Genetic Differentiation among Sheep Populations from Near-sea Mainland in East Asia*

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ABSTRACT : Using the method of "random sampling in typical colonies of the central area of the habitat", 60 Small-tailed Han sheep were obtained in Jining city, Shangdong province. The variations of Small-tailed Han sheep at 12 structural loci encoding blood proteins were detected by several electrophoresis techniques and their gene frequencies were then estimated. The same data of four other sheep populations from Near-sea Mainland in East Asia were cited for the analysis of genetic differentiation. The average heterozygosities of five populations, namely Kharkhorin sheep, Ulaanbaatar sheep, Small-tailed Han sheep, Hu sheep and Cham Tribe sheep were 0.3447, 0.3285, 0.3157, 0.3884 and 0.2300, respectively. The coefficient of gene differentiation among four populations, Kharkhorin sheep, Ulaanbaatar sheep, Small-tailed Han sheep and Hu sheep, was 0.045557, and that between these four breeds and Cham Tribe sheep was 0.088005, indicating that the level of gene differentiation among the former four sheep populations of Mongolian group was comparatively lower than that between Cham Tribe sheep and other four sheep populations. The origin of Cham Tribe sheep deserve further research. The documentary research on the evolution of Small-tailed Han sheep and Hu sheep from Mongolian sheep was further verified by the biochemical experiments in the study. It was reasonably deduced that Hu sheep, Small Tailed Han sheep and Cham Tribe sheep were decreasingly influenced by the bloodline of Mongolian sheep. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 10 : 1360-1365)***

Key Words : Sheep, Structural Loci, Genetic Differentiation

INTRODUCTION

The Mongolian Plateau is generally recognized as one of the birthplaces of sheep in East Asia (Xie, 1985). Small-tailed Han sheep, Hu sheep and Cham Tribe sheep, which are raised by the Cham Tribe in Vietnam, are native sheep in easternmost Asian regions from north to south. Revealing the phylogenetic relationships and the levels of genetic differentiation among sheep breeds in the typical agricultural areas, and current Mongolian sheep contributes to tracing the spreading routes of domestic sheep in East Asia, and provides a basis for the evaluation, conservation and utilization of the genetic resources of these sheep.

In this study, we genetically examined Small-tailed Han sheep according to the same electrophoresis techniques reported by Tsunoda et al. (1988,1998,1999) and Yokohama et al. (1987) using 12 structural loci and estimated the gene frequencies at each structural locus. The same data of four other sheep populations, Kharkhorin sheep and Ulaanbaatar sheep in Mongolia, Hu sheep in China, and Cham Tribe sheep in Vietnam, were comparatively analyzed to explore the relationships of genetic differentiation among populations and to supplement an important link for the intensive research of phylogenetic relationships of sheep in the world.

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MATERIALS AND METHODS

Materials, sampling method and multiloci electrophoresis

Using the method "random sampling in typical colonies of the central area of the habitat", 60 Small-tailed Han sheep were sampled in Jining city, Shandong province. Blood samples of Small-tailed Han sheep were collected and treated by the methods described by Sun et al. (2002) and Geng et al. (2003) for electrophoresis. Starch gel electrophoresis, polyacrylamide gel electrophoresis, cellulose acetate-membrane electrophoresis, horizontal polyacrylamide gradient gel electrophoresis, and polyacrylamide gel isoelectric focusing were used to examine the variations of these 12 structural loci: alkaline phosphatase (Alp), arylesterase (Ary-Es), leucine aminopeptidase (Lap), hemoglobin-β (Hb-β), X-protein (X-p), malate dehydrogenase (ME), catalase (Cat), transferrin (Tf), albumin (Al), lysine (Ly), red cell esterase D (Es-D) and carbonic anhydrase (CA) following the methods reported by Tsunoda et al. (1988,1998,1999) and Yokohama

