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Original Paper

Analysis of genetic polymorphism in six meat sheep breeds and genetic distances between them

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ABSTRACT: The genomes of 6 sheep populations (Dorset, Texel, Black-Suffolk, Tan sheep, Small-tailed Han sheep, Tan and Small-tailed Han hybrid sheep) were screened using 7 microsatellite DNA markers to estimate the genetic diversities and genetic distances among these populations. About 105 alleles were detected at 7 loci in 6 populations. The average observed and expected heterozygosity ranged from 0.2901 to 0.4534 and from 0.8007 to 0.8737 in 6 sheep populations. The expected heterozygosity of each population was much higher than the observed heterozygosity. The mean polymorphism information content (*PIC*) value of populations ranged from 0.7188 to 0.8546. The coefficient of gene differentiation (F_{st}) between populations was very low (6.98%). The percentage of inbreeding coefficient for all populations (F_{it}) was 56.02%, while within breeds (F_{is}) it was 52.72%. Assuming that heterosis could be estimated on the basis of genetic distances between examined breeds, the hybrid of Tan and Small-tailed Han sheep could be designated as the best female parent, followed by Tan sheep and Small-tailed Han sheep.

Keywords: sheep; microsatellite DNA; genetic polymorphism; heterosis

Tan sheep and the hybrid of Tan and Small-tailed Han sheep (TH hybrid sheep) are important local populations in China, which are particularly suitable for the local environment. The high performance traits in Dorset, Texel and Black-Suffolk sheep are exogenous. So it is important to establish a cross hybridisation system for pachy-lamb production. In the development of molecular biology technology, microsatellites have been increasingly used as the marker of choice because of their high degree of polymorphism, random distribution across the genome and possibility of automated scoring of genotypes. 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 (Moore et al., 1991; Buchanan et al., 1994; Ellegren et al., 1997). Microsatellite DNA is used to anticipate the heterosis in sheep breeds (Zhang et al., 2006), analyzed hereditary constitution and genetic diversity in goat and cattle breeds (Sun et al., 2000; Li et al., 2008). In this study, the heterosis of Small-tailed Han sheep, Tan sheep, TH hybrid

sheep crossed with exogenous meat sheep breeds was assessed by 7 microsatellite DNA markers to study the genetic diversity and genetic distance in 6 sheep populations and to design a breeding strategy aimed at providing a relevant forecast for the improvement of production through crossing and establishing excellent matches.

MATERIAL AND METHODS

A total of 349 individuals from 6 sheep populations including Dorset sheep (*n* = 72), Texel sheep (*n* = 74) and Black-Suffolk sheep (*n* = 73) from the Sizheng biological company, China; Tan sheep (*n* = 48), Small-tailed Han sheep (*n* = 48) and a hybrid of Tan and Small-tailed Han sheep (*n* = 37) from the Ningxia province of China were studied. 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 followed by the standard phenol-chloroform extraction protocol according to

Table 1. The observed number of alleles sampled at 7 microsatellite loci in 6 sheep populations

Loci	Dorset	Texel	Black-Suffolk	Tan sheep	Han Sheep	TH hybrid
OarAE101	14	11	10	6	11	10
OarFCB11	14	12	10	9	12	11
MAF70	14	13	9	8	7	7
MAF33	14	16	16	10	14	13
MCM38	10	11	13	6	10	8
BM6526	10	11	11	11	12	9
BMS1714	11	7	5	9	11	8

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 7 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, 1.0–2.4 µl 25 mmol MgCl$_2$, 0.3 µl 5 U Taq DNA polymerase, 2 µl 10 × buffer. PCR amplification conditions were as follows: 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, 45–66°C annealing for 30 s and extension at 72°C for 30 s, and final extension at 72°C 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 7 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 (F_{is}), coefficient of gene differentiation between populations (F_{st}) and inbreeding coefficient of all populations (F_{it}), 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 F_{st} were firstly calculated by FSTAT among populations, and then the average number of effective migrants exchanged per generation (*Nem*) was calculated by the following formula:

$$Nem = (1 - F_{st})/(4F_{st})$$

Based on allele frequency, genetic distance among populations (DC genetic distance and DA genetic

