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Full Length Research Paper

Analysis of genetic polymorphism and genetic distance among four sheep populations

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The genomes of 4 sheep populations {Yuanqu white Tan sheep (YWT), Baozhongchang white Tan sheep (BWT), black Tan sheep (BT) and small-tailed Han sheep (Han)} were screened using 10 microsatellite DNA markers to estimate the genetic diversities and genetic distances among these populations. Small-tailed Han sheep was the reference group. About 167 alleles were detected at 10 loci in 4 populations. The average observed and expected heterozygosity ranged from 0.1771 to 0.4576 and from 0.8294 to 0.9083, respectively in 10 loci. The expected heterozygosity of each population was much higher than the observed heterozygosity. The mean polymorphism information content (PIC) value of populations ranged from 0.7723 to 0.7946. The coefficient of gene differentiation (Fst) between populations was high (8.93%). The percentage of inbreeding coefficient for all populations (Fit) was 67.4%, while within breeds (Fis) it was 64.2%. Constructing four dendrograms based on D_A and D_C genetic distance using UPGMA and NJ method, it was shown that the relationship between two white Tan sheep populations was the closest, then between white Tan sheep population and black Tan sheep population, small-tailed Han sheep population was the farthest when compared with other three populations.

Key words: Sheep, microsatellite DNA, genetic polymorphism, genetic distance.

INTRODUCTION

Tan sheep is an important indigenous sheep breed in Ningxia of China, which is selected by natural and artificial selection in specified ecological environment over a long period of time. Ningxia black Tan sheep is formerly known as Ningxia black sheep which has been feed on for more than 80 years. According to data, the Ningxia black Tan sheep was a descendant of cross-breeding between black Tibetan sheep and white Tan sheep. Black Tan sheep was breeded to get hereditary stability population by long-term directional selection and local acclimatization (Gong et al., 2002). The amount of black Tan sheep reared is declining, which was less than 1000 by 2007, so, there is danger of extinction. Microsatellite markers have been proved to belong to the most powerful tools for genetic diversity evaluations and estimations of genetic distances among closely related populations of ruminant species (Buchanan et al., 1994; Ellegren et al., 1997). In this study with small-tailed Han sheep as a reference, the genetic diversity, the genetic evolutionary relationship, the level of differentiation and the systematically status of black Tan sheep were assessed by 10 microsatellite DNA markers in four sheep populations. The aim was to provide a strategy for protecting and developing the fine genetic resource.

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MATERIALS AND METHODS

A total of 271 individuals from 4 sheep populations including white Tan sheep (n = 158) were from Yuanqu farm (n = 88) and Baozhongchang farm (n = 70) of Yanchi county of Ningxi province, black Tan sheep (n = 65) were from black Tan sheep farm of Yanchi county of Ningxi province, small-tailed Han sheep (n = 48) were from Yanquan farm of Ningxi province. A random sampling method was used in the typical colonies, auri-tissue samples were collected in ethanol. Genomic DNA was extracted using proteinase K digestion

Table 1. Characterization of the ten microsatellite loci and conditions of PCR.

Locus	Chromosome location	Primer sequence (5'-3')	Annealing temperature (℃)	MgCl (mmol/L)
OarAE101	6	TTCTTATAGATGCACTCAAGCTAGG TAAGAAATATATTTGAAAAAACTGTATCTCCC	60	2.4
OarFCB11	2	GGCCTGAACTCACAAGTTGATATATCTATCAC GCAAGCAGGTTCTTTACCACTAGCACC	66	2.0
MAF70	4	GCAGGACTCTACGGGGCCTTTGC CACGGAGTCACAAAGAGTCAGACC	64	1.0
MAF33	9	GATCATCTGAGTGTGAGTATATACAG GACTTTGTTTCAATCTATTCCAATTTC	60	1.8
MCM38	18	TGGTGAATGGTGCTCTCATACCAG CAGCCAGCAGCCTCTAAAGGAC	64	2.4
BM6526	26	CATGCCAAACAATATCCAGC TGAAGGTAGAGAGCAAGCAGC	53	1.0
BMS1714	25	TTTATCCCAAGAGGTTCCACC AGGTGCTTGCAGTGAATCTG	45	1.0
OarFCB193	11	TTCATCTCAGACTGGGATTCAGAAAGGC GCTTGGAAATAACCCTCCTGCATCCC	63	1.5
OarFCB48	17	GAGTTATGTACAAGGATGACAAGAGGCAC GACTCTAGAGGATCGCAAAGAACCAG	62	1.5
OarFCB304	19	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	63	1.5

