# Studies on the Genetic Relationships of Sheep Populations from East and South of Central Asia

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**ABSTRACT**: Hu sheep was sampled randomly from Huzhou city, Zhejiang province, China. Of the 11 genetic markers from the blood examined by starch-gel and cellulose acetate electrophoresis, polymorphisms in Hu sheep were found for 10 loci, i.e. post-albumin (Po), transferring (Tf), alkaline phosphatase (Alp), leucine aminopeptidase (Lap), arylesterase (Ary-Es), hemoglobin-β (Hb-β). X-protein(X-p), carbonic anhydrase (CA), catalase (Cat) and lysine (Ly). The same data except for Po locus were collected from another 14 sheep breeds from China and other countries, in order to ascertain their genetic relationships with one another and with the Hu sheep. The sheep populations from the east and south of Central Asia can be classified into three genetic groups: "Mongolian sheep", "South Asian sheep" and "European sheep". The Hu sheep belong to the "Mongolian sheep" group. (Asian-Aust. J. Anim. Sci. 2002. Vol. 15, No. 10: 1398-1402)

Key Words: Sheep, East and South of Central Asia, Genetic Relationships

## INTRODUCTION

The phylogenetic classification of domemestic animal populations is one of the bases for determining the scope of a breed, estimating the possibility of the potential distribution of special gene resources in a specific population, judging the possibility of identical genes (or gene groups) controlling similar body shapes in different populations, analysing the population characteristic of gene coadaptation, predicting heterosis, and devising breeding strategy (Chang et al., 1995). Hu sheep is the indigenous breed of Zhejiang province, China. It is distributed along the Taihu valley, and it is famous for its high fertility and beautiful lambskin. It is regarded as a "secret breed" in China (Zheng, 1980).

With regards to sheep, there are rich sheep resources in China. There were a few reports about some sheep populations in Asia (Tsunoda et al., 1988, 1990, 1992, 1995, 1998, 1999), but apart from Yunnan sheep, no information is available about Chinese sheep breeds. The genetic relationships of sheep breeds from the east and south of Central Asia is not completely understood.

This paper attempts to describe the genetic constitution and origin of Hu sheep-a "secret breed" in China and the genetic relationships among the sheep breeds of the east and south of Central Asia, so as to provide a basis for the development of the sheep husbandry in this area.

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## MATERIALS AND METHODS

### Materials, sampling method

The Hu sheep studied were from Lianshi Town of Huzhou city in Zhejiang province of China. 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 have traceable genetic relationship. Some external morphological characteristics of Hu sheep were also investigated.

# Collecting and treatment of blood samples

8 ml of blood was taken from the cervical vein of each animal and put into a centrifuge tube using heparin as an anticoagulant. The blood serum is separated by centrifugation (3,000 rpm, 5-10 min) and transferred into a capped test tube. After that the same volume of 0.85% NaCl as the remaining blood cells was added and the tube was centrifuged for two or three times (3.000 rpm, 5-10 min). After the last centrifugation, the upper layer of serum was discarded and the same volume (or 1.5 times) distilled water was added into the remaining blood cells. After numbering, the tube was kept in a refrigerator (below -20°C).

# Multiloci electrophoresis

Starch gel electrophoresis was used to determine the variations of 10 loci:albumin (Al), post-albumin (Po), transferrin (Tf), alkaline phosphatase (Alp), leucine aminopeptidase (Lap), arylesterase (Ary-Es), hemoglobin- $\beta$  (Hb- $\beta$ ), X-protein (X-p), carbonic anhydrase (CA) and catalase (Cat): lysine (Ly) was tested for by cellulose acetate electrophoresis. in which 6 loci: Al. Po, Tf, Alp. Lap and Ary-Es were typed from serum: 5 loci: Hb- $\beta$ . X-p. CA, Cat and Ly were typed from red cell hemolysate. The

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methodologies used and the types of the variations present were determined according to the methods universally accepted in the countries neighboring China (Tsunoda et al., 1988, 1992).

#### Statistical analysis

Gene frequencies for the polymorphic traits were computed by the gene counting method for Al, Po. Tf. Hb- $\beta$ . CA and Cat loci and by the square root method for Alp. Lap. Ary-Es, X-p and Ly loci.

The following two formulae were used to calculate the reliability that ensures the estimate not to deviate from the true value by more than 0.5 times ( $\beta$ ) and the relative deviation when the reliability reaches 0.9545( $\eta$ ).

$$\beta = \int_0^{\lambda} \frac{2e^{-\lambda^2}}{\sqrt{2\pi}} d\lambda \qquad \eta = 2 \left[ V(p)^{\frac{1}{2}} \right] P^{-1}$$

In the above formula, P and V(p) stand for gene frequency and gene variance, respectively:  $\lambda$  is the standard deviation of the estimate and is suitable for the first formula when its standard deviation is

$$\lambda = 0.5 \div [V(p)]^{\frac{1}{2}}$$

## Genetic relationship clustering

The population information and the gene frequencies for 10 loci in 15 populations (breeds) are shown in

Tables 1 and 3.

