

Introduction about Indigenous Goat Breed Resources in Chongqing Municipality and Genetic Relationship Research about These Goat Breeds with Other Goat Breeds in Adjacent Provinces

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Abstract: Chongqing contains five goats breed resources representing special economic and ecological characteristics. These goat breeds' characteristics were researched and genetic relationships with other goats in adjacent provinces were ensured by analyzed mitochondrial DNA (mtDNA) D-loop sequences. The results indicated the goat breed resources in Chongqing have especial production performance in growth, meat quality and fecundity. The five goats breeds in Chongqing had high mtDNA diversity but the differences among breeds were significant. The near genetic distance of Youzhou dark goat and Banjiao goat indicated Youzhou dark goat may be a subline of Banjiao goat. The results of this study contribute to the knowledge of the genetic structure and correctly protecting and utilizing these goat breeds.

Key words: Goat, breed characteristics, mitochondrial DNA, genetic relationship, growth, meat quality

INTRODUCTION

Chongqing is the only municipality directly under the central government in Western China, located on the upper reaches of the Yangtze river. It has mild climate, abundance rainfall, large area's mountain and hill. In history because of inconvenient traffic, many local excellent goat breeds have emerged. Banjiao goat and Chuandong white goat had been indigenous goat breeds in the 1970s. In Oct, 2009, Ministry of Agriculture of the People's Republic of China approved Youzhou dark goat, Yudong black goat and Dazu black goat in Chongqing to be national livestock genetic resources. So, Chongqing has five goat breeds. In this study, these five goat breeds were introduced and their genetic relationship with others goat breeds adjacent provinces were analyzed.

Youzhou dark goats (Fig. 1) mainly live in karst landform area of Youyang county in Chongqing have dark color hair. Viewable mucous membranes are dark including eyes, nose, mouth, anus and vulva but eye rings are black. All goats have beard and horns. Dorsal keel has a strip of black line. This goat has high medicinal value. The ages of sex maturation are both 5-6 months. The average body weight of adult ram is 35.2 ± 2.4 kg ($n = 67$) adult ewe is 33.3 ± 3.5 kg ($n = 321$). The slaughter rate of adult goat is $>48\%$ ($n = 67$). The average year rate of the primiparity ewes is $117.0 \pm 2.6\%$ ($n = 432$) and the pluriparity ewes is $195.0 \pm 1.8\%$ ($n = 321$).



Fig. 1: a) The ram and b) the ewe of Youzhou dark goat

Yudong black goats (Fig. 2) mainly live in Wuling mountain area including Fuling district, Fengdu county,



Fig. 2: a) The ram and b) the ewe of Yudong black goat

Wulong county, Qianjiang district, Pengshui county and Youyang county. This goat breed has pure black hair, small habius. The ages of sex maturation of the ram and the ewe are 5-7 and 4-6 months, respectively. The average body weight of adult rams is 39.5 ± 8.3 kg ($n = 85$) adult ewe is 34.3 ± 6.4 kg ($n = 433$). The slaughter of the adult ram and ewe is $>49\%$ ($n = 78$). The average year rate of the primiparity ewe is $136.4 \pm 2.3\%$ ($n = 445$) and the pluriparity ewe is $194.3 \pm 2.8\%$ ($n = 433$).

Dazu black goats (Fig. 3) live in Dazu county of Chongqing. Dazu black goat population is absolute black color pattern, the majority have strong horns and beards, the extension direction of the horn is latero-rear, the shape of the horn just likes Chinese number 八. Dazu black goat has antero-lateral extending slender ears. The habius of Dazu black goat is huge and likes a rectangle. Manitruncus is developed, wide and deep. The average weight of the adult ram is 59.5 ± 1.8 kg ($n = 89$), the ewe is 40.2 ± 1.4 kg ($n = 456$). The ages of sex maturation of the ram and the ewe are 7-8 and 5-6 months, respectively. The main characteristic about Dazu black goat is high fecundity that manifests in outstanding superfoetation trait: three foetus in 2 years, the average year rate of the primiparity ewe is $218.0 \pm 1.2\%$ ($n = 523$) and the pluriparity ewe is $272.2 \pm 2.4\%$ ($n = 456$).

