H	0.3081±0.244/0.9095±0.024	0.3253±0.228/0.9184±0.013	0.1519±0.192/0.8906±0.077
PIC	0.2458±0.205/0.9024±0.028	0.2637±0.191/0.9116±0.017	0.1031±0.138/0.8821±0.087
Ne	1.6581±0.775/11.7723±3.270	1.6816±0.779/12.4538±2.170	1.2109±0.352/11.6104±4.591

polymorphism (PIC) (Bostein et al., 1982), Number of effective allele (Ne) (Kimura. et al., 1974) were calculated for each population. The standard genetic distances of Nei (Ds) (Nei, 1972) were calculated from the allele frequencies.

RESULTS

Structural and microsatellite loci characterization

The allele frequency data for 7 microsatellite and 14 structure loci are shown in Table 2. The number of alleles observed at a single microsatellite locus ranged from 19 (MAF70) to 36 (OarFCB304), with an average number of alleles per locus of 26.3. For each population, there were at least 7 alleles per locus. This showed that microsatellite markers could give a lot of genetic information. Of the 14 structural gene loci, polymorphisms were seen at 12 loci in Hu and Tong sheep populations (Al and EsD loci did not show polymorphism). In the Yantse River Delta white goat population, polymorphisms were seen at only 7 loci (Al, Po, Ary-Es, Hb-β, CA, Cat and Ly did not show polymorphism).

Diversity analysis

Diversity measures were calculated on the basis of allele frequencies. Table 3 shows mean heterozygosity, information content of polymorphism and number of effective alleles for each population. The diversity measures calculated based on microsatellite markers were obvious higher than those based on structural loci. When comparing the diversity indices of the three populations, Tong sheep was highest, while Yantse River Delta white goat was lowest.

One-Way ANOVA indicates that Hu sheep and Tong sheep only had significant greater PIC than Yantse River Delta white goat ($p<0.05$). T-test on all indices between

Table 4. The standard genetic distances among 3 populations (upper right: based on structural loci data, lower left: based on microsatellite marker data)

	Hu	Tong	YRD
Hu	0.0000	0.0268	0.2411
Tong	0.2321	0.0000	0.2487
YRD	1.2313	1.0921	0.0000

microsatellite and structural loci showed significant difference ($p<0.01$).

Ds genetic distances based on structural loci data ranged from 0.0268 to 0.2487, while those calculated on the basis of microsatellite markers ranged from 0.2312 to 1.2313. Thus, the latter values were much larger than the former (see Table 4).

DISCUSSION AND CONCLUSION

Barker et al. (1997) analyzed genetic diversity of Asian water buffalo using two kinds of genetic markers. As expected, the microsatellite loci showed very high levels of genetic diversity. This study show that heterozygosity for each population ranged from 0.8906-0.9184. These estimates are approximately three times of those derived from assay of 14 structural loci in the same three populations, which show an expected heterozygosity for each population ranging from 0.1519-0.3081. Our study has demonstrated far greater genetic variability at microsatellite compared with structural markers. This higher variability of microsatellite DNA is an obvious advantage over protein variability when applied to inter population or inter species genetic differentiation studies if the same loci can be screened. So, this observation indicates the superiority of microsatellite over protein markers for many population genetic studies.

Tsunoda et al. (1999) divided the Asian sheep into three groups: Mongolian group, Indo-Pakistani group and Tibetan group. Hu sheep is generally recognized as belonging to the Mongolian group (Sun et al., 2002; Geng et al., 2003). Hu sheep and Tong sheep are famous breeds in agricultural area of China. According the origin, breeding history (Lei, 1999) and Yang et al. (2002), there were closed genetic relationships among Tong sheep, Hu sheep and Mongolia sheep populations. This study shows that the genetic distances between Hu sheep and Tong sheep were 0.2312 (based on microsatellite loci) and 0.0268 (based on structural loci) and confirms that just as Hu sheep, Tong sheep could belong to the Mongolian group.

Our observations with sheep and goat also show that the conservation of microsatellite markers between these two related species existed in all microsatellite loci detected in this study. With regard to the distribution of microsatellite alleles in three populations, the allele co-shared percentages between sheep and goat were more than 50% for locus Oar FcB48 and MAF70, and less than 50% for locus Oar FcB11, MAF33, Oar AE101, OarFCB128 and Oar FCB304. Thus genetic differentiation between species leads to inter specific microsatellite polymorphism allowing OarFCB11, MAF33, OarFCB128, OarAE101 and OarFCB304 to be used as representative loci for research on genetic differentiation between sheep and goat.

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