Table 1. A summary of 5 sheep populations used in this study

Population	Abbr.	Number	Sampling location	Data source
Kharkhorin sheep	Kh	99	Kharkhorin area of Central Mongolia	Tsunoda et al., 1999
Ulaanbaatar sheep	Ub	97	Ulaanbaatar area of Central Mongolia	Tsunoda et al., 1999
Cham Tribe sheep	Ct	34	The Ninh Son district in Ninh Thuan province of Vietnam	Tsunoda et al., 1998
Hu sheep	Hu	63	Huzhou city in Zhejiang province of China	Sun et al., 2002; Geng et al.,2003
Small-tailed Han sheep	St	60	Jining city in Shandong province of China	This study, 2002-2003

et al. (1987). The types of variations were determined according to the standards universally accepted and the standard samples supplied by Dr. Tsunoda whose papers were cited in the references.

Statistical analysis

The allele frequencies (P) were computed by the square root method for the dominant loci (Alp, Ary-Es, Lap, X-p, Al and Ly) and by the gene counting method for the co-dominant loci (Hb-β, ME, Cat, Tf, EsD and CA) (Tsunoda et al., 1988,1998,1999; Lee, 1995). The relative deviation (η) of the gene frequency was calculated by formula (1) when the reliability (β) reached 0.9545, which was calculated by formula (2). The reliability estimate ensures that the gene frequency will not deviate from the true value more than 0.5 times.

$$\eta=2[V(P)^{1/2}]\cdot P^{-1} \quad (1)$$

$$\beta=\int_0^{\lambda}\frac{2e^{-\frac{\lambda^2}{2}}}{\sqrt{2\pi}}d\lambda \quad (2)$$

In the above formula, P stands for gene frequency and V(P) its variance; V(P)=P·(1-P)/[2(n-1)]; n stands for sample size; λ is the standardized deviation of the estimate of the gene frequencies and was suitable for formula (2) when its standardized deviation was $\lambda=0.5\ P/[V(P)]^{0.5}$ (Chang, 1998).

The average heterozygosity (H) within a population:

$$H=1-\frac{1}{m}\sum_{j=1}^{m}\sum_{i=1}^{k}X_{ji}^2$$

Where m stands for the number of all loci, k the number of all alleles of a locus, X_{ji} the gene frequency of the i*th* allele of the j*th* locus (Nei, 1978).

The standard genetic distance (D) among populations (Nei, 1972):

$$D=-\ln I \quad I=J_{XY}/\sqrt{J_X J_Y}$$

$$J_X=\frac{1}{m}\sum_{j=1}^{m}\sum_{i=1}^{k}X_{ji}^2 \qquad J_Y=\frac{1}{m}\sum_{j=1}^{m}\sum_{i=1}^{k}Y_{ji}^2$$

$$J_{XY}=\frac{1}{m}\sum_{j=1}^{m}\sum_{i=1}^{k}X_{ji}Y_{ji}$$

In the above formulae, m stands for the number of all loci, k the number of all alleles of a locus, X_{ji} and Y_{ji} the frequency of the i*th* allele of the j*th* locus of population X and Y, respectively.

Gene diversity in the total populations (Ht), gene diversity within the populations (Hs) and the coefficient of gene differentiation among the populations (G_{ST}) were calculated using the DISPAN computer program (Ota, 1993).

Fuzzy cluster was carried out according to the gene frequencies at 12 structural loci in 5 populations. The following formula was used to describe the fuzzy consistency relationship matrix of the similarities between populations and to compose the fuzzy similarity relations. Thus a population cluster was achieved (Chang, 1995,1998).

$$\mu_{\tilde{R}}(X,Y)=\frac{1}{2}\ln(J_{X,Y}/\sqrt{J_X J_Y})+1$$

In the above formula, J_X, J_Y and J_{XY} stand respectively for the probability average over loci of the same alleles obtained at random from population X, population Y and both populations X, Y at the same time. $\mu_{\tilde{R}}(X,Y)$ stands for the membership function under the fuzzy consistency relation $\tilde{R}$ between populations X and Y.

Quotation of source materials

The information of all the populations was shown in Table 1. The gene frequencies of 12 structural loci in the cited populations were reported by other researchers (Tsunoda et al., 1998, 1999; Sun et al., 2002; Geng et al., 2003) with the same techniques.