Table 2. The effective number of alleles (*Ne*) at 7 microsatellite loci in 6 sheep populations

Loci	Dorset	Texel	Black-Suffolk	Tan sheep	Han Sheep	TH hybrid
OarAE101	9.5602	5.9708	4.3459	2.5291	4.2275	2.7825
OarFCB11	11.2644	6.2745	7.7952	5.1892	5.3581	7.3405
MAF70	10.6557	5.7830	2.7922	3.3508	3.4887	4.2188
MAF33	8.6108	10.1840	7.7739	6.9248	8.2889	6.8622
MCM38	6.9531	6.6699	5.1020	2.6074	4.5973	4.6703
BM6526	5.2683	9.5069	2.3013	8.5333	8.7273	7.0933
BMS1714	5.2390	4.2456	3.5140	5.1834	6.8776	5.2755
Mean	8.2216	6.9478	5.7839	4.9026	7.9379	5.4633

Table 3. The polymorphism information content (*PIC*) of different loci of 6 sheep populations

Loci	Dorset	Texel	Black Suffolk	Tan sheep	Han Sheep	TH hybrid	Mean
OarAE101	0.8863	0.8131	0.7353	0.5319	0.7311	0.6159	0.7189
OarFCB11	0.9044	0.8244	0.8579	0.7847	0.7921	0.8489	0.8354
MAF70	0.8985	0.8067	0.6138	0.6609	0.6661	0.7254	0.7286
MAF33	0.8732	0.8940	0.8580	0.8390	0.8686	0.8395	0.8621
MCM38	0.8406	0.8334	0.7793	0.5584	0.7518	0.7558	0.7532
BM6526	0.7932	0.8854	0.8809	0.8715	0.8750	0.8430	0.8582
BMS1714	0.7859	0.7274	0.6670	0.7854	0.8381	0.7870	0.7651
Mean	0.8546	0.8263	0.7703	0.7188	0.7890	0.7736	

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, 1996).

RESULTS

Microsatellite loci polymorphism

A total of 105 alleles were obtained in 6 sheep populations, demonstrating that they were highly polymorphic in all populations. The number of alleles per locus varied from 13 (BMS1714) to 20 (MAF70) with the average value 15.67 in each population. At least 5 alleles per locus were observed in each population. This showed that microsatellite markers could offer abundant genetic information. The numbers of alleles for 7 microsatellite loci in 6 sheep populations and effective number of alleles (*Ne*) are presented in Tables 1 and 2, respectively.

The mean polymorphism information content (*PIC*) varied from 0.7189 to 0.8621 (Table 3), all the selected loci could provide enough genetic information indicating that the genetic diversity of 6 sheep populations was high. The highest *PIC* value (0.8621) existed at MAF70 locus and the lowest *PIC* (0.7189) existed at OarAE101 locus.

The average of observed and expected heterozygosity for 6 sheep populations was 0.3838 and 0.8177, respectively (Table 4). Dorset sheep showed the highest observed heterozygosity (0.4534) while Black-Suffolk showed the lowest (0.2901). The expected heterozygosities in all populations were higher than the observed ones, showing that homozygous individuals were more than common, and inbreeding was serious in the tested populations.

The values of the three fixation indices, F_{it}, F_{st} and F_{is}, in Table 5 indicated that inbreeding was high among the populations. The mean of genetic differentiation among breeds, measured as the F_{st} value, was 6.98%, thus 93.02% of the total genetic diversity in the 6 sheep populations resulted from differences among individuals, indicating a close relationship among populations. The value of F_{st} among the 6 sheep populations was extremely significant $P < 0.001$ (Table 6). The highest value of F_{st} (0.0991) existed between Black-Suffolk and TH hybrid sheep but their *Nem* value was low (3.0370). The lowest value of F_{st} (0.0386) and the highest value of *Nem* (6.2320) existed between Tan sheep and Han sheep.