These data were reported by other researchers who used the same techniques as we did for Hu sheep. Only 10 loci but not Po were included because these were the loci examined in all the other populations. Based on these data the genetic distance was computed by Ward's method and clustering was done by the Hierarchy Clustering Method (Chang, 1995).

## **RESULTS**

The accuracy and reliability of the estimates of gene frequencies of Hu sheep are shown in Table 2.

Of the 11 loci tested, 10 showed polymorphisms. It can be seen from table 2 that the reliability of the estimates of gene frequencies for 25 alleles reached 0.95 except that for  $Po^F$ ,  $Tf^A$ ,  $Tf^F$ , Hb- $\beta^A$  and  $CA^F$ , and their reliabilities were 0.5222, 0.7478, 0.5222, 0.6212 and 0.899, respectively.

# Genetic relationships

Figure 1 shows the genetic relationships among the 15 sheep populations.

From Figure 1 we can see that the 15 sheep populations from the east and south of Central Asia are clearly classified into three groups: one group consists of the Mongolian sheep (Ub, Kh), Yunnan sheep and Hu sheep, which can be described as "Mongolian sheep"; the other group consists of the Nepalese native sheep (Bar, Kag), Bangladeshi native sheep (Ban) and Vietnamese sheep (Viet), which can be described as "South Asian sheep"; another group consists of five European sheep (breeds in Japan) and a Nepalese sheep

Table 1. Information on the 15 populations studied

Number	Population	Native area of location	Abbr	Reference		
1	Yunnan sheep	Lufeng and Lunan in Yunnan province of China	Yunnan	K. Tsunoda et al., 1995		
2	Hu sheep	Huzhou city in Zhejiang province of China	Hu sheep	this study, 2001		
3	Baruwal sheep	Solu area. Kathmandu city and Ghara area of Nepal	Bar	K. Tsunoda et al., 1992, 1995		
4	Kagi sheep	neep Kathmandu city and Chitlang farm of Nepal		K. Tsunoda et al., 1992, 1995		
5	Lampuchhre sheep Narayangath, Somnath-parasi and Lumbini area of Nepal		Lam	K. Tsunoda et al., 1992, 1995		
6	Bhyanglung sheep	Kathmandu city and Ghara area of Nepal	Bhy	K. Tsunoda et al., 1992, 1995		
7	Bangladeshi sheep	eshi sheep  Jessore-Khulna、 Mymensingh and Noakhali area of Bangladesh		K. Tsunoda et al., 1988		
8	Kharkhorin sheep	Kharkhorin area of Central Mongolia	Kh	K. Tsunoda et al., 1999		
9	Ulaanbaatar sheep	Ulaanbaatar area of Central Mongolia	Ub	K. Tsunoda et al., 1999		
10	Vietnamese sheep	Vietnam	Viet	K. Tsunoda et al., 1998		
11	Suffolk sheep	European sheep breed in Japan	Suf	K. Tsunoda et al., 1990		
12	Cheviot sheep	European sheep breed in Japan	Che	K. Tsunoda et al., 1990		
13	Corriedale sheep	European sheep breed in Japan	Cor	K. Tsunoda et al., 1990		
14	Finnish Landrace sheep	European sheep breed in Japan	Fin	K. Tsunoda et al., 1990		
15	Border Leicester sheep	European sheep breed in Japan	Bor	K. Tsunoda et al., 1990		

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Table 2. Estimates of gene frequencies and their reliabilities and precision

