Genetic Differentiation between Sheep and Goats Based on Microsatellite DNA

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ABSTRACT : The 7 sheep microsatellite markersOarFCB48, OarAE101, MAF33, OarFCB11, MAF70, OarFCB304 and OarFCB128, which were located on chromosomes 2, 4, 6, 9, 17 and 19, were selected to PCR in Hu sheep, Tong sheep and their closely related species,the goat. They were studied with the amplifying result of 7 microsatellite sites of Hu Sheep, Tong Sheep and goats, the data of allele number and range of allele's size of amplifying were analyzed with ANOVA. The results showed that there were no significant differences ($p<0.05$) in microsatellite DNA sites among 3 populations. Concerning the conservation of microsatellites in closely related species, selecting microsatellite sites located on the chromosome where the Robertsonian fusion was caused between sheep and goat, may be used in research into genetic differentiation and evolutionary relationships between sheep and goats. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 583-587)***

Key Words : Sheep, Goat, Microsatellite DNA, Genetic Differentiation

INTRODUCTION

The karyotype of sheep and goats is 2n=54 and 60, respectively. They belong to the genera *Ovis* and *Capra* of the family*Caprinae*. Archaeological and morphological research indicates that the sheep and the goat originated from the same ancestor: *Rupicaprids, goat-antelopes* in the Pleistocene era. Cellular genetic research showed that the sheep and the goat were evolved from a common ancestor: *Rupicaprids* and the karyotype of the goat is similar to the ancestral form (Li et al., 2000). It can be seen that the two species have a close relationship, but it is still necessary to study their genetic differentiation using on modern molecular technology. There are some reports about genetic differentiation between the sheep and the goat (Upholt et al., 1977; Li et al., 2000), but there are no reports on genetic differentiation of the two species based on microsatellite DNA. In the last 10 years, research on polymorphic marker-microsatellite DNA markers has been greatly advanced because of new techniques, especially PCR. The usefulness of microsatellites for the analysis of genetic relationships among closely related populations has been documented by numerous studies (Buchanan et al., 1994; Bancroft et al., 1995; Arranz et al., 1998; Joseph et al., 1999).The first genetic linkage map of the sheep genome was published in 1995 (Crawford et al., 1995), the second genetic linkage map of the sheep genome was published by de Gortari in 1998 and the first genetic linkage map of the goat genome was published by Vaiman in 1996 (Wu, 1999). This paper is concerned with Hu sheep, Tong sheep and their closely related speciesthe goat. We discuss the probability of studying the genetic differentiation between sheep and goats based on 7 sheep microsatellites, so as to provide a basis for the data bank of sheep (goat) microsatellites, and also put forward theoretical grounds for genetic differentiation among closely related species using microsatellite DNA.

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Received November 14, 2002; Accepted February 19, 2004

MATERIALS AND METHODS

Materials and sampling

The Hu and Tong sheep studied were from Lianshi Town of Huzhou city in Zhejiang province and Baishui country in Shannxi province of China, respectively. The sample size was 63 and 65 respectively. Blood sampling was performed by the "Random sampling in typical colonies of central area" method and we tried to avoid sampling two (or more) individuals that had traceable genetic relationships. Some external morphological characteristics were also investigated (Sun et al., 2002). At the same time, 49 Yangtse River Delta White Goats were sampled by the same method as the contrast population from the suburb of Yangzhou city in Jiangsu province of China.

Microsatellites, PCR conditions and fragment analysis

The genomic DNA was separated according to procedures described by Sun (2002). The 7 sheep microsatellites studied and their characteristics are shown in Table 1. Each 20 μl reaction contained: 0.4 μl dNTP (10 m mol/l), 2 μl 10×buffer, 25 m mol/l $MgCl_2$(shown in Table 1), 1 μl GT and CA primer (8 μmol/μl) (2 μl for OarFCB11), 0.2 μl of Taq polymerase (5 U/μl) and 2 μl template DNA (50 ng/μl), then super purified water was added. After an initial denaturation at 94°C for 5 min, the

Table 1. Microsatellite primer sequences, chromosome assignment and part of PCR conditions of the microsatellites used in this study

