PHILIPP AGRIC SCIENTIST
Vol. 100 No. 2, 150–155
June 2017

ISSN 0031-7454

Genetic Diversity among Yellow Cattle Populations (*Bos taurus*) in the Loess Plateau of Western China

Shengguo Zhao*, Li Liu, Yuan Cai and Jianping Wu

College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China
*Author for correspondence; email: zhaosg@gsau.edu.cn, zhaoshengguo0628@hotmail.com; Tel.: +(86) 931 7631225; Mobile: +(86) 13909469015; Fax: +(86) 931 7632468

Maternally inherited mitochondrial DNA (mtDNA) has been used extensively to determine genetic diversity and to guide genetic resource conservation. It is hypothesized that local populations of Chinese Yellow Cattle (*Bos taurus*) in the Loess Plateau of Western China were produced from Zaosheng cattle, and estimates of genetic diversity in Zaosheng and other derived populations are needed to assist in getting more detailed information about genetic resource conservation. Samples from Qinchuan cattle (QC, n = 171), Zaosheng cattle (ZS, n = 184), Pingliang native cattle (PL, n = 112), and Guyuan native cattle (GY, n = 75) were analyzed using mtDNA D-loop analytical techniques. A total of 140 variable sites and 244 haplotypes were identified. Among the QC, ZS, PL and GY populations, the diversity of haplotypes (0.946 ± 0.012, 0.976 ± 0.005, 0.966 ± 0.010, and 0.975 ± 0.009, respectively), the average number of nucleotide differences (16.312, 13.685, 14.503, and 13.778, respectively) and nucleotide diversity (0.02661, 0.02236, 0.02370, and 0.02248, respectively) were determined. There were 202 unique haplotypes found in four populations: 56 in QC, 71 in ZS, 42 in PL, and 33 in GY. Results from this research indicated that the genetic diversity of QC was lower than that of ZS, PL and GY. Results also suggested that, based on number of shared haplotypes, Qinchuan, Pingliang, and Guyuan cattle were descended from Zaosheng cattle and gradually formed three distinct maternal branches with Pingliang and Guyuan apparently maintaining the genetic diversity of Zaosheng. Identification of unique haplotypes within these populations provided a basis for further cattle genetic resource assessment of diversity and conservation of native cattle populations in the Loess Plateau region of western China.

Key Words: Chinese Yellow Cattle, genetic diversity, genetic resource conservation, mtDNA D-loop

Abbreviations: GY – Guyuan native cattle, mtDNA – mitochondrial DNA, PL – Pingliang native cattle, PRC – People's Republic of China, QC – Qinchuan cattle, ZS – Zaosheng cattle

INTRODUCTION

Since the domestication of cattle around 10,000 years ago, humans have improved traits through selection of animals. Selection progress was slow until the application of quantitative genetics and artificial insemination in the 20th century (Felius 1995). Breeds were developed based on geographic, historical, and evolutionary backgrounds, and defined by specific phenotypic characteristics (Choi et al. 2012). In addition, breeds developed useful adaptation to their production environment through natural selection. Notter (1999) noted that matching germplasm resources to production or management environment is important in animal genetic resource management. The characterization of these indigenous, economically important genetic resources has become an issue of increasing importance both in the scientific community and in production agriculture. Researchers and farmers in many countries are increasingly aware that their indigenous livestock and plant material may have unique and potentially valuable genetic distinctiveness. Notter (1999) reported that there is evidence that favorable alleles exist even in less productive genetic resources (breeds). Genetic diversity is a critical component of fitness strategies in organisms (Ouburg et al. 2009) and is important in the maintenance of viable populations. Lower genetic diversity was associated with lower disease resistance in *Drosophila melanogaster* and it is reasonable to hypothesize that this association would occur in other genera and species (Spielman et al. 2004).

