

## Polymorphisms of two Y chromosome microsatellites in Chinese cattle

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(Received 15 December 2005; accepted 18 April 2006)

**Abstract** – Two Y chromosome specific microsatellites *UMN2404* and *UMN0103* were genotyped and assessed for polymorphisms in a total of 423 unrelated males from 25 indigenous Chinese cattle breeds. Consistently, both microsatellites displayed specific indicine and taurine alleles in each bull examined. The indicine and taurine alleles were detected in 248 males (58.6%), and 175 males (41.4%), respectively, although these frequencies varied amongst different breeds examined. The indicine alleles dominated in the southern group (92.4%), while the taurine alleles dominated in the northern group (95.5%). Hainan Island was possibly the site for the origin of Chinese zebu, and Tibetan cattle were probably independently domesticated from another strain of *Bos primigenius*. The geographical distribution of these frequencies reveals a pattern of male indicine introgression and a hybrid zone of indicine and taurine cattle in China. The declining south-to-north and east-to-west gradient of male indicine introgression in China could be explained by historical data, geographical segregation and temperature and weather conditions.

**Chinese cattle / taurine / indicine / genetic introgression / Y chromosome**

### 1. INTRODUCTION

China is rich in genetic resources of cattle breeds, with 28 indigenous and other cattle breeds [15]. These cattle breeds have been divided into three major groups on the basis of their geographical distribution, morphological characteristics and sex chromosome polymorphisms: the northern group in northern China, the central area group in the middle and lower areas of the Yellow River and the Huaihe River, and the southern group in southern China [2, 15].

Attempts have been made to trace the origins and phylogenetic relationships of indigenous Chinese cattle through the analysis of morphology, protein loci

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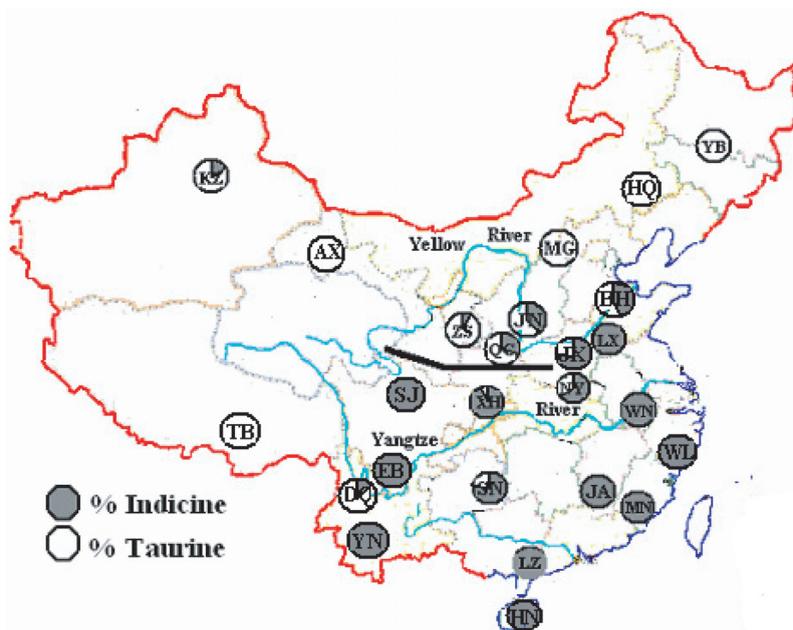
and mtDNA RFLP. The common view is that Chinese cattle breeds could be classified into four origins: *Bos taurus*, *Bos indicus*, *Bos javanicus*, *Bos gaurus*, and even *Bos grunniens* [5, 12, 21]. Nevertheless, some western scientists hold the view that Chinese native cattle breeds are admixtures of *Bos taurus* and *Bos indicus* [13, 14]. The study of sex chromosome morphology and mtDNA D-loop region sequence data have indicated that the taurine (*Bos taurus*) had a higher genetic influence on the development of the northern group of breeds than the indicine (*Bos indicus*), while the indicine had a higher genetic influence on the development of the southern group of breeds. Both the taurine and indicine contributed to the formation of the central area group of cattle breeds [2, 8, 9].