The highest DA (0.2629, 0.3098, and 0.2874) and DC (0.4484, 0.4824, and 0.4716) genetic distances

Table 4. The observed heterozygosity and expected heterozygosity of 6 sheep populations

Populations	*Ho*	*He*
Dorset	0.4534	0.8736
Texel	0.4213	0.8514
Black-Suffolk	0.2901	0.8007
Tan sheep	0.3655	0.7617
Han Sheep	0.3699	0.8219
TH hybrid	0.4023	0.7967
Mean	0.3838	0.8177

Table 5. The results of *F*-statistics for each of the 7 loci across 6 sheep populations

Loci	F_{is}	F_{st}	F_{it}
OarAE101	0.5471	0.1137	0.5986
OarFCB11	0.5488	0.0537	0.5692
MAF70	0.3653	0.1103	0.4273
MAF33	0.5421	0.0374	0.5592
MCM38	0.6629	0.0783	0.6892
BM6526	0.4455	0.0328	0.4637
BMS1714	0.5929	0.0666	0.6200
Mean	0.5272	0.0698	0.5602

were observed between Dorset, Texel and Black-Suffolk on the one hand and TH hybrid sheep on the other (Table 7). Similarly, the smallest DA (0.2042, 0.2396, and 0.2255) and DC (0.3930, 0.4242, and 0.4139) genetic distance was observed between the three introduced breeds and Han sheep.

Based on the genetic distances, when Tan sheep or Small-tailed Han sheep was used as female parent, Texel could be designated as the best male parent, followed by Black-Suffolk and Dorset. When TH hybrid sheep was used as female parent, Black-Suffolk could be designated as the best male parent, followed by Texel and Dorset.

In a two-way cross, when Tan sheep and Small-tailed Han sheep were used as female, the best match was male Texel, then Black-Suffolk and Dorset. In a three-way cross, the best match for female TH hybrid sheep was Black-Suffolk, then Texel and Dorset.

Constructing a phylogenetic tree

Based on DA and DC genetic distances, UPGMA trees were constructed (Figures 1 and 2). The 6 sheep populations were divided into 2 groups: one group included Texel, Black-Suffolk and Dorset, and the other group comprised Tan sheep and Han sheep and TH hybrid sheep.

DISCUSSION AND CONCLUSION

Genetic polymorphism and variation

The selected microsatellite loci in this study were at different chromosomes, they are highly polymorphic (Bolstein et al., 1980). At least 5 alleles per locus were detected in each population, and polymorphism information contents were very high at each locus. The high polymorphism at these microsatellite loci in sheep populations demonstrated the usefulness of microsatellite loci in genetic research.

The mean polymorphism information content (*PIC*) and mean expected heterozygosity (*He*) showed abundant genetic diversity among the 6 sheep populations. The mean observed heterozygosity (*Ho)* was lower than that detected by Arranz
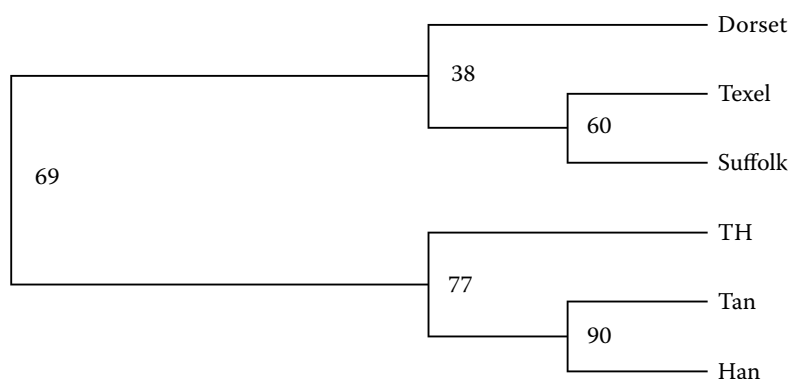

Figure 1. Dendrogram of 6 sheep populations based on DA distance using the UPGMA method

Table 6. Pairwise estimates of F_{st} (below diagonal) and *Nem* (above diagonal) of 6 sheep populations

Populations	Dorset	Texel	Black-Suffolk	Tan sheep	Han Sheep	TH hybrid
Dorset		4.3800	4.1890	3.2640	4.4920	2.6570
Texel	0.0540***		3.4910	3.7200	3.2430	2.4510
Black-Suffolk	0.0563***	0.0668***		2.8850	3.0370	2.2730
Tan sheep	0.0711***	0.0630***	0.0797***		6.2320	4.1320
Han Sheep	0.0527***	0.0716***	0.0761***	0.0386***		3.7500
TH hybrid	0.0860***	0.0926***	0.0991***	0.0571***	0.0625***	

all F_{st} were significant ***$P < 0.001$

Table 7. The DA distance (below diagonal) and DC distance (above diagonal) of 6 sheep populations