Locus	Phenotype	N	Allele	P	Vp	λ	β	η
Al	С	60	$\mathrm{AL}^{\mathtt{C}}$	1	0	0	1	0
$P_{\hat{0}}$	FS	58	$P_0^F$	0.0167	1.368×10 <sup>-4</sup>	0.7137	0.5222	1.4012
	SS	2	$P_{\scriptscriptstyle 0}^{\scriptscriptstyle S}$	0.9833	1.368×10 <sup>-4</sup>	42.021	1	0.0238
Tf	AB	5	$Tf^{i}$	0.0417	$3.330 \times 10^{-4}$	1.1425	0.7458	0.875
	BC	7	$Tf^{\mathcal{B}}$	0.15	$1.063 \times 10^{-3}$	2.3006	0.9786	0.434
	CD	25	$T\!f^C$	0.30	1.75×10 <sup>-3</sup>	3.5858	1	0.279
	BD	3	$\mathit{Tf}^{\mathcal{D}}$	0.3417	$1.875 \times 10^{-3}$	3.9466	1	0.253
	DE	11	Tf <sup>E</sup>	0.15	$1.063 \times 10^{-3}$	2.3006	0.9786	0.434
	EF	1	$Tf^{F}$	0.0166	$1.368 \times 10^{-4}$	0.7137	0.5222	1.4012
	CF	l						
	CE	3						
	BE	3						
	DD	l						
Alp	B(+)	38	$\mathbf{B}^{-}$	0.3945	$1.990 \times 10^{-3}$	4.423	1	0.2261
	B(-)	22	B-	0.6055	$1.990 \times 10^{-3}$	6.788	1	0.1473
Es	Es(+)	33	Es <sup>-</sup>	0.3292	$1.840 \times 10^{-3}$	3.837	1	0.2606
	Es(-)	27	Es.	0.6708	$1.840 \times 10^{-3}$	7.818	I	0.1279
Lap	A	41	Lap <sup>A</sup>	0.4373	$2.05 \times 10^{-3}$	4.827	I	0.2072
	В	19	Lap <sup>B</sup>	0.5627	$2.05 \times 10^{-3}$	6.211	l	0.1610
Нь-β	AB	3	Α	0.0238	$1.84 \times 10^{-4}$	0.8763	0.6212	1.1412
	BB	60	В	0.9762	$1.84 \times 10^{-4}$	35.943	1	0.0278
X-p	$X(\pm)$	28	X	0.2546	$1.506 \times 10^{-3}$	3.289	1	0.3049
	X(-)	35	X	0.7454	$1.506 \times 10^{-3}$	9.603	1	0.1041
CA	FS	10	$CA^F$	0.0794	5.801×10 <sup>-4</sup>	1.648	0.899	0.6067
	SS	53	CAS	0.9206	5.801×10 <sup>-4</sup>	19.111	1	0.0523
Cat	BB	16	Cat <sup>B</sup>	0.4444	$1.960 \times 10^{-3}$	5.016	1	0.1994
	BC	24	Cat <sup>C</sup>	0.5556	$1.960 \times 10^{-3}$	6.27	1	0.1595
	cc	23						
Ly	Ly(+)	58	$\mathrm{Ly}^{\mathrm{A}}$	0.7183	$1.606 \times 10^{-3}$	8.962	1	0.1116
•	Ly(-)	5	Lya	0.2817	$1.606 \times 10^{-3}$	3.515	1	0.2845

breed (Bhy), which can be described as "European sheep". We also find that the Hu sheep clusters closely with the Mongolian sheep.

# **DISCUSSION AND CONCLUSIONS**

# Sampling method

Chang et al. (1988) have discussed the method of genetic resources inspection. The accuracy depends on the sample and loci tested. In this study we adopted the "Random sampling in typical colonies of central area" method. The higher reliability and precision obtained for gene frequency estimates for 11 loci of Hu sheep proved that this method is effective.

The breeds in the east and south of Central Asia can be divided into three groups "Mongolian sheep", "South Asian sheep" and "European sheep"

Tsunoda et al. (1999) divided the Asian sheep into three groups: Mongolian group. Indo-Pakistani group and Tibetan group. Our study is only limited to the 15 populations breeding in east and south of Central Asia. Whether the two results are identical or not needs further studies.

The dendrogram of genetic relationships indicates that Hu sheep clusters closely with Mongolian sheep and this can also be supported by the known history of sheep breeding

Mongolian sheep were brought into the Taihu valley when the Nan-Song Dynasty left their former capital-Bianjing (i.e., Kaifeng) to Linan (i.e., Hangzhou) (Editorial Committee of the "Breeds of Domestic Animal and Poultry in China" et al., 1989). Thus this study confirms the origin of the Hu sheep.