The Y-specific region constituting about 95% of the Y chromosome is immune to recombination and inherited "en bloc" as a haplotype, which ensures that original combinations of mutational events along male lineages are preserved as single linked unambiguous haplotypes. Y chromosome-specific microsatellites of bovines are effectively haploid and paternally inherited [7, 10, 11]. Two Y-specific microsatellites *UMN2404* and *UMN0103* have been reported to be polymorphic in some domestic cattle breeds, which were also used for the construction of a first generation radiation hybrid map for the bovine Y chromosome [10, 11]. The purposes of this work were to genotype the two Y-specific microsatellites and assess for their polymorphisms in indigenous Chinese cattle breeds.

## 2. MATERIALS AND METHODS

A total of 423 unrelated males from 25 indigenous breeds representing the three major groups of Chinese cattle (northern group, central area group, southern group) were sampled. These sampled bulls were from a fair geographical distribution, as shown in Figure 1 and Table I. As a control, microsatellite sizes of taurine (Angus, introduced from England) and indicine (Brahman, from USA) were established by molecular methods. In addition a small number of females (1–3) from each breed were screened to ensure Y-chromosome specificity of the markers.

Genomic DNA was isolated from muscle tissue and whole blood using the normal method of phenol-chloroform extraction [16]. The primers of *UMN2404* (forward primer 5'-GGTACAATTGAAAATATG-3', reverse primer 5'-TGTACCTACACTGATATGTT-3') and *UMN0103* (forward primer 5'-ACACAGAGTATTCACCTGAG-3', reverse primer 5'-ATTTACCTGGGTCAAAGCAC-3') were developed by Liu *et al.* [10]. PCR was carried



**Figure 1.** Frequency of taurine and indicine Y chromosome alleles in Chinese cattle breeds. In black, indicine Y-chromosome, and in white, taurine. KZ, Kazakh; AX, Anxi; ZS, Zaosheng; MG, Mongolia; HQ, Horqing; YB, Yanbian; QC, Qinchuan; JN, Jinnan; BH, Bohai Black; JX, Jiaxian Red; LX, Luxi; SJ, Sanjiang; XH, Xuanhan; NY, Nanyang; WN, Wannan; TB, Tibetan; EB, Ebian; SN, Sinan; JA, Ji'an; WL, Wenling hump; DQ, Diqing; MN, Minnan; YN, Yunnan hump; LZ, Leizhou; HN, Hainan. The bold line indicates the Qinling Mountains.

out on a PTC-200 thermocycler (MJ Research Inc.) in 15  $\mu$ L reaction volume containing 20 ng of DNA, 0.4  $\mu$ M of each primer, 50  $\mu$ M of each dNTP (Tiangen Biotech Co., Ltd, Beijing, China), 0.6 U of Taq DNA polymerase (Sino-American Biotechnology Co., Luoyang, Henan province China), 1 $\times$ PCR buffer (10 mM Tris-HCl [pH 9.0 at 25  $^{\circ}$ C]; 1.5 mM MgCl<sub>2</sub>; 50 mM KCl; 0.1% Triton X-100). The amplification cycle of *UMN2404* included denaturation at 95  $^{\circ}$ C for 5 min, then followed by 36 cycles at 94  $^{\circ}$ C for 30 s, 54  $^{\circ}$ C for 30 s, 72  $^{\circ}$ C for 30 s, and a final extension step at 72  $^{\circ}$ C for 5 min. *TouchDown* PCR was carried out to amplify *UMN0103* under the following conditions: 94  $^{\circ}$ C for 3 min, followed by 40 cycles of 94  $^{\circ}$ C for 30 s, 65–55  $^{\circ}$ C for 30 s and 72  $^{\circ}$ C for 30 s. Annealing temperatures were decreased from 65 to 55  $^{\circ}$ C with the decrements of 0.5  $^{\circ}$ C successively for each of the first 21 cycles.

The specific taurine or indicine alleles were distinguished by polyacrylamide gel (8%) electrophoresis, visualized using silver stain and analyzed

