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Genetic Diversity and Relationship of Yunnan Native Cattle Breeds and Introduced Beef Cattle Breeds

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In this study, random amplified polymorphic DNA (RAPD) analysis was used to estimate genetic diversity and relationship in 134 samples belonging to two native cattle breeds from the Yunnan province of China (DeHong cattle and DiQing cattle) and four introduced beef cattle breeds (Brahman, Simmental, MurryGrey, and ShortHorn). Ten primers were used, and a total of 84 bands were scored, of which 63 bands (75.0%) were polymorphic. The genetic distance matrix was obtained by proportions of shared fragment. The results indicate that the Yunnnan DeHong cattle breed is closely related to the Brahman (Bos indicus), and the Yunnan DiQing cattle breed is closely related to the Simmental, ShortHorn, and MurryGrey (Bos taurus) breeds. Our results imply that Bos indicus *and* Bos taurus *were the two main origins of Yunnan native cattle. The results also provide the basic genetic materials for conservation of cattle resources and crossbreeding of beef cattle breeds in South China.*

KEY WORDS: native cattle breeds; beef cattle breeds; RAPD; genetic diversity; relationship.

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INTRODUCTION

Understanding the origin and genetic variation of cattle breeds (or populations) and the relationship among breeds may help in the conservation of cattle resources and the development of cattle breeding programs.

The cattle gene pool in China is rich and extensive, with 28 cattle breeds and many other local cattle populations (Qiu *et al*., 1988). Chinese cattle have been divided into three groups based on morphological characteristics, Y-chromosome structure and blood protein polymorphisms: the Mongolian group in North China, the Huanghuai group in the middle and lower Yellow river (Huanghe) and the Huaihe river basin, and the Changzhu group in the area of the Changjiang (Yangtze) and Zhujiang rivers of South China. It is suggested that the Mongolian group originated from *Bos namadicus*, the Asian variety of *Bos primigenius*, while the Huanghuai group is a hybrid of Mongolian cattle and zebu (Chen *et al.*, 1990; Namikkawa *et al*., 1995). The origin of the Changzhu group is much more complicated, with speculation that it might originate from the *Bos genus* varieties, such as zebu, *Bos banteng*, and even *Bos gaurus* (Chen *et al*., 1990).

To reveal the origin and genetic variation in cattle of the Changzhu group of South China, we studied the mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) of 11 native and 1 cultivated cattle breed from Yunnan, Guizhou, Hainan, and Guangdong provinces of South China, which belong to the Changzhu group. The study showed that there is abundant mtDNA genetic diversity in the Yunnan native cattle population, especially in the Dehong and Diqing cattle breeds (Yu *et al.*, 1999). mtDNA RFLP only discloses genetic variation of mitochondrial DNA, however, and it is necessary to obtain more evidence from nuclear DNA variation to reveal more deeply the genetic diversity and origin of Yunnan native cattle populations.

Random amplified polymorphic DNA (RAPD) is often used to detect nuclear DNA polymorphisms (Williams *et al.*, 1990). It has also been applied in genetic research of various animal and plant species successfully, as well as in the characterization, genetic diversity, and origination of bovine populations and the relationship between them (Bardin *et al*., 1992; Gwakisa *et al*., 1994; Kemp and Teale, 1994).

In the present study, using RAPD we examine the nuclear DNA polymorphisms of two Yunnan native cattle breeds (DeHong and DiQing cattle breeds) and four introduced beef cattle breeds (Brahman, Simmental, ShortHorn, and Murry-Grey, which all have clear genetic backgrounds—Brahman is *Bos indicus* and the others are *Bos taurus*). The purpose of the study is to gain more knowledge about the origin and genetic variation of Yunnan native cattle populations, and to provide the basic genetic materials for the conservation of cattle resources and the breeding programs of beef cattle in South China.