RESULTS

Gene frequency at 12 structural loci in Small-tailed Han sheep

Of 12 structural loci analyzed, Alp, Ary-Es, Lap, Hb-β, X-p, ME, Cat and Tf were polymorphic, and the rest were monomorphic (Table 2). Apart from B+, Tf^B and Tf^F (0.6914, 0.7428 and 0.5199, respectively), the reliability

Table 2. Sampling estimates of gene frequencies at structural loci in Small-tailed Han sheep

Locus	Allele	P	V (P)	η	β
Alp	B^{+}	0.0351	2.9709×10^{-4}	0.9821	0.6914
	B^{-}	0.9649	2.9709×10^{-4}	0.0357	1
Ary-Es	Es^{+}	0.4748	2.1874×10^{-3}	0.1970	1
	Es^{-}	0.5252	2.1874×10^{-3}	0.1781	1
Lap	Lap^{A}	0.6784	1.9138×10^{-3}	0.1290	1
	Lap^{B}	0.3216	1.9138×10^{-3}	0.2721	0.9998
Hb-β	Hb-β^{A}	0.2917	1.7509×10^{-3}	0.2869	0.9995
	Hb-β^{B}	0.3583	1.9485×10^{-3}	0.2464	1
	Hb-β^{X}	0.3500	1.9280×10^{-3}	0.2509	0.9999
X-p	X	0.2254	1.4796×10^{-3}	0.3413	0.9966
	x	0.7746	1.4796×10^{-3}	0.0993	1
ME	ME^{F}	0.5500	2.0975×10^{-3}	0.1665	1
	ME^{S}	0.4500	2.0975×10^{-3}	0.2035	1
Cat	Cat^{B}	0.5833	2.0598×10^{-3}	0.1556	1
	Cat^{C}	0.4167	2.0598×10^{-3}	0.2178	1
Tf	Tf^{A}	0.1293	9.8756×10^{-4}	0.4861	0.9604
	Tf^{B}	0.0431	3.6178×10^{-4}	0.8826	0.7428
	Tf^{C}	0.2500	1.6447×10^{-3}	0.3244	0.9980
	Tf^{D}	0.2414	1.6064×10^{-3}	0.3321	0.9974
	Tf^{E}	0.3190	1.9056×10^{-3}	0.2737	0.9998
	Tf^{F}	0.0172	1.4828×10^{-4}	1.4159	0.5199
Al	Al^{C}	1	0	0	1
Ly	Ly^{A}	1	0	0	1
EsD	EsD^{S}	1	0	0	1
CA	CA^{S}	1	0	0	1

Table 3. Gene diversity and the coefficients of gene differentiation among sheep populations

Locus	Ht		Hs		G_{ST}	
	4 populations	5 populations	4 populations	5 populations	4 populations	5 populations
Alp	0.333276	0.280904	0.300906	0.240725	0.097125	0.143036
Ary-Es	0.471428	0.423466	0.462171	0.369736	0.019636	0.126880
Lap	0.496539	0.496516	0.459195	0.466641	0.075207	0.060169
Hb-β	0.623460	0.650804	0.602044	0.577400	0.034351	0.112790
X-p	0.241951	0.278382	0.221952	0.256841	0.082655	0.077378
ME	0.479216	0.460209	0.465419	0.437737	0.028791	0.048829
Cat	0.499538	0.490952	0.484972	0.424902	0.029159	0.134535
Tf	0.783210	0.760912	0.767693	0.724619	0.019812	0.047697
Al	0.005137	0.004112	0.005097	0.004078	0.007745	0.008257
Ly	0.324679	0.316915	0.295470	0.293211	0.089964	0.074796
EsD	0.002497	0.001998	0.002487	0.001990	0.003755	0.004004
CA	0.038912	0.031256	0.036548	0.029238	0.060756	0.064545
All loci	0.358320	0.349702	0.341996	0.318926	0.045557	0.088005

* 4 populations: Kharkhorin sheep, Ulaanbaatar sheep, Hu sheep, Small-tailed Han sheep.
5 populations: Kharkhorin sheep, Ulaanbaatar sheep, Hu sheep, Small-tailed Han sheep and Cham Tribe sheep.

estimates (β) of the gene frequencies not deviating more than 0.5 times were over 96%. Therefore, these data might serve as a basis for the genetic analyses.