**Table I.** Number and frequency (%) of indicine and taurine alleles in Chinese cattle breeds and cattle groups.

Groups	Breeds	Geographical distribution	Number of Y alleles		Frequency of Y alleles		
			Indicine	Taurine	Indicine	Taurine	
Northern	Anxi (AX)	Northwestern region of the Gansu province	0	15	0	100	
	Mongolia (MG)	Inner Mongolia, Shanxi and the Hebei province, and Northeast China	0	7	0	100	
	Horqing (HQ)	Inner Mongolia	0	13	0	100	
	Yanbian (YB)	Jiling, Liaoning and the Heilongjiang province and inner Mongolia	0	22	0	100	
	Tibetan (TB)	Southern region of Tibet	0	8	0	100	
	Zaosheng (ZS)	Northeast region of converging regions of Ningxia and the Gansu province	2	27	6.9	93.1	
	Kazakh (KZ)	Northern region of the Xinjiang province	3	15	16.7	83.3	
	Total numbers and frequencies		5	107	4.5	95.5	
	Central Area	Qinchuan (QC)	Guanzhong region of the Shaanxi province	10	21	32.3	67.7
		Jinnan (JN)	Southern region of the Shanxi province	5	8	38.5	61.5
Bohai Black (BH)		Northwest region of the Shandong province	8	11	42.1	57.9	
Nanyang (NY)		Southern region of the Henan province	18	10	64.3	35.7	
Jiaxian Red (JX)		Jiaxian and Baofeng counties of the Henan province	12	4	75	25	
Luxi (LX)		Southwestern region of the Shandong province	19	0	100	0	
Total numbers and frequencies			72	54	57.1	42.9	
Southern	Diqing (DQ)	Diqing region of the Yunnan province	2	9	18.2	81.8	
	Sinan (SN)	Northeast region of the Guizhou province	13	4	76.5	23.5	
	Xuanhan (XH)	Converging regions of Shaanxi, Sichuan and the Hubei province	12	1	92.3	7.7	
	Sanjiang (SJ)	Northern region of the Sichuan province	8	0	100	0	
	Ebian (EB)	Liangshan region of the Sichuan province	14	0	100	0	
	Wannan (WN)	Regions in the Anhui province along the Yangtze River	32	0	100	0	
	Wenling (WL)	Wenling city of the Zhejiang province	11	0	100	0	
	Ji'an (JA)	Southern region of the Jiangxi province	9	0	100	0	
	Minnan (MN)	Southern region of the Fujian province	22	0	100	0	
	Yunnan hump (YN)	Southern region of the Yunnan province	10	0	100	0	
	Leizhou (LZ)	Converging regions of Guangdong and the Heinan province	21	0	100	0	
	Hainan (HN)	Northern region of the Heinan province	17	0	100	0	
	Total numbers and frequencies		171	14	92.4	7.6	
All breeds			248	175	58.6	41.4	

by employing the Discovery Series Quantity One Software Version 4.3.1 (Bio-Rad Laboratoris Inc.). The frequency of the alleles was determined by direct counting.

### 3. RESULTS

The microsatellites *UMN2404* and *UMN0103* were assessed for polymorphisms in 17 unrelated bulls by Liu *et al.* [10]. *UMN2404* displayed a typical ladder-like band ranging from 85 bp to 112 bp and *UMN0103* presented fewer bands ranging from 124 bp to 136 bp [10]. The Chinese cattle are divided into two major morphological groups, the humped indicine and humpless taurine types. Accordingly, the *UMN2404* microsatellite showed specific taurine (Angus) (104 bp, 91 bp) and indicine (Brahman) (120 bp, 110 bp, 85 bp) alleles in these indigenous breeds. Interestingly, *UMN0103* also displayed specific taurine (155 bp, 140 bp) and indicine (136 bp, 125 bp) alleles consistent with *UMN2404* in each bull examined. The bulls of five breeds (Anxi, Tibetan, Mongolia, Horqing and Yanbian cattle) were totally genotyped as taurines, while all the individuals in ten breeds (Luxi, Sanjiang, Ebian, Wannan, Wenling hump, Ji'an, Minnan, Yunnan hump, Leizhou and Hainan cattle) were genotyped as indicines. The frequencies of the taurine and indicine specific alleles in the indigenous Chinese cattle breeds are shown in Figure 1 and Table I.

Amongst the 25 indigenous Chinese cattle breeds (423 males), the indicine alleles were higher, comprising 58.6% of the alleles observed (Tab. I). Seven breeds of the northern cattle group studied were from ten provinces. The frequency of taurine alleles in the northern group was predominant, comprising 95.5% of the animals examined. Only two indicine bulls were observed in 29 Zaosheng cattle (ZS) and three in 18 Kazakh cattle (KZ). Twelve breeds of the southern group sampled from 11 provinces were examined in this study. In over a total of 185 bulls from the southern group, the taurine alleles were detected in one Xuanhan (XH) male, 9 Diqing males (DQ) and four Sinan bulls (SN) (7.6%), while the indicine ones were observed in 171 sires of the 12 breeds from the southern group (92.4%). The frequency and geographical distribution of taurine and indicine alleles among cattle breeds from the central area group were also not homogenous. The taurine alleles were detected in 67.7% of the Qinchuan bulls (QC) examined, 61.5% of the Jinnan males (JN), 57.9% of Bohai Black cattle (BH), 35.5% of the Nanyang sires (NY) and 25% of the Jiaxian Red animals (JX), but none were observed in the animals of the Luxi breeds (LX).