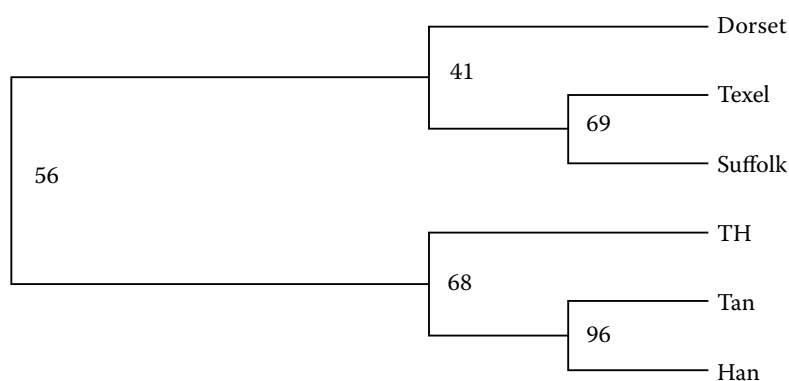| Populations	Dorset	Texel	Black-Suffolk	Tan sheep	Han Sheep	TH hybrid
Dorset		0.4042	0.3968	0.4276	0.3930	0.4484
Texel	0.2087		0.3787	0.4599	0.4242	0.4824
Black-Suffolk	0.1962	0.1950		0.4354	0.4139	0.4716
Tan sheep	0.2334	0.2665	0.2476		0.3331	0.3882
Han Sheep	0.2042	0.2396	0.2255	0.1395		0.4017
TH hybrid	0.2629	0.3098	0.2874	0.1886	0.2055	

Figure 2. Dendrogram of 6 sheep populations based on DC distance using the UPGMA method

et al. (1998) and Chu et al. (2002). The expected heterozygosities of 6 sheep populations were higher than the observed heterozygosities, showing that homozygous individuals were closer due to inbreeding among populations. 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 6 sheep populations were highly significant ($P < 0.001$) showing the highest genetic differentiation among the 6 sheep populations, similar results were reported by Buchanan et al. (1994) and Li et al. (2002). Coefficients of genetic differentiation between indigenous populations and exogenous populations were higher than among populations, and gene migration between indigenous populations and exogenous populations was lower than in indigenous populations, the reason is the geographical distribution. According to the F_{st} and *Nem* values the highest genetic variation existed between Black-Suffolk and TH hybrid sheep while the lowest genetic variation existed between Tan sheep and Han sheep.

Anticipation of crossing effect

Previous studies showed that Suffolk (Black-Suffolk) and Small-tailed Han sheep were an ideal cross group for heterosis and crossbreeding experiment (Yan et al., 2003; Zhang et al., 2006). In this study, based on the genetic distances we found that cross-breeding between Black-Suffolk males and TH hybrid sheep female was highly productive.

The effects of crossing on Small-tailed Han sheep with exogenous meat sheep confirmed our above-mentioned conclusion partly, when we used Black-Suffolk as male parent, the effects of crossing were better. While Texel had higher heterosis than

Black-Suffolk and that was in contrast to results reported by Zhang (2006), it is due to inbreeding in the Black-Suffolk sheep population and long-term selection for some productive traits.

In addition, there were reports on the hybridization of Small-tailed Han sheep crossed with the 3 introduced meat sheep (Yan et al., 2003; Zhang et al., 2006), however, relatively, the use of Tan sheep and TH hybrid sheep as crossing female parents was scarce, and there was no report on the comparison among Small-Tailed Han sheep, Tan sheep and TH hybrid sheep. Three-way cross sheep possessed not only Tan sheep's characteristics of standing rough breeding and adaptation to the local climate but also Han sheep's features of multiplets and perennial empathema, meanwhile, which improved defects of Tan sheep's unplump body and low production. In this study we concluded that TH hybrid sheep was the best female parent for cross-breeding with other breeds based on their production traits.

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