followed by the standard phenol-chloroform extraction protocol according to Mullenbach et al. (1989). The quantity and quality of DNA were measured with a spectrophotometer at 260/280 nm using an Eppendorf BioPhotometer.

The panel of 10 sheep microsatellites was selected (Table 1); primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services CO, Ltd., Shanghai, China. PCR was carried in 20 µl volume containing 100 ng template, 1 µl 8 pmol/µl each primer, 0.4 µl 10 mmol/µl dNTP, 1.0 to 2.4 µl 25 mmol $MgCl_2$, 0.3 µl 5 U Taq DNA polymerase, 2 µl 10 × buffer. PCR amplification conditions were as follows: 94℃ for 5 min, followed by 30 cycles of denaturation at 94℃ for 30 s, 53 to 66℃ annealing for 30 s and extension at 72℃ for 30 s, and final extension at 72℃ for 10 min. The amplified fragments were electrophoresed on 10% polyacrylamide gels in 1×TBE with 90 to 150 V of running voltage, gels were detected by silver staining. The fragment sizes were calculated by Kodak Digital Science ID Image Analysis Software. The genotype of each individual animal at 10 different loci was recorded by direct counting.

Allelic frequencies were analysed by GeneClass software, effective number of alleles (Ne) was calculated by GENEPOP (V3.3) software (Raymond and Rousset, 2001) and polymorphism information content (PIC) was calculated according to Botstein et al. (1980). Genetic differentiation among populations was measured using 3 fixation indices (Wright, 1978), inbreeding coefficient within each population (Fis), coefficient of gene differentiation between populations (Fst) and inbreeding coefficient of all populations (Fit), and all indices were computed by FSTAT (V2.9.3.2) (Goudet and FSTAT, 2002). Island model (Slatkin, 1993) was used to analyze gene flow among populations, the values of Fst were firstly calculated by FSTAT among populations, and then the average number of effective migrants exchanged per generation (Nem) was calculated using the following formula:

$$Nem = (1 - Fst)/(4Fst)$$

Based on allele frequency, genetic distance among populations (DC genetic distance and DA genetic distance) was calculated by Population (1.2.28) software (Olivier L.). Based on DA and DC genetic distances, UPGMA (unweighted pair group method with arithmetic mean) phylogenic tree was constructed (Nei et al., 1983; Takezaki and Nei, 1996).

Table 2. Effective number of alleles (N_e), observed number of alleles (N_o), observed heterozygosity (H_o) and expected heterozygosit (H_e) of 10 microsatellite DNA in four sheep populations.