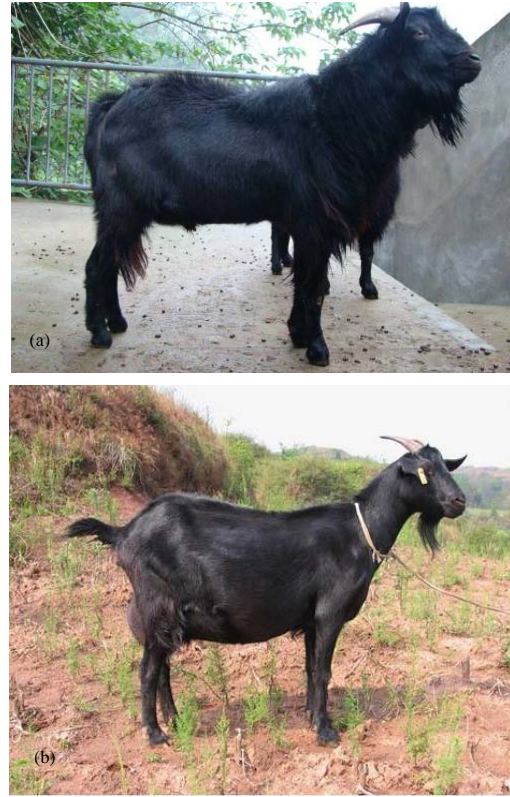


Fig. 3: a) The ram and b) the ewe of Yudong black goat

The ewe has big and well-developed breasts. Dazu black goat has good adaptability displaying that they can normally grow and propagate in humid heat environment, efficiently resist disease and rear coarse breeding.

Banjiao goats (Fig. 4) mainly live in Chengkou county, Wuxi county, Wulong county of Chongqing municipality and Wanyuan county of Sichuan province. This goat breed is famous for its long and applanation horns in the ram and the ewe. The majority has white hair. The ages of sex maturation of the ram and the ewe are both 6-7 months. The average year rate of the primiparity ewe is $124.4 \pm 2.7\%$ ($n = 321$) and the pluriparity ewe is $184.4 \pm 3.3\%$ ($n = 298$). The average body weight of adult ram is 40.5 ± 4.3 kg ($n = 98$) adult ewe is 30.3 ± 6.2 kg ($n = 434$). The slaughter of the adult ram and ewe is $>55.6\%$ ($n = 72$).

Chuangdong white goats (Fig. 5) mainly live in Hechuan district, Yunyang county, Fengdu county, Wushan county, Wanzhou district of Chongqing. This breed has two kinds: one is big individuals that distribute in Hechuan district, the other is small individuals that live in three Gorges reservoir area. The big kind has white hair, horns, long and muscular limbs. The ram has short and muscular neck and the ewe has long and thin neck. The average body weight of the adult ram is 33.4 ± 9.4 kg

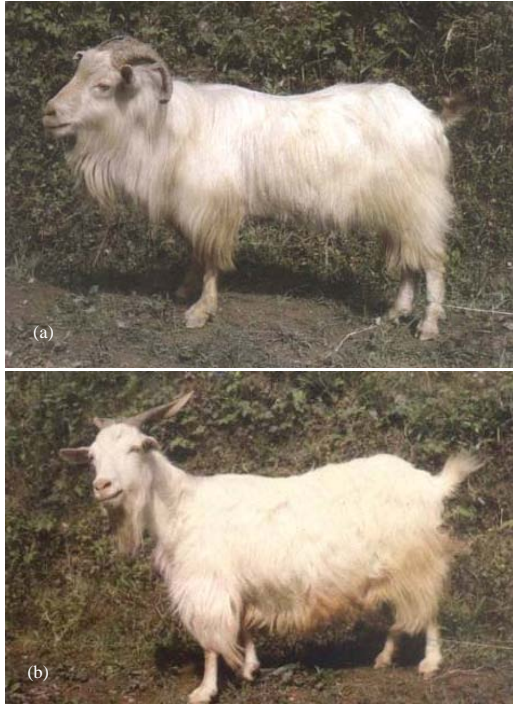


Fig. 4: a) The ram and b) the ewe of Banjiao goat



Fig. 5: a) The ram and b) the ewe of Chuandong white goat

(n = 87), the adult ewe is 30.8 ± 6.3 kg (n = 232). The ages of sex maturation of the ram and the ewe are