Site	Chromosome assignment	Primer sequences	Anneal temp. (°C)	$MgCl_2$ amount (μl)
OarFCB 11	2	(CA strand): GGCCTGAACTCACAAGTTGATATATCTATCAC (GT strand): GCAAGCAGGTTCTTTACCACTAGCACC	63	1.8
OarFCB 128	2	(CA strand): CAGCTGAGCAACTAAGACATACATGGCG (GT strand): ATTAAAGCATCTTCTCTTTATTTCCTCGC	60	1.0
OarFCB 304	19	(CA strand): CCCTAGGAGCTTTCAATAAAGAATCGG (GT strand): CGCTGCTGTCAACTGGGTCAGGG	61	1.5
OarFCB 48	17	(CA strand): GAGTTATGTACAAGGATGACAAGAGGCAC (GT strand): GACTCTAGAGGATCGCAAAGAACCAG	53	1.6
MAF 70	4	(CA strand): GCAGGACTCTACGGGGCCTTTGC (GT strand): CACGGAGTCACAAAGAGTCAGACC	63.5	1.0
MAF 33	9	(CA strand): GATCATCTGAGTGTGAGTATATACAG (GT strand): GACTTTGTTTCAATCTATTCCAATTTC	58	1.5
OarAE 101	6	(CA strand): TAAGAAATATATTTGAAAAAACTGTATCTCCC (GT strand): TCCTTATAGATGCACTCAAGCTAGG	57	1.0

Table 2 (i). Estimates of gene frequencies of the microsatellite DNA sites

OarFCB 48				OarAE 101				MAF 33			
Allele	Hu	Tong	Goat	Allele	Hu	Tong	Goat	Allele	Hu	Tong	Goat
127	0.0000	0.0122	0.0000	75	0.0000	0.0000	0.0526	110	0.0000	0.0000	0.1200
141	0.0125	0.0122	0.0000	77	0.0000	0.0000	0.0263	112	0.0000	0.0000	0.4800
145	0.0125	0.0122	0.0000	79	0.0000	0.0000	0.0263	114	0.0000	0.0000	0.0800
147	0.0250	0.0366	0.0000	85	0.0000	0.0172	0.0000	116	0.0250	0.0000	0.1600
149	0.1125	0.0976	0.0000	87	0.0000	0.0000	0.0263	120	0.0125	0.0125	0.0000
151	0.0750	0.0732	0.0000	93	0.0588	0.0517	0.0263	122	0.0125	0.0000	0.0600
153	0.0375	0.0488	0.0227	95	0.1176	0.0690	0.0263	124	0.0250	0.0750	0.0600
155	0.0375	0.0976	0.0681	97	0.2059	0.1724	0.0263	126	0.1500	0.1500	0.0400
157	0.0500	0.0610	0.0909	99	0.1765	0.1034	0.0000	128	0.0500	0.0875	0.0000
159	0.0375	0.0366	0.1136	101	0.0588	0.0690	0.0000	130	0.0125	0.0250	0.0000
161	0.0250	0.0366	0.0227	103	0.1176	0.0862	0.0789	132	0.0500	0.0500	0.0000
163	0.0500	0.0244	0.0909	105	0.1029	0.0862	0.0526	134	0.0250	0.0500	0.0000
165	0.0125	0.1098	0.0227	107	0.1176	0.1207	0.1052	136	0.1750	0.0750	0.0000
167	0.0875	0.0122	0.0455	109	0.0000	0.0517	0.1052	138	0.1250	0.1625	0.0000
169	0.1375	0.0732	0.0455	111	0.0294	0.0517	0.0526	140	0.0750	0.1125	0.0000
171	0.0500	0.0610	0.1136	113	0.0147	0.0690	0.0789	142	0.0750	0.0500	0.0000
173	0.1000	0.0854	0.0000	115	0.0000	0.0344	0.0263	144	0.0000	0.0125	0.0000
175	0.0125	0.0732	0.1136	117	0.0000	0.0000	0.0526	146	0.0375	0.0000	0.0000
177	0.0375	0.0122	0.0227	119	0.0000	0.0172	0.0789	148	0.0000	0.0250	0.0000
179	0.0125	0.0244	0.0909	121	0.0000	0.0000	0.0263	150	0.0500	0.0500	0.0000
181	0.0250	0.0122	0.0227	123	0.0000	0.0000	0.0526	152	0.0500	0.0375	0.0000
185	0.0000	0.0000	0.0227	125	0.0000	0.0000	0.0263	154	0.0125	0.0250	0.0000
187	0.0125	0.0000	0.0681	127	0.0000	0.0000	0.0263	156	0.0125	0.0000	0.0000
189	0.0125	0.0000	0.0227	131	0.0000	0.0000	0.0263	158	0.0125	0.0000	0.0000
197	0.0250	0.0000	0.0000					160	0.0125	0.0000	0.0000

PCR was performed with 30 cycles: denaturation at 94°C for 1 min. anneal at temperatures in Table 1 for 1 min. extension at 72°C for 1 min; the final cycle was followed by an extension at 72°C for 5 min. Amplified fragments were analyzed on 8% denaturation-polyacrylamide gel and detected with EB. Fragment sizes were calculated by Kdak Digital Science ID Image Analysis Software according to pBR322/Msp I Marker.