Population	YWT	BWT	BT	Han	Mean±SD	Total
N_a						
OarAE101	7	8	7	11	8.2500±1.8930	21
OarFCB11	11	7	5	12	8.7500±3.3040	20
MAF70	9	9	8	12	9.5000±1.7321	14
MAF33	9	7	9	12	9.2500±2.0616	17
MCM38	6	6	6	13	7.7500±3.5000	14
BM6526	7	7	7	10	7.7500±1.5000	22
BMS1714	9	7	7	12	8.7500±2.3629	22
OarFCB193	11	11	11	5	9.5000±3.0000	12
OarFCB48	8	8	9	5	7.5000±1.7321	12
OarFCB304	9	9	8	10	9.0000±0.8165	13
Mean±SD	8.6000±1.6465	7.9000±1.4491	7.7000±1.7029	10.2000±2.8983	8.6000±2.1697	
N_e						
OarAE101	2.3931	4.1263	5.0358	6.8776	4.6082±1.8683	5.8600
OarFCB11	8.0583	3.6842	3.5135	5.2543	5.1276±2.1051	9.5527
MAF70	7.8579	7.7655	7.2038	5.3581	7.0463±1.1620	10.6769
MAF33	7.3229	4.8732	7.3542	9.6674	7.3044±1.9577	10.9001
MCM38	5.2148	5.3581	5.2193	8.2915	6.0209±1.5152	7.4187
BM6526	3.2717	3.9437	4.4615	5.0205	4.1744±0.7453	6.7109
BMS1714	5.8733	3.9789	3.5077	8.7273	5.5218±2.3690	8.2328
OarFCB193	7.7712	7.3906	5.2064	3.2751	5.9108±2.0892	7.7691
OarFCB48	7.4070	6.9454	6.2500	2.2801	5.7206±2.3425	7.5189
OarFCB304	8.2559	5.9611	5.3212	6.6017	6.5350±1.2608	8.9371
Mean±SD	6.3426±2.0965	5.4027±1.5365	5.3073±1.3308	6.1354±2.3636	5.7970±1.8612	
N_a-N_e						
OarAE101	4.6069	3.8737	1.9642	4.1224	3.6418±1.1591	15.1400
OarFCB11	2.9417	3.3158	1.4865	6.7457	3.6224±2.2267	10.4473
MAF70	1.1421	1.2345	0.7962	6.6419	2.4537±2.7985	3.3231
MAF33	1.6771	2.1268	1.6458	2.3326	1.9456±0.3389	6.0999
MCM38	0.7852	0.6419	0.7807	4.7085	1.7291±1.9874	6.5813
BM6526	3.7283	3.0563	2.5385	4.9795	3.5757±1.0551	15.2891
BMS1714	3.1267	3.0211	3.4923	3.2727	3.2282±0.2041	13.7672
OarFCB193	3.2288	3.6094	5.7936	1.7249	3.5892±1.6798	4.2309
OarFCB48	0.5930	1.0546	2.7500	2.7199	1.7794±1.1194	4.4811
OarFCB304	0.7441	3.0389	2.6788	3.3983	2.4650±1.1843	4.0629
Mean±SD	2.2574±1.4399	2.4973±1.1517	2.3927±1.4801	4.0646±1.7140	2.8030±1.5873	
H_o						
OarAE101	0.0682	0.2429	0	0.3542	0.1663±0.1617	0.1476
OarFCB11	0.2614	0.2429	0.2462	0.3542	0.2762±0.0526	0.2694
MAF70	0.3636	0.3571	0.3385	0.2708	0.3325±0.0425	0.3395
MAF33	0.4545	0.3000	0.4615	0.2979	0.3785±0.0919	0.3889
MCM38	0.1250	0.3143	0.0308	0.5814	0.2629±0.2429	0.2256
BM6526	0.3068	0.2000	0.4000	0.1702	0.2693±0.1051	0.2778
BMS1714	0.1818	0.4286	0.1077	0.4792	0.2993±0.1822	0.2804
OarFCB193	0.5227	0.4714	0.4308	0.3542	0.4448±0.0711	0.4576
OarFCB48	0.1364	0.1143	0.2923	0.1875	0.1826±0.0793	0.1771
OarFCB304	0.2727	0.3286	0.2769	0.1875	0.2664±0.0584	0.2731

Table 2 Continue

Mean±SD	0.2693±0.1474	0.3000±0.1060	0.2585±0.1634	0.3237±0.1323	0.2879±0.1360	
H_e						
OarAE101	0.5821	0.7577	0.8014	0.8546	0.7490±0.1181	0.8294
OarFCB11	0.8759	0.7286	0.7154	0.8097	0.7824±0.0750	0.8953
MAF70	0.8727	0.8712	0.8612	0.8134	0.8546±0.0280	0.9063
MAF33	0.8634	0.7948	0.8640	0.8966	0.8547±0.0428	0.9083
MCM38	0.8082	0.8134	0.8084	0.8794	0.8274±0.0348	0.8652
BM6526	0.6943	0.7464	0.7759	0.8008	0.7544±0.0458	0.8510
BMS1714	0.8297	0.7487	0.7149	0.8854	0.7947±0.0773	0.8785
OarFCB193	0.8713	0.8647	0.8079	0.6947	0.8097±0.0817	0.8713
OarFCB48	0.8650	0.8560	0.8400	0.5614	0.7806±0.1465	0.8670
OarFCB304	0.8789	0.8322	0.8121	0.8485	0.8429±0.0282	0.8881
Mean±SD	0.8142±0.0990	0.8014±0.0538	0.8001±0.0524	0.8045±0.1035	0.8050±0.0779	