both 5-6 months. The average year rate of the pluriparity ewe is $202.2 \pm 2.1\%$ (n = 198). The slaughter of the adult ram and ewe is $>48.1-50.2\%$ (n = 76). The majority of the small kind has white hair and horns. Its neck and limbs are short. The average body weight of the adult ram is 19.2 ± 1.8 kg (n = 145), the adult ewe is 20.9 ± 3.2 kg (n = 347). The ages of sex maturation of the ram and the ewe are both 3-4 months.

The average year rate of the pluriparity ewe is $166.2 \pm 1.8\%$ (n = 368). The slaughter of the adult ram and ewe is $>48.1-50.2\%$ (n = 76). Mitochondrial DNA (mtDNA) has represented the most informative genomic element to study genetic relationship of domestic goat, mainly because mtDNA displays a simple maternal inheritance without recombination and relatively rapid evolution rate (MacHugh and Bradley, 2001; Liu *et al.*, 2006). The mtDNA D-loop or control region of goat breeds in Southwest China has been analyzed by DNA sequencing technology (Liu *et al.*, 2006; Chen *et al.*, 2005; Zhang *et al.*, 2007; Zhao *et al.*, 2011). But all goat breeds of Chongqing municipality were not included, so the genetic relationship between the five goat breeds and other goat breeds in adjacent provinces remains uncertain. In this study, mtDNA D-loop sequences were used to investigate the genetic relationship of these goat breeds.

MATERIALS AND METHODS

Animals: The 12 goat breeds were collected in this study including five goat breeds (Youzhou dark goat, Yudong black goat, Dazu black goat, Banjiao goat and Chuandong white goat) in Chongqing municipality, three breeds (Nanjiang brown goat, Lezhi black goat and Yingshan black goat) came from Sichuan province, three breeds (Guizhou black goat, Guizhou white goat and Qianbei grey goat) came from Guizhou province and Guishan black goat in Yunnan province (Fig. 6). Every goat population, respectively came from central breeding farm and there was no blood relationship in every individual of any goat population.

Methods: Blood samples were collected from 154 experimental goat individuals. DNA utilized in PCR amplification was extracted from blood according to the methods of Sambrook and Russell (2011).

The pair primers were designed on the positions 15, 429-16, 640 of the complete goat mitochondrial sequence (GenBank accession number AF533441). The goat mtDNA D-loop primer sequences were as follows: GF: 5'-CAGTCGAACATCCCTACATTATTATTGG-3' and GR: 5'-TTAGTCTTATTGATTTGGAGGGCGTTA-3'. The PCR cycling protocol was as described by Liu *et al.*

(2006): 5 min at 94°C, 35 cycles of 94°C for 60 sec, 66°C annealing for 60 sec, 72°C for 60 sec with a final extension at 72°C for 10 min and storage at 4°C. The amplified products were purified using the QIAquick PCR purification kit (QIAGEN) according to the manufacturer's instructions.

Another pair of internal primers was designed to sequence the amplified products: F: 5'-TAC AATCAATACACCTG-3' and R: 5'-ATTACGTTTATG CTGGATT-3'. The PCR products were directly sequenced on a ABI 3700 PRISM DNA Sequencer (Applied Biosystems) by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd.



Fig. 6: Geographical locations of the 12 goat breeds in China. The two-letter or three-code letter besides the black point in the figure corresponds to the populations sampled as follows: Youzhou dark goat, YZD; Yudong black goat, YDB; Dazu black goat, DZB; Nanjiang brown goat, NJ; Lezhi black goat, LZB; Yingshan black goat, YSB; Guizhou black goat, GZB; Guizhou white goat, GZW; Qianbei grey goat, QBG; Guishan black goat, GSB

Data analysis: All 154 sequences of mtDNA were aligned using the Clustal X2 program (Larkin *et al.*, 2007). Phylogenetic and molecular evolutionary analysis were conducted using MEGA Version 4. The phylogenetic tree was constructed using the Neighbor-Joining Method (Saitou and Nei, 1987) under the Kimura 2-Parameters Model (Kimura, 1980) with 1000 replicates in the Bootstrap test with the consensus sequence of every breed. The DnaSP programme, Version 5.10.01 (Librado and Rozas, 2009) was used for estimating haplotype and nucleotide diversity.