Genetic variation within a population

The average heterozygosities of Kharkhorin sheep, Ulaanbaatar sheep, Small-tailed Han sheep, Hu sheep and Cham Tribe sheep were 0.3447, 0.3285, 0.3157, 0.3884 and 0.2300, respectively. The estimates indicated that the degree of genetic variation within Hu sheep was highest and that within Cham Tribe sheep lowest.

Gene differentiation

The coefficients of gene differentiation (G_{ST}) among all the five sheep populations ranged from 0.004004 to 0.143036 with a mean value of 0.088005 (Table 3). The G_{ST} among the four sheep populations except Cham Tribe sheep population varied within a range of 0.003755-0.097125

Table 4. Standard genetic distances (below diagonal) and fuzzy similarity relations (above diagonal) among five sheep populations

	Kh	Ub	St	Hu	Ct
Kh	-	0.9946	0.9865	0.9922	0.9642
Ub	0.0109	-	0.9865	0.9922	0.9642
St	0.0271	0.0515	-	0.9865	0.9642
Hu	0.0157	0.0262	0.0456	-	0.9642
Ct	0.0717	0.0907	0.0725	0.1086	-

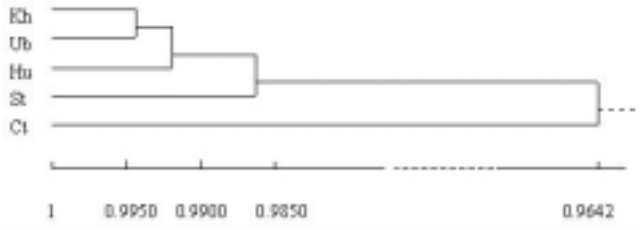

Figure 1. Dendrogram of fuzzy clustering showing the relationships among five sheep populations.

with a mean value of 0.045557. The gene diversity in the total populations (Ht) was very close to the gene diversity within the populations (Hs). The values of these two parameters were relatively high over the 7 loci both in the five populations and the four populations. In case of Al, EsD, and CA loci, the values of these two parameters were very low. This could be easily interpreted as the gene at each of these three loci was fixed or nearly fixed in 5 populations.

Genetic distance and fuzzy cluster

The standard genetic distances and fuzzy similarity relation matrix were presented in Table 4. The smallest standard genetic distance (0.0109) was observed between Kharkhorin sheep and Ulaanbaatar sheep and the largest (0.1086) between Hu sheep and Cham Tribe sheep. Based on fuzzy similarity relation matrix, the relationship tree among 5 sheep populations was constructed (Figure 1). Kharkhorin sheep and Ulaanbaatar sheep were grouped firstly. Hu sheep clustered secondly. Small-tailed Han sheep clustered on the value level of 0.9865. Cham Tribe sheep last clustered together with above four sheep populations. All these sheep populations shared the same origin in earlier generations.

DISCUSSION

The average heterozygosity is a good measurement of genetic variation within a population. The values of average heterozygosity allowed us to establish the degree of genetic variation within the population. The average heterozygosities, which were computed according to 35 allele frequencies at 12 structural loci, indicated that the degree of genetic variation of Hu sheep was highest, Kharkhorin sheep next and Cham Tribe sheep lowest, which were closely related to the effective population size, selective level and method. This finding conformed to reality and resembled the other reported results (Tsunoda et al., 1998,1999; Sun, 2002).