Danulation	Al Tr												Alp					
Population	A	В	С	Χ	А	В	С	D	Е	F	G	Н	I	J	K	$\mathrm{B}^{\scriptscriptstyle{\dagger}}$		
Yuman	0	0	I	0	0.0714	0.2143	0	0.0286	0.6857	0	0	0	0	0	0	0.2829		
Hu sheep	0	0	1	0	0.0417	0.15	0.3	0.3417	0.15	0.0166	0	0	0	0	0	0.3945		
Bar	0	0	1	0	0.0116	0.0233	0	0	0.9651	0	0	0	0	0	0	0		
Kag	0	0	1	0	0	0	0.1585	0.0122	0.7927	0.0122	0.0122	0.0122	0	0	0	0.05		
Lam	0	0	1	0	0	0	0.2955	0.1136	0.5909	0	0	0	0	0	0	0		
Bhy	0	0	1	0	0.0732	0.061	0.3658	0.1219	0.3537	0.0122	0.0122	0	0	0	0	0.5583		
Ban	0.197	0	0.803	0	0.039	0.013	0.178	0	0.757	0.013	0	0	0	0	0	0.02		
Kh	0	0	1	0	0.0859	0.1263	0.3131	0.1768	0.197	0.0404	0	0.005	0.0101	0.0101	0.0353	0.215		
UЪ	0	0	1	0	0.1237	0.0567	0.3608	0.134	0.268	0.0258	0.0103	0.0052	0	0	0.0155	0.2005		
Viet	0	0	1	0	0	0	0.5441	0	0.3824	0.0735	0	0	0	0	0	0		
Suf	0	0	1	0	0.122	0	0.265	0.187	0.135	0.291	0	0	0	0	0	0.334		
Che	0	0	1	0	0	0.052	0.276	0.586	0.069	0.017	0	0	0	0	0	0.475		
Cor	0	0	1	0	0.175	0.042	0.15	0.425	0.142	0.066	0	0	0	0	0	0.051		
Fin	0	0	1	0	0.038	0	0.482	0.404	0.038	0.038	0	0	0	0	0	0.097		
Bor	0	0	1	0	0.260	0.020	0.420	0.22	0.080	0	0	0	0	0	0	0.041		
D 1.0	Alp Es		La	ар Нь <sup>-β</sup>			x-p C			CA	CA Ca		ıt Ly		Ly			
Population	В.	Es*	Es.	A	В	A	В	С	X	x F	r s	A	В	С	А	a		
Yunnan	0.7171	0.1894	0.8106	0.4145	0.5855 0	.1571 0	.5857 0.	2572 0.0	0438 0.9	562 (	) 1	0	0.75	71 0.242	9 0.707.	2 0.2928		
Hu sheep	0.6055	0.3292	0.6708	0.4373	0.5627 0	.0238 0.	.9762	0 - 0.2	2546 0.7	454 0.0	794 0.92	906 0	0.44	44 0.555	6 0.718	3 0.2817		
Bar	1	0.318	0.682	0.9535	0.0465 0	.1395 0.	.0930 0.	7675 0.0	0.9 0.11	883 (	) 1	0	0.74	42 0.255	8 1	0		
Kag	0.95	0.2349	0.7651	0.8438	0. <b>1562</b> 0	.1707 0.	7195 0.	1098 0.2	2037-0.7	963 (	) 1	0	0.96	34 0.03 <del>6</del>	6 0.779	1 0.2209		
Lam	l	0.0707	0.9293	0.7868	0.2132 0	.4546 0.	.5227 O.	0227 0.4	1359 0.5	641 0.04	454 0.9 <mark>5</mark>	46 0	0.93	18 0.068	2 1	0		
Bhy	0.4417	0.5583	0.4417	0.4156	0.5844 0	.7805 0.	.0122 0.	2073	0	1 0	) 1	0	0.97	56 0.024	4 0.779	1 0.2209		
Ban	0.98	0.089	0.911	0.719	0.281 0	.1908 0.	.8092	0 0.	372 0.0	6 <b>28</b> 0.0	07 0.9	93 0	0.41	4 0.586	6 0.771	0.229		
Kh	0.785	0.414	0.586	0.414	0.586_0	.1515.0	.5152 0.	3333-0.0	0.518 0.9	482 (	) 1	0	0.54	55 0.454	5 0.770	6 0.2294		
UЪ		0.3039								686 (		0		6 0.63				

0

0

0

0

0

0

0.69

0.608

0.4444

0.900

0.2724 0.7276

0.71

0.965

0.796

-0.882

0.29

0.035

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1



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0.8971 0.1029 0.8285 0.1715

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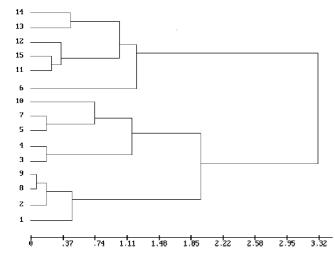
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1

0.965

0.983

0.43

0.917

0.646

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0.666 - 0.095

0.525

0.949

0.903

0.959

0.035

0.017

0.57

0.083

0.4577 0.5423 0.6029 0.3971

0.31

0.392

0.556

0.904 0.502 0.498 0.074 0.926

0.354

0.553 0.447 0.100

Viet

Suf

Che

Cor

Fin

Bor

Figure 1. The dendrogram of genetic relationships of 15 sheep population from the east and south of Central Asia.

1: Yunnan, 2: Hu sheep, 3: Bar, 4: Kag, 5: Lam, 6: Bhy, 7: Ban, 8: Kh, 9: Ub, 10: Viet, 11: Suf, 12: Che, 13: Cor, 14: Fin, 15: Bor.

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