RESULTS AND DISCUSSION

Sequence polymorphism: The 68 polymorphic sites were found in 154 individuals. The average polymorphic sites of Yudong black goats were the lowest in all 12 goat breeds while Banjiao goats were the highest. The 118 different haplotypes were found in all experimental individuals. Haplotype proportion of Banjiao goats and Guishan black goats were 100% while Yudong black goats' haplotype proportion was the lowest (58.20%). Haplotype diversity value ranged from 0.9111-1.0000 in all breeds, haplotype diversity value of Yudong black goats was the lowest (0.9111) but the value were the highest in Banjiao goats and Guishan black goats. Nucleotide diversity value ranged from 0.0079-0.0217, the value in Chuandong white goats and Nanjiang brown goats were low (Table 1). The results indicated the genetic diversity of the five goat breeds in Chongqing was evident, Yudong black goats' average polymorphic sites, Haplotype proportion and haplotype diversity value were both low while Banjiao goats were both high.

Genetic relationship: In five goat breeds, Youzhou dark goat was the nearest (0.0004) with Banjiao goat in genetic distance (Table 2 and Fig. 7). The genetic distance of

Table 1: The sequences polymorphism for each goat breed in this study

Breeds	Abbreviation	Geographic distribution	No. of sample	No. of single-vari sites	No. of parsim-info sites	No. of haplotypes	Haplotype proportion (%)	Haplotype diversity	Nucleotide diversity
Youzhou dark goat	YZD	Chongqing	11	12	34	8	72.73	0.9236±0.0342	0.0177
Yudong black goat	YDB	Chongqing	17	4	26	10	58.20	0.9111±0.0340	0.0161
Dazu black goat	DZB	Chongqing	17	37	46	11	67.70	0.9265±0.0471	0.0179
Banjiao goat	BJ	Chongqing	10	26	47	10	100.00	1.0000±0.0447	0.0195
Chuandong white goat	CDW	Chongqing	9	4	35	7	77.78	0.9167±0.0920	0.0079
Nanjiang brown goat	NJ	Sichuan	10	13	47	9	90.00	0.9778±0.0540	0.0123
Lezhi black goat	LZB	Sichuan	21	14	39	15	71.42	0.9524±0.0451	0.0217
Yingshan black goat	YSB	Sichuan	17	5	35	12	70.58	0.9485±0.0357	0.0145
Guizhou black goat	GZB	Guizhou	8	4	17	5	62.50	0.9168±0.0573	0.0165
Qianbei grey goat	QBG	Guizhou	10	16	29	8	80.00	0.9333±0.0773	0.0188
Guizhou white goat	GZW	Guizhou	10	6	40	9	90.00	0.9778±0.0540	0.0164
Guishan black goat	GSB	Yunnan	14	19	57	14	100.00	1.0000	0.0185