Table I. Sources of DNA Samples Used in the Present Study

Breed	Sampling locality	No. of samples
Yunnan native cattle		
DeHong cattle	DeHong prefecture of Yunnan province	32
DiQing cattle	DiQing prefecture of Yunnan province	25
Introduced beef cattle		
Brahman	Yunnan Beef Cattle and Pasture Research Center (introduced from Australia)	23
MurryGrey	Yunnan Beef Cattle and Pasture Research Center (progenies of introduced beef cattle from Australia)	9
Simmental	Yunnan Breeding Stock Farm (introduced from Canada and Australia)	23
ShortHorn	Yunnan Breeding Sheep Farm (introduced from America)	22
Outgroup species		
Zhongdian Yak	Zhongdian county, Diqing prefecture, Yunnan province	2
Mithan	Gongshan county, Lincang prefecture, Yunnan province	2
Total		138

MATERIALS AND METHODS

DNA Samples

Blood samples of 138 individuals were collected from Yunnan province (Table I). Among them, 77 were from breeds that have been introduced from foreign countries. Mithan (*Bos frontalis*) and ZhongDian Yak (*Bos grunniens*) from Yunnan province were selected as outgroups.

RAPD–PCR Reaction

Each 10-μl RAPD reaction mix comprised: 10 mmol/L Tris–HCl, pH = 8.3; 2.0 mmol/L $MgCl_2$; 2.0 mmol/L BSA; 2.5 mmol/L dNTP; 0.2 μmol/L primer (10 bp); 25 ng template DNA; 1 U Taq polymerase. The reaction mixtures were overlayed with a drop of paraffin oil before covering.

Amplifications were performed in the same programmable thermal controller with the following temperature cycling: one cycle at 94°C for 3 min; 94°C denaturing 1 min, 36°C annealing 1 min, 72°C extension 1.5 min for 40 cycles; and one cycle at 72°C for 5 min. Amplification products were loaded and run on a 1.5% agarose gel stained with ethidium bromide for 1.5–2 h at 5 V/cm.

Data Analysis

Only distinct, repeatable prominent amplified bands were scored. Nei's formulas (Nei, 1979) were used to calculate the proportion of shared fragments (F) and the genetic distance (D) between breeds. The genetic diversity index (H_0) of breeds was calculated by the formula of Shannon Index (Wachria *et al*., 1995). The UPGMA and NJ trees of breeds were constructed using the Mega2.0 software package (Kumar *et al.*, 2001).

RESULTS AND ANALYSIS

Genetic Diversity of the Six Cattle Breeds

Ten random primers (10 bp) detected clear polymorphism in this study, 3 of them (OPA-19, OPF-05, OPH-11) were selected by us from 30 primers, and the others were used because they had been used to detect polymorphism in *Bos* in previous studies (Gwakisa *et al*., 1994; He, 1999). Each of the 10 primers produced a variable number of RAPD fragments in six breeds, as shown in Table II.

All the primers used in the study gave fingerprints ranging from 0.25 to 2.00 kb, which corresponded to the results on breeds in many other studies (e.g. Gwakisa *et al*., 1994; He, 1999). The number of amplified bands ranged from 6 to 12. A total of 84 bands were scored, of which 63 bands were polymorphic (Table II). The average frequency of polymorphism was 75.0%. However, the frequencies were not identical among all different breeds. The highest frequency was 73.0%, which was detected in DeHong cattle. The frequencies detected in Brahman, Simmental, and DiQing cattle were 68.3, 68.3, and 65.1%, respectively. The lowest frequency (41.2%) was detected in the MurryGrey breed.

The genetic diversity indexes (H_0) of the two Yunnan native cattle breeds and the four introduced beef cattle breeds are shown in Table III. This shows that except for the MurryGrey breed ($H_0 = 0.7053$), the other five cattle breeds have a higher H_0 (ranging from 0.9530 to 0.8175). Our previous study on mtDNA RFLP showed that the average sequence distance value (π) of the Yunnan native cattle population greatly exceeds that of other Southern cattle populations in China (Yu *et al*., 1999). These results suggest that the Yunnan native cattle population has a rich genetic diversity both in nuclear and mitochondrial DNA.

The reason for the lower H_0 in the MurryGrey breed is probably related to the increase of the coefficient of inbreeding in the MurryGrey group. The nine samples used in the study were all collected from living progenies of the original MurryGrey population that was introduced from Australia in 1984.