Statistical analysis

Allele frequencies were computed by the gene counting method. Heterozygosity (H). polymorphism information content (PIC) (Bostein et al., 1980) and effective allele number (Ne) (Kimura et al.. 1974) were calculated using the SAS package (Sun, 2002). The Nei's genetic distances were calculated using PAPP (Guo et al.. 1996). The results of 7 sheep microsatellite primers amplifying in goat and sheep were analyzed with ANOVA procedure using the SPSS package.

RESULTS

From Table 2-7, we saw that the PIC and H of each microsatellite site is more than 0.7. the genetic information

Table 2 (ii). Estimates of gene frequencies of the microsatellite DNA sites

OarFCB 11				MAF 70				OarFCB 304				OarFCB 128			
Allele	Hu	Tong	Goat	Allele	Hu	Tong	Goat	Allele	Hu	Tong	Goat	Allele	Hu	Tong	Goat
120	0.0405	0.0351	0.0000	133	0.0119	0.0000	0.0000	126	0.0000	0.0000	0.0172	91	0.0000	0.0000	0.0667
124	0.0135	0.0238	0.0000	135	0.0119	0.0469	0.0000	128	0.0000	0.0000	0.0172	93	0.0000	0.0250	0.1333
126	0.0000	0.0714	0.0000	137	0.0595	0.0625	0.0625	130	0.0000	0.0000	0.3450	95	0.0000	0.0000	0.0667
128	0.0405	0.1548	0.0000	139	0.1667	0.2031	0.1042	132	0.0000	0.0000	0.0517	97	0.0000	0.0250	0.1333
130	0.0270	0.0833	0.0000	141	0.0952	0.0625	0.0208	134	0.0000	0.0000	0.0517	99	0.0313	0.1000	0.1000
132	0.0000	0.0833	0.0000	143	0.0952	0.0312	0.0625	136	0.0000	0.0000	0.0517	101	0.0313	0.0500	0.0000
134	0.0541	0.0714	0.0000	145	0.0119	0.0625	0.1042	138	0.0000	0.0000	0.0862	103	0.0000	0.0500	0.0000
136	0.0541	0.0357	0.0476	147	0.0238	0.0156	0.0208	140	0.0156	0.0000	0.1207	105	0.0000	0.0750	0.0000
138	0.0405	0.0714	0.0000	149	0.0476	0.0312	0.0833	142	0.0000	0.0000	0.0517	107	0.0938	0.0750	0.0000
140	0.0811	0.0357	0.0238	151	0.0000	0.0156	0.0416	144	0.0000	0.0000	0.0517	109	0.0313	0.1000	0.0333
142	0.0270	0.0357	0.0238	153	0.0595	0.1719	0.1875	146	0.0000	0.0000	0.0517	111	0.01563	0.0000	0.0000
144	0.0541	0.0952	0.0000	155	0.0952	0.0781	0.0625	148	0.0156	0.0000	0.0172	113	0.0938	0.0750	0.0000
146	0.0946	0.0595	0.0000	157	0.1548	0.0938	0.0208	150	0.0000	0.0000	0.0345	115	0.0938	0.0500	0.0000
148	0.0405	0.0357	0.2143	159	0.0833	0.0469	0.1042	152	0.0000	0.0000	0.069	117	0.0313	0.1000	0.0000
150	0.0405	0.0238	0.1904	161	0.0238	0.0156	0.0416	156	0.0156	0.0000	0.0172	119	0.0000	0.0750	0.0000
152	0.0135	0.0119	0.0952	163	0.0476	0.0156	0.0416	158	0.0000	0.0761	0.0000	121	0.1250	0.0750	0.0000
154	0.0541	0.0119	0.0476	165	0.0119	0.0000	0.0208	160	0.0156	0.0761	0.0000	123	0.0938	0.0000	0.0000
156	0.0541	0.0000	0.0238	167	0.0000	0.0156	0.0208	162	0.0938	0.0326	0.0517	125	0.0313	0.0500	0.0000
158	0.0405	0.0357	0.0000	169	0.0000	0.0312	0.0000	164	0.1406	0.0761	0.0690	127	0.0313	0.0000	0.0000
160	0.0270	0.0238	0.0000					166	0.1719	0.0978	0.0517	129	0.0313	0.0250	0.0333
162	0.0405	0.0000	0.0000					168	0.0938	0.0326	0.0000	131	0.0625	0.0000	0.0667
164	0.0946	0.0000	0.1190					170	0.0469	0.0326	0.0000	133	0.0313	0.0250	0.0000
166	0.0541	0.0000	0.0714					172	0.0156	0.0435	0.0000	135	0.0000	0.0000	0.1000
168	0.0000	0.0000	0.0714					174	0.0000	0.0326	0.0000	137	0.0000	0.0250	0.0667
170	0.0135	0.0000	0.0476					176	0.0625	0.0217	0.0345	139	0.0000	0.0000	0.0333
174	0.0000	0.0000	0.0238					178	0.0469	0.0109	0.0345	141	0.0313	0.0000	0.0000
								180	0.0625	0.0435	0.0172	143	0.0000	0.0000	0.0333
								182	0.0781	0.0870	0.0172	153	0.0000	0.0000	0.0667
								184	0.0156	0.0761	0.0000	159	0.0000	0.0000	0.0667
								186	0.0156	0.0000	0.0000				
								188	0.0156	0.1087	0.0000				
								190	0.0156	0.0543	0.0000				
								192	0.0469	0.0435	0.0000				
								194	0.0156	0.0326	0.0000				
								196	0.0000	0.0109	0.0000				
								198	0.0000	0.0109	0.0000				