Taleb 2: Genetic distance of 12 goat breeds

Breeds	CDW	BJ	NJ	YZD	GZW	QBG	GZB	YDB	LZB	YSB	GSB	DZB
CDW	-	-	-	-	-	-	-	-	-	-	-	-
BJ	0.0022	-	-	-	-	-	-	-	-	-	-	-
NJ	0.0015	0.0018	-	-	-	-	-	-	-	-	-	-
YZD	0.0025	0.0004	0.0017	-	-	-	-	-	-	-	-	-
GZW	0.0025	0.0008	0.0012	0.0008	-	-	-	-	-	-	-	-
QBG	0.0036	0.0016	0.0036	0.0017	0.0002	-	-	-	-	-	-	-
GZB	0.0036	0.0013	0.0025	0.0008	0.0017	0.0008	-	-	-	-	-	-
YDB	0.0021	0.0008	0.0011	0.0008	0.0017	0.0025	0.0017	-	-	-	-	-
LZB	0.0052	0.0041	0.0058	0.0041	0.0050	0.0058	0.0050	0.0050	-	-	-	-
YSB	0.0018	0.0025	0.0008	0.0025	0.0033	0.0041	0.0033	0.0017	0.0049	-	-	-
GSB	0.0045	0.0075	0.0092	0.0075	0.0066	0.0075	0.0083	0.0029	0.0036	0.0017	-	-
DZB	0.0048	0.0092	0.0108	0.0092	0.0100	0.0092	0.0086	0.0031	0.0020	0.0025	0.0027	-

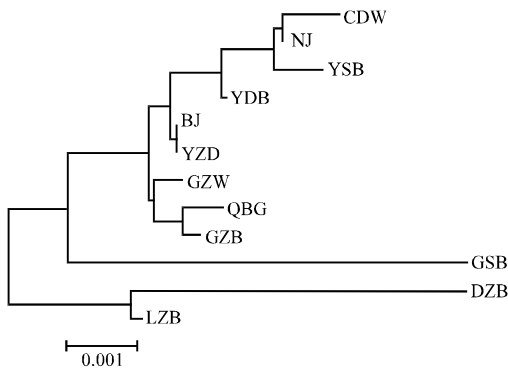


Fig. 7: Neighbor-Joining (NJ) tree about mtDNA D-loop consensus sequence of 12 goat breeds

Yudong black goat with Banjiao goat and Youzhou dark goat were both 0.0008, it indicated a near genetic relationship in three goat breeds. At the same time, Chuandong white goat and Dazu black goat with the three goat breeds were farther, respectively. The genetic distance of Chuandong white goat and Nanjiang brown goat was nearer.

In three goat breeds of Sichuan province, Nanjiang brown goat and Yingshan black had a near genetic distance. The three goat genetic breeds in Guizhou province including Guizhou white goat, Guizhou black goat and Qianbei grey goat had a near genetic relationship. Guishan black goat of Yunnan province with others goat breeds had a farther genetic relationship.

Different mtDNA diversity of domestic goats in Chongqing: The five goat breeds in Chongqing had different mtDNA diversity. Haplotype proportion and haplotype diversity of Banjiao goats were the highest. But Yudong black goats had the lowest polymorphic sites, haplotype proportion and haplotype diversity in all 12 goat breeds. The finding indicated that Yudong black goats had poor genetic diversity, so protecting and expanding the population quantity should be done for Yudong black goat.

But compared with other genetic diversity studies on Chinese domestic goat breeds (Li *et al.*, 2002; Liu *et al.*, 2006; Chen *et al.*, 2005; Zhao *et al.*, 2011), the value of haplotype proportion, haplotype diversity and nucleotide diversity of Yudong black goat were still high. The high genetic diversity observed in a breed could be explained by maternal effective of multiple wild ancestor, overlapping generations, mixing of populations from different geographical locations, natural selection favoring heterozygosis or subdivision accompanied by genetic drift (Naderi *et al.*, 2007; Agha *et al.*, 2008). The finding indicated that the five goat breeds of Chongqing vary in their genetic potential for the production of meat, disease resistance, heat tolerance and fecundity.

Genetic relationship: The genetic distance of Youzhou dark goat and Banjiao goat was near which indicated the origin of Youzhou dark goat was concerned with Banjiao goat. It means Youzhou dark goat may be a subline of Banjiao goat. The clustering relationship of three goat breeds in Guizhou province, Chuandong white and Nanjiang brown goat, Lezhi black goat and Dazu black goat reflected the geographical position was important to the genetic relationship among goat breeds.

CONCLUSION

Chongqing in China has abundance of goat breed resources every goat breed has especial production performance in growth, meat quality and fecundity. Different goat breed has different mtDNA diversity. Youzhou dark goat may be a subline of Banjiao goat. The results of this study contribute to the knowledge of the genetic structure and correctly protecting and utilizing these goat breeds.

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