Table II. Amplified Results of RAPD With 10 Primers

Primer	5′ → 3′ Primer sequence	No. of RAPD	No. of polymorphisms	Frequency of polymorphism (%)	Polymorphism within Breed[a]					
					BM	DH	MG	DQ	SM	SH
OPA-19	CAA ACG TCG G	6	6	100.0	4	6	2	4	3	5
OPB-11	GTA GAC CCG T	11	9	81.8	5	6	1	4	3	4
OPC-13	AAG CCT CGT C	8	5	62.5	4	2	2	2	4	3
OPD-01	ACC GCG AAG G	12	8	66.7	7	4	7	7	7	6
OPF-05	CCG AAT TCC C	6	3	50.0	2	3	2	2	2	1
OPF-08	GGG ATA TCG G	10	8	80.0	7	8	3	8	8	6
OPH-11	CTT CCG CAG T	6	5	83.3	4	2	1	3	1	2
OPN-05	ACT GAA CGC C	8	7	87.5	4	7	2	4	5	2
OPN-11	TCG CCG CAA A	8	5	62.5	3	3	4	3	4	3
ILO1127	CCG CGC CGG T	9	7	77.8	3	5	2	4	6	4
Total		84	63		43	46	26	41	43	36
Frequency of polymorphism (%)			75.0		68.3	73.0	41.2	65.1	68.3	57.1

[a]BM: Brahman; DH: DeHong cattle; MG: MurrayGrey; DQ: DiQing cattle; SM: Simmental; SH: ShortHorn.

Table III. Genetic Diversity of Six Cattle Breeds

Primer	Brahman	DeHong	MurryGrey	DiQing	Simmental	ShortHorn
OPA-19	0.9416	0.8906	0.5221	0.8883	0.7957	1.1513
OPB-11	0.8464	0.6817	0.1955	0.7892	0.8630	0.9163
OPC-13	0.6010	0.1200	0.6365	0.4004	0.7159	0.5304
OPD-01	1.3751	0.6555	2.3204	1.1889	2.0031	1.6538
OPF-05	0.5098	0.6869	0.4709	0.2910	0.2790	0.1850
OPF-08	2.0241	1.9359	0.7354	1.9386	1.5785	1.3621
OPH-11	0.8996	0.6712	0.1178	0.5892	0.0425	0.2478
OPN-05	0.7558	1.2130	0.4389	1.1046	0.4745	0.4663
OPN-11	0.7483	0.6197	1.1015	0.8395	0.6952	0.8772
ILO1127	0.8287	1.1471	0.5144	0.8157	0.9837	0.7845
Average	0.9530	0.8622	0.7053	0.8845	0.8431	0.8175

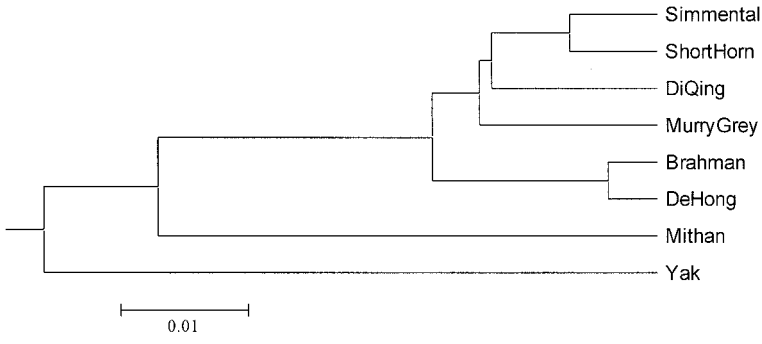
The Relationship Among Yunnan Native Cattle and Introduced Beef Cattle Breeds

The genetic distances (*D*) among the six cattle breeds, the mithan, and the Zhong-Dian yak are shown in Table IV. The phylogenetic trees were drawn by the UPGMA and NJ methods with scaled branch lengths (Fig. 1). The topology of the UPGMA tree and the NJ tree is almost the same except that the positions of DiQing cattle and the MurryGrey breed are swapped around.