Previous cytogenetic research on the indigenous Chinese cattle breeds showed that the male indicine Y chromosome was present in 100% of Hainan (HN), Wenling hump (WL), Ebian (EB), NY and LX males; 75% of JX cattle; 25% of QC bulls; and in 22.2% of JN breeds, but it was absent in Mongolia (MG) and Tibetan cattle (TB) [2, 3, 6, 19, 20]. The analysis of these data and our results indicate that the frequency of the indicine Y chromosome among Chinese cattle breeds ranged from 0 to 100%. As shown in Figure 1, the geographical distribution of these two types of alleles reveals a declining south-to-north and east-to-west gradient of male indicine introgression in China.

#### 4. DISCUSSION

The origin, domestication and migration of indigenous Chinese cattle are much more complicated due to their complex and extensive geographical distribution. In this work, we genotyped the Y chromosome microsatellites *UMN2404* and *UMN0103*, which consistently showed specific indicine and taurine alleles in 25 Chinese cattle breeds. The assessment of *UMN2404* and *UMN0103* for their polymorphisms revealed the geographical distribution and frequency of male taurine and indicine in Chinese cattle. The declining south-to-north and east-to-west gradient of male indicine introgression in China could be explained by historical data, geographical segregation and temperature weather conditions.

Among the 423 Chinese bulls analyzed, 248 were genotyped as indicine while the remaining belonged to the taurine type. The frequencies of the taurine and indicine alleles differed in the regional matrilineal pool of cattle in China. For instance, the indicine alleles dominated in the southern group (92.4%; 171/185) and still took a higher percentage in the central area group (57.1%; 72/126), while the taurine alleles dominated in the northern group (95.5%; 107/112) (Tab. I). This pattern was consistent with the findings based on the analysis of mtDNA RFLP and mtDNA D-loop sequence diversity, suggesting that cattle from South China received more genetic contribution from the indicine [8, 21].

The earliest domestic cattle in North China were the progenies of the *Longhorn* of *Bos taurus* from Western Europe, which were distinguished from *bachryseros* and *primigenius* of *Bos taurus*, and appeared between 3766 and 3122 years B.P. (*before present*) with the introgression of Mongolian into the huge and extensive area to the north of the Great Wall [4, 5, 13]. These taurine cattle gradually adapted to the local environmental conditions and expanded

southward but the Qinling Mountains and temperature and weather constituted the natural barriers to their expansion. Therefore, the highest percentages of taurine were examined in the cattle from the northern group, with 100%, 100%, 100%, 100%, 93.1% and 83.3% of taurine alleles in AX, MG, HQ, YB, ZS and KZ, respectively. Meanwhile, the lower percentages of indicine introgression in KZ and ZS resulted from the introduction of zebu from North Africa and the hybridization between African zebu and indigenous taurine through the Silk Road of ancient China [4, 5].

Interestingly, the eight Tibetan bulls examined were totally genotyped as taurine, which indicated Tibet was not only a habitat of yak, but also a habitat of taurine in China. This result was consistent with the research based on cytogenetic analysis by Chen *et al.* [6], who suggested that Tibetan cattle were probably the progenies of another strain of *Bos primigenius*. Similarly, the research of Tu and Zhang [18] indicated that Tibetan cattle are one of the strains of *Bos taurus* independently domesticated from local wild cattle. Based on the study of morphological features, blood protein polymorphisms and cytogenetic analysis, Qiu *et al.* [15] proposed that Tibetan cattle should be included in the northern cattle group, and in this work we analyzed Tibetan cattle in the northern cattle group.