Table 3. The polymorphism information content (PIC)of different loci of 4 sheep populations

Population	YWT	BWT	BT	Han	Mean±SD
OarAE101	0.5501	0.7316	0.7753	0.8381	0.7238±0.1238
OarFCB11	0.8642	0.7021	0.6644	0.7889	0.7549±0.0896
MAF70	0.8591	0.8575	0.8454	0.7921	0.8385±0.0315
MAF33	0.8483	0.7662	0.8491	0.8873	0.8377±0.0510
MCM38	0.7808	0.7877	0.7811	0.8677	0.8043±0.0424
BM6526	0.6598	0.7128	0.7410	0.7757	0.7223±0.0490
BMS1714	0.8092	0.7225	0.6671	0.8750	0.7685±0.0920
OarFCB193	0.8584	0.8508	0.7892	0.6415	0.7850±0.1005
OarFCB48	0.8497	0.8393	0.8225	0.5139	0.7564±0.1620
OarFCB304	0.8663	0.8135	0.7881	0.8311	0.8248±0.0328
Mean±SD	0.7946±0.1067	0.7784±0.0597	0.7723±0.0650	0.7811±0.1176	0.7816±0.0877

RESULTS

Microsatellite loci polymorphism

The numbers of alleles for 10 microsatellite loci in 4 sheep populations and effective number of alleles (Ne) are presented in Table 2. A total of 167 alleles were obtained in 4 sheep populations and it demonstrated that they were highly polymorphic in all sheep populations. The number of alleles per locus varied from 12 (OarFCB193, OarFCB48) to 22 (BM6526, BMS1714). At least 8.6 alleles per locus were observed in each population. The values of N_a and N_e are shown in Table 2.

The average of observed and expected heterozygosity for 4 sheep populations was 0.2879 and 0.8050, respectively (Table 2). Small-tailed Han sheep showed the highest observed heterozygosity (0.3237), while the black Tan sheep showed the lowest (0.2585). The expected heterozygosities of all populations were higher than the observed ones, the expected heterozygosity of all populations and all loci were about 0.8, which showed it to be highly heterozygous. The values of observed heterozygosity and expected heterozygosity were highly different, showing that homozygous individuals were more than common, and inbreeding was severe in the tested population.

The mean polymorphism information content (PIC) varied from 0.7223 to 0.8385 (Table 3), all the selected loci could provide enough genetic information indicating that the genetic diversity of 4 sheep populations was high. The highest PIC value (0.8385) existed at MAF70 locus and the lowest PIC (0.7223) existed at BM6526 locus.

The values of the three fixation indices, F_{it}, F_{is} and F_{st} in Table 4 indicated that inbreeding was high among the

Table 4. The results of F-statistics for each of the 10 microsatellite loci of 4 sheep populations.

Locus	F_{it}	F_{is}	F_{st}
OarAE101	0.8068	0.7780	0.1298
OarFCB11	0.6945	0.6471	0.1345
MAF70	0.6323	0.6109	0.0549
MAF33	0.5865	0.5572	0.0662
MCM38	0.7003	0.6823	0.0568
BM6526	0.6920	0.6431	0.1370
BMS1714	0.6650	0.6234	0.1104
OarFCB193	0.4867	0.4507	0.0657
OarFCB48	0.7869	0.7661	0.0890
OarFCB304	0.7008	0.6839	0.0534
Total	0.6740	0.6420	0.0893

The significant test for F_{is}, F_{st} and F_{it} were highly significant ($p<0.001$).