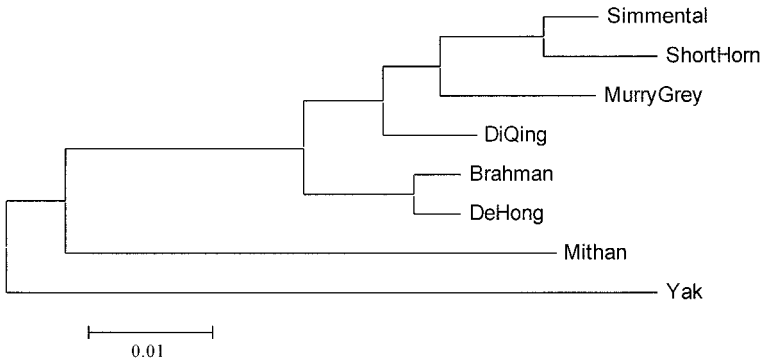
In the hypothetical phylogenetic trees, DeHong cattle and Brahman are most closely related; they form a branch, which belongs to the *Bos indicus* type cattle—zebu. The ShortHorn, Simmental, and DiQing cattle and the MurryGrey breed have a closer relationship. They form another branch, which belongs to *Bos taurus* type cattle—taurine (Fig. 1). It appears that DeHong cattle have the nearest relationship with *Bos indicus*, and DiQing cattle have a nearer relationship with *Bos taurus*. The results support our previous viewpoint that DeHong cattle are much more closely related to zebu, and DiQing cattle are closer to taurine (Yu *et al.*, 1999).

Table IV. Genetic Distance Among Cattle Breeds, Mithan, and Yak

	Brahman	DeHong	MurryGrey	DiQing	Mithan	Yak	Simmental	ShortHorn
Brahman	0							
DeHong	0.00768	0						
MurryGrey	0.03482	0.03875	0					
DiQing	0.03656	0.02928	0.02759	0				
Mithan	0.07349	0.06795	0.07924	0.07091	0			
Yak	0.08744	0.09393	0.10103	0.08110	0.09726	0		
Simmental	0.03549	0.03572	0.02465	0.02637	0.08208	0.10412	0	
ShortHorn	0.03578	0.03770	0.03162	0.02582	0.09699	0.10900	0.01373	0



a)



b)

Fig. 1. The UPGMA and NJ trees of breeds were constructed using the Mega2.0 software package (Kumar *et al.*, 2001): (a) The UPGMA dendrogram, (b) NJ dendrogram.

It has also provided the basic genetic evidence for crossbreeding of new beef cattle breeds and cross improving of native cattle in South China.

From our phylogeny trees, the mithan is quite different from other cattle populations, which was also reported and discussed in our previous study on blood protein polymorphisms (Nie *et al.*, 1999).

The Origin of Yunnan Native Cattle Population

The origin of native cattle in Yunnan province is still an open question. It is now popularly accepted that both taurine and zebu animals have hybridized to form the Yunnan native cattle population (Chen *et al.*, 1990). Later, on the basis of

allozyme, Y-chromosome, morphological, and mtDNA RFLP data, Yunnan native cattle were suggested to result from a combination of taurine, zebu, *Bos banteng*, *Bos gaurus*, and even *Bos grunniens* breeds, by which taurine and zebu bloods were mixed with Yunnan native cattle as popularly accepted now (Chen *et al.*, 1990; Lan *et al*., 1993; Nie *et al*., 1999; Yu *et al*., 1999). The data from nuclear DNA variation in this study also show that there has been both taurine and zebu influence in the origin of the Yunnan cattle population.

In our previous study, a new mtDNA haplotype IV was detected in DeHong cattle breeds in Yunnan province. It suggested that DeHong cattle might arise from an independent domestication event, probably from another *Bos indicus* population, because the divergence of haplotype I (*Bos indicus*) and IV occurred about 268,000–535,000 years ago, much earlier than the 10,000 years' history of cattle husbandry. Because of the limitations of the RAPD technique, the time of divergence of DeHong cattle and Brahman (*Bos indicus*) cattle cannot be speculated in this study. Therefore the origin of Yunnan native cattle is complicated and further studies are needed.

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