The humped zebu inhabited in the vast and huge areas of the Yangtze River and the Zhujiang River as early as 5500 years B.P. and they might originate from the *Bibos* genus, such as zebu, *Bos bateng* and even *Bos gaurus*, which were also the progenitors of the zebu from Southeastern Asia [4, 5, 13, 15]. In this sense, the predominantly higher percentages of indicine alleles detected in the southern group agreed with the massive distribution of Chinese zebu in south China. Here the Qinling Mountains and temperature and weather also constituted the main natural barriers to the northward expansion of Chinese zebu. The results of research on six types of blood protein (Hb, Alb, Pa, Tf, Ptf and Akp) polymorphisms of 27 thousand cattle distributed in the northern and southern flank regions along the Qinling Mountains revealed that gene-migration had occurred among QC, Xizhen cattle (XZ) and XH [1]. Thus, the small amount of taurine Y chromosome transmission into XH was due to the gene-exchanging between QC and XH. Unexpectedly, much higher taurine alleles (81.8%) were observed in Diqing cattle. This could be explained by the similarity of the habitat of Diqing cattle to that of Tibetan cattle. The Diqing region where Diqing cattle inhabit is located in the northwest region of the Yunnan province and is a southern extension of the Tibet-Qinghai plateau, which is on average 2800–3300 meters above sea level. Diqing cattle are humpless and show taurine-like morphological features, and have adapted

to the cold weather and high altitude. Therefore, this unique habitat also constituted a natural barrier to the introgression of indicine into Diqing cattle. The existence of taurine alleles in SN was due to some individuals of SN originating from hybridization between *Bos taurus* and *Bos indicus* [17,21].

Chen *et al.* [5] suggested that the indicine cattle from the eastern part of the central area have characteristics resembling those of zebu from Southeast Asia by coat color, body stature and blood protein polymorphisms, while those from the western and central parts have characteristics resembling those of the zebu from Africa and West Asia. In our study both the taurine and indicine alleles were observed in the cattle from the central area group, while the percentage of indicine was higher, comprising 57.1% of the individuals studied. Furthermore, a declining east-to-west gradient of male indicine introgression was evident, with 100%, 75%, 64.3%, 38.5% and 32.3% of indicine alleles in LX, JX, NY, QC and JN, respectively. The genetic influence of indicine to LX, JX and NY was significantly greater than that to QC, JN and BH; conversely, the influence of taurine to QC, JN and BH was greater than that to LX, JX and NY. This result was consistent with the gradient described by Chen *et al.* [2] and Lai *et al.* [8].

Moreover, a hybrid zone in the central area of China between the two types of alleles was also revealed in this work. Our results indicate that the influence of indicine from South China, detouring the Qinling Mountains and dispersing along a south-to-north direction, has reached the Guanzhong region of the Shaanxi province, the Jinnan region of the Shanxi province and the northwest area of the Shandong province, with the indicine alleles present in QC, JN and BH, respectively. It is possible that the gradual adaptation of indicine to the relatively cold and dry weather of northern China will facilitate their northward and westward dispersion.

Finally, the declining south-to-north and east-to-west gradient of the male indicine introgression pattern in China indicated that the Hainan Island to the southernmost continent of China was possibly the site for the origin of Chinese zebu in paternal lineage. Our result is supported by the research on the gene frequency of T<sup>F</sup> in Hainan cattle [4, 5].

This work, for the first time, establishes a pattern of male indicine introgression in China using two polymorphic Y chromosome specific markers. The extent of male indicine influence on indigenous Chinese cattle clearly emerged from our results (Fig. 1). It appears that relative geographical segregation, environmental and temperature and weather conditions have prevented the spread of the indicine Y alleles throughout China.

## ACKNOWLEDGEMENTS

We thank Dr. Zhiqing Zhang for providing blood and muscle tissue samples of Qinchuan, Nanyang and Luxi cattle. We are also thankful to Prof. Lihong Su, Ms. Shenrong Hu, Dr. Chunlei Zhang and other colleagues working in the College of Animal Science and Technology, Northwest A&F University, for their support of the experiment and suggestions to this paper. Still we are grateful to Prof. Paul Bach (Xi'an Foreign Language University, China) and Dr. Zhiying Zhang (Gemin X Biotechnologies Inc., Quebec, Canada) for their help in editing the language and comments on the manuscript. This research was supported by grants from the National Natural Science Foundation of China (No. 30471238), Project Supporting of Excellent Researchers of Northwest A&F University (No. 01140101) and Creativity Education Project of Graduate Students of Northwest A&F University (No. 05YCH018).

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