Table 5. Pairwise estimates of F_{st} (below diagonal) and N_{em} (above diagonal) of 4 sheep populations.

Population	YWT	BWT	BT	Han
YWT		8.4306	2.9675	1.5044
BWT	0.0288		2.8750	1.5525
BT	0.0777	0.0800		1.4495
Han	0.1425	0.1387	0.1471	

All F_{st} in tables 2-6 are highly significant ($p<0.001$).

populations. The values of F_{it}, F_{is} and F_{st} were 0.674, 0.642 and 0.0893, respectively which were extremely significant ($P<0.01$). The mean of genetic differentiation among breeds, measured as the F_{st} value, was 8.93%, thus 91.07% of the total genetic diversity in the 4 sheep populations resulted from differences among individuals, indicating a close relationship among populations or migration among sheep populations.

The value of F_{st} among the 4 sheep populations was extremely significant ($P<0.001$) (Table 5). The highest value of F_{st} (0.1471) existed between small-tailed Han sheep and black Tan sheep, but their Nem value was low (1.4495). The lowest value of F_{st} (0.0288) and the highest value of *Nem* (8.4306) existed between Yanqu white Tan sheep and Baozhongchang white Tan sheep.

Genetic distance and constructing a phylogenetic tree

The DA and DC genetic distances are shown in Table 6 among the 4 sheep populations. The highest DA (0.6888, 0.6485 and 0.6807) and DC (0.7323, 0.7039 and 0.7251) genetic distances were observed between Tan sheep and Small-tailed Han sheep. Similarly, the smallest DA (0.1341) and DC (0.2898) genetic distances were observed among two white Tan sheep populations.

Based on the DA and DC genetic distances, NJ trees were constructed (Figure 1). The results show that the two white Tan sheep are the closest, followed by white Tan sheep and black Tan sheep. The farthest distance is between Small-tailed Han sheep and Tan sheep.

DISCUSSION

The mean PIC and mean He showed abundant genetic diversity among the 4 sheep populations. The mean observed heterozygosity (Ho) was lower than that detected by Chun et al. (2002) and Arranz et al. (1998). The expected heterozygosities of 4 sheep populations were higher than the observed heterozygosities, showing that the homozygous were closer due to inbreeding among populations. Moreover, some introduced breeds crossed with Tan sheep were in order to improve hybridization, leading to homozygous with fewer and a serious degradation of breed. Further study should be performed in future with a greater number of microsatellites in order to obtain more accurate results.

The value of F_{st} among the 4 sheep populations were highly significant ($P<0.001$) showing the highest genetic differentiation among the 4 sheep populations, similar results were reported by Hou et al. (2005) and Buchanan et al. (1994). According to the F_{st} and *Nem* values, the

Table 6. The D_A distance (below diagonal) and D_C distance (above diagonal) of 4 sheep populations.

Population	YWT	BWT	BT	Han
YWT		0.2898	0.4732	0.7323
BWT	0.1341		0.4723	0.7039
BT	0.2889	0.2914		0.7251
Han	0.6888	0.6485	0.6807	

highest genetic variation existed between Tan sheep and small-tailed Han sheep, while the lowest genetic variation existed among two white Tan sheep.The results of genetic clustering verified the origin of sheep populations and the blood relationship, similar results were reported by Sun et al. (2007). Baozhongchang white Tan sheep breed tend to be protected and developed, however, Yuanqu white Tan sheep is more related with the actual production.

In the actual production situation, there must be a connection between two white Tan sheep. From the analysis of gene flow, we can also see that there is a strong gene flow between the two. Black Tan sheep is a relatively closed population and gene flow is relatively infrequent with white Tan sheep. However, the results show that a certain communication of gene flow existed between white and black Tan sheep populations. In conclusion, genetic diversity existied between white and black Tan sheep populations, but the level of diversity was very low. Small-tailed Han sheep was as the reference group, the genetic relationship was much closer than between white and black Tan sheep populations. Further research is needed to certify whether black Tan sheep can be an independent breed when compared with whit Tan sheep.

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