Table 3. Polymorphism information content (PIC) and Heterozygosity (H) of each microsatellite site

Site	PIC			H		
	Hu sheep	Tong sheep	Goat	Hu sheep	Tong sheep	Goat
OarFCB 48	0.9240	0.9252	0.9138	0.9284	0.9296	0.9195
OarAE 101	0.8520	0.8987	0.9343	0.8664	0.9061	0.9378
MAF 33	0.8985	0.8986	0.6869	0.9056	0.9059	0.7144
OarFCB 11	0.9409	0.9198	0.8636	0.9438	0.9246	0.8753
MAF 70	0.8926	0.8856	0.8992	0.9000	0.9008	0.9063
OarFCB 304	0.9036	0.9291	0.9374	0.9101	0.9331	0.9406
OarFCB 128	0.9055	0.9242	0.9068	0.9120	0.9288	0.9133

was abundant; and we also found that more than 7 alleles could be detected at each sheep microsatellite site in 3 populations. According to the protocol for the estimation of the global animal breeds distance, Barker put forward the selection standard for microsatellite DNA: only if there are more than 4 can the microsatellite site be used (Barker et al., 1994). At the same time, we compared the genetic distances obtained from 7 sheep microsatellites and found that the distance between goat and sheep was longer than the distance between Hu sheep and Tong sheep, thus, OarFCB48, OarAE101, MAF33, OarFCB11, MAF70, OarFCB304 and OarFCB128 could be used in the research of the genetic differentiation between sheep and goats. And we also could see from the tables the difference in gene amount and gene frequencies in the same microsatellite site in the different breeds (species), which could show the difference in heredity among them., This also indicated that the 7 microsatellite sites could be used to distinguish the breeds and species. The common alleles were the most antique and conservative, the other alleles were the result of insertions, deletionsand mutations.

Table 8-9 also shows the conservation of each microsatellite among 3 breeds (species): there were no significant differences in allele number of amplifying ($p>0.05$) and allele size of amplifying ($p>0.05$) in each microsatellite site.

Table 4. Effective number of alleles of each microsatellite DNA site

Site	Hu sheep	Tong sheep	Goat
OarFCB 48	13.9738	14.2043	12.4183
OarAE 101	7.4839	10.6474	16.0637
MAF 33	10.5960	10.6312	3.5014
OarFCB 11	17.7772	13.2683	8.0218
MAF 70	10.0796	9.4382	10.6676
OarFCB 304	11.1285	14.9520	16.8237
OarFCB 128	11.3673	14.0351	11.5385

Table 5. Mean polymorphism information content (mean PIC), mean heterozygosity (mean H) and mean effective number of alleles (mean Ne) of microsatellite DNA sites in 3 populations