The mean coefficient of genetic differentiation (G_{ST}) among all the studied populations was 0.088005, indicating that 8.8005% of the total variability was due to the differences among 5 sheep populations, and the remainder of the total variability due to the differences within 5 sheep populations. The G_{ST} among all the populations was nearly two times the G_{ST} among the four sheep populations, Kharkhorin sheep, Ulaanbaatar sheep, Hu sheep and Small-tailed Han sheep, suggesting that the high value obtained was mainly affected by the inclusion of Cham Tribe sheep. That was, there was a considerable genetic differentiation between Cham Tribe sheep and other four sheep. The values of gene diversity in the total populations (Ht) were parallel to the values of gene diversity within the populations (Hs). The mean values of these two parameters were similar and relatively high both in the five populations and the four populations, indicating that the populations presented genetic variations and the diversity was high both among the sheep populations and within the populations in the mass.

It was discovered that Cham Tribe sheep were genetically closer to Kharkhorin sheep and Small-tailed Han sheep (D=0.0717, 0.0725, respectively) than to the two other sheep populations (D=0.0907, 0.1086, respectively). This result reminded us of the developmental history of the Cham Tribe in Vietnam. Vietnam is the neighbor country of China. The Cham people in Vietnam have a long history of close cultural exchange with the Chinese people. It almost has a population of 125,000 at present, 50,000 of whom reside in Vietnam (Editorial committee of "Word Ocean", 1987). The northeast part of the Cham's residence was successively governed by Xiang Prefecture of Qin Dynasty, Zhao Clan South Yue Court and Ri Nan Prefecture of Han Dynasty from 3 B.C. to A.D. 2 (Ban, A.D. 89; Si, A.D. 306; Tan, 1982). During that time they directly introduced cattle, sheep and ironware from the Central Plains in many separate events (Ban, A.D. 89). This literature showed that the partial founder population in the Central Plains, i.e. Mongolian sheep forming current Small-tailed Han sheep, possibly made a contribution to the bloodline of Cham Tribe sheep. However, Tsunoda et al. (1999) divided the Asian sheep populations into three groups: Mongolian

group, Indo-Pakistan group and Tibetan group, and proposed that Cham Tribe sheep belonged to the Indo-Pakistan group. No details about the origin of Cham Tribe sheep were available. Tsunoda et al. (1998) suggested that Cham Tribe sheep might have been imported with the goats from Pakistan. From our analyses based on this study and related literature, we suggest that the lineage of Cham Tribe sheep was possibly affected by the lineage of several breeds to varying degrees and great differences exist between Cham Tribe sheep and sheep of the Mongolian group. In future, additional polymorphic structural loci and other kinds of genetic markers should be used to confirm the origin of Cham Tribe sheep.

That the degree of genetic similarity between Mongolian sheep and Hu sheep was higher than that between the former and Small-tailed Han sheep demonstrated that Hu sheep could not have derived from Small-tailed Han sheep, and these two sheep populations in Chinese agricultural areas commonly shared the bloodline of Mongolian sheep. We could find out that the history of Hu sheep was longer than that of Small-tailed Han sheep according to the documentary research (Xie, 1985). This study confirmed this finding.

The results of gene differentiation, genetic distance and fuzzy cluster demonstrated that Hu sheep derived from Mongolian sheep. This demonstration was identical with the conclusions of Sun et al. (2002) and Geng et al. (2003). Our results also demonstrated Small-tailed Han sheep evolved from Mongolian sheep, and this demonstration was consistent with its known history of breeding (Editorial section of "Sheep and Goat in China", 1989) and the viewpoint that "sheep breeds across China, except in the Qinghai-Tibet Plateau and its neighboring areas, originate from Mongolian sheep" (Xie, 1985).

The analysis of the genetic relationship was performed by means of the genetic distance method of Nei (1972) and fuzzy clustering (Chang, 1995). The standard genetic distances of the pairs Kh-Hu, Kh-St, and Kh-Ct gradually increased, showing their relationships gradually became more distant, as did the pairs Ub-Hu, Ub-St and Ub-Ct (Table 4). Along with the above analyses of gene differentiation and fuzzy clustering, we thus deduced that the relationships among the two Mongolian sheep populations and either of Hu sheep, Small-tailed Han sheep or Cham Tribe sheep became gradually more distant, that is, they were decreasingly influenced by the bloodline of Mongolian sheep.

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