	Hu sheep	Tong sheep	Goat
mean PIC	0.9024	0.9116	0.8821
mean H	0.9095	0.9184	0.8906
mean Ne	11.7723	12.4538	11.6104

DISCUSSION

Sun (2002) also used 20 biochemical markers to detect the Tong sheep, Hu sheep and goats, and concluded that the microsatellite sites provided more accurate values and more abundant information than protein markers, for example, the PIC, H and Ne of each protein marker was smaller than those of microsatellite sites. The 20 biochemical markers indicated that the genetic distance between Hu sheep and Tong sheep, Hu sheep and goats, Tong sheep and goats was 0.0268, 0.2411, 0.2476, respectively, which is also smaller than those of the 3 populations based on microsatellite sites, but the dendrogram of the 3 populations based on the microsatellites is similar to that of the 3 populations based on biochemical markers. Thus, the microsatellites might be a better indicator than protein polymorphisms of evolutionaryrelationships among populations or between closely related species.

Nei thought that the time polymorphism alleles existed in a population were longer than the time the breed existed, so that gene diversity (heterzygosity) in one locus in one breed is relative with the gene diversity of its closely related species (Nei et al., 1983). For the conservation of microsatellite profile sequences, microsatellites could be used in many species, the microsatellite primer of one breed might be used in its relative species, and for example, the sheep microsatellite could amplify in goats, which provided the possibility of expediting the obtaining of microsatellite primers and speeding up the comparative genome map. Because of the slow research progress of goat microsatellites, the first genetic linkage map of the goat published by Vaiman (Wu et al., 1999) only reported 10 goat microsatellite sites. So in this study we adopted sheep microsatellite to study the genetic diversity of goats.

Table 6. The standard genetic distance among 3 populations

	Hu sheep	Tong sheep	Goat
Hu sheep	0.0000	0.2321	1.2313
Tong	0.2321	0.0000	1.0921
Goat	1.2313	1.0921	0.0000

In general, during the evolution of mammalian chromosomes, the difference in chromosomes of closely related species was caused by the Robertsonian translocation (fusion or fission) near the centromere. Comparing the G band type between *Capra* and *Ovis* could yield significant homology. Some M chromosomes or SM chromosomes of *Ovis*: M1, M2, M3 and M4 were caused from the Robertsonian fusion of *Capra* 1/5, 3/10, 4/9 and 11/17 chromosomes. Thus, it was believed that the sheep and the goat originated from the same ancestor, the goat karyotype was similar to the ancestral, the telocentric chromosome of goat caused the Robertsonian fusion, the number of goat chromosomes was reduced from 2n=60 to 2n=54 and differentiated into the sheep karyotype (Chang, 1995).

Concerning the conservation of microsatellites and the similar origin of closely related species, selecting microsatellite sites located on the chromosome where the

Table 7. The comparison of 7 microsatellite primers amplifying in Goat, Hu sheep and Tong sheep

Site	Hu sheep		Tong sheep		Goat	
	Allele numbers	Size	Allele numbers	Size	Allele numbers	Size
OarFCB 48	23	141-197	21	127-181	17	153-189
OarAE 101	10	93-113	14	85-119	21	75-131
MAF 33	20	116-160	16	120-154	7	110-126
OarFCB 11	22	120-170	19	120-160	13	136-174
MAF 70	16	133-165	17	135-169	16	137-167
OarFCB 304	20	140-194	20	158-198	22	126-18
OarFCB 128	17	99-141	17	93-137	14	91-159

Table 8. The comparison of microsatellite allele number and range of allele's size in Goat, Hu sheep and Tong sheep Allele number of amplifying

	Sum of squares	df	Mean square	F	P value
Between groups	25.524	2	12.762	0.745	0.489
Within groups	308.286	18	17.127		
Total	333.810	20			

삭제됨: BASED ON MICROSATELLITE DNA

Table 9. The comparison of microsatellite allele number and range of allele's size in goat, Hu sheep and Tong sheep Allele size of amplifying

	Sum of squares	df	Mean square	F	P value
Between groups	34.667	2	17.333	0.096	0.909
Within groups	3244.571	18	180.254		
Total	3279.238	20			

Figure 1. Electrophoresis photograph of some microsatellite sites.

Robertsonian fusion occurred between sheep and goat. may be used in the study of genetic differentiation and evolutionary relationships between sheep and goats.

ACKNOWLEDGEMENTS

This work was supported by the International Cooperation Item of the National Natural Science Foundation of China (30310103007).

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