There may be an artificial reduction of genetic diversity in domesticated livestock because of the

ubiquitous importation and use of exotic breeds in crossbreeding with local breeds. Such practices have not only decreased productivity through the introduction of unadaptable genetics to specific production environments, but they have also served to dilute the genetics necessary for adaptation to those environments. Consequently, it is imperative that the genetics of indigenous breeds be both characterized and preserved for use in increasing the efficiency of producing protein for humans and for other resources to assist in responding to the effects of global climate change on livestock production. Highly informative nuclear and cytoplasmic DNA molecular markers would allow estimation of levels of within-breed diversity, as well as the elucidation of genetic relationships with reported European and Latin American breeds (Delgado et al. 2012). Genomics can be used to provide estimates of genetic diversity in specific parts of the genome and facilitates conservation strategies based on specific genetic diversity (Windig and Engelsma 2010).

Maternally inherited mitochondrial DNA (mtDNA) has been used extensively to determine genetic diversity and to guide genetic resource conservation (e.g., Troy et al. 2001; Kikkawa et al. 2003; Kim et al. 2003; Cai et al. 2007); and mtDNA has been used to infer regions of domestication and to determine the number of maternal lineages and their geographic origins in macroevolution studies (Di Lorenzo et al. 2015). In this respect, mtDNA has been a very valuable tool in understanding the origin and domestication of cattle. It has been used extensively to determine origin and diversity of taurine cattle (*Bos taurus*) (Kantanen et al. 2009). Taurine mitochondrial diversity could be evaluated with two major mitochondrial haplotype clusters as reference, each group being usually named as European consensus (Eucons) and African consensus (Afcons) (Bradley et al. 1996; Ginja et al. 2010). These groups represent the central haplotypes of phylogenetic networks presented to date, to which a number of peripheral haplotypes coalesce (Troy et al. 2001; Miretti et al. 2004).

Many valuable attributes of Chinese Yellow Cattle have been characterized in recent years, especially those breeds in Western China. This practice has been detrimental to local Yellow cattle populations. Germplasm from Dengchuan, Tangjiao and Jinan cattle is at present largely unavailable. Populations of Zhoushan, Fuzhou, Pinglu Mountain, Bohai black, Guangfeng, Zaobei, and Leiqiong breeds have been severely reduced by crossbreeding; Zhoushan, Fuzhou, and Pinglu Mountain cattle are now nearly extinct. Additionally, Mongolian, Qinchuan, Nanyang, Luxi, Minnan and other local cattle breeds have been gradually replaced with exotic breeds in Chinese beef production systems (Chen and Cao 2000).

Thus, the objectives of this study were (1) to evaluate the genetic diversity and population genetic structure of native Chinese Yellow Cattle in the Loess Plateau of western China using the hypervariable D-loop region of mtDNA, and (2) to provide a genetic characterization that may serve as a scientific basis for future genetic resource conservation and management strategies of native Chinese Yellow Cattle in western China.

MATERIALS AND METHODS

Sampling

Blood samples were collected from 542 native cattle sampled from four populations to represent current blood lines and broad categories of body types in producer households and breeding enterprises in Baoji Prefecture of Shanxi province (Qinchuan, QC, n = 171), Qingyang Prefecture (Zaosheng, ZS, n = 184), Pingliang Prefecture (Pingliang, PL, n = 112) of Gansu province, in producer households and breeding enterprises in the Baoji Prefecture of Shanxi province and Guyuan prefecture (Guyuan, GY, n = 75) of Ningxia Hui Autonomous Region, respectively, which comprise the Loess Plateau. Samples were collected in tubes with anticoagulant (Heparin sodium) stored on ice, and transported to the laboratory where they were stored at −70 °C until analyzed. Additionally, to enhance reliability, 13 mtDNA D-loop sequences from Qinchuan cattle (DQ166083, DQ166084, DQ166085, DQ166086, DQ166087, DQ166088, DQ166089, AY521107, AY521108, AY521109, AY521110, AY521111 and AY902395), and 4 mtDNA D-loop sequences of Zaosheng cattle (DQ166063, DQ166064, DQ166065 and DQ166066) were downloaded from Genbank (www.ncbi.genbank.org).

Sequencing of the Bovine mtDNA D-Loop Region

Total DNA was extracted from blood samples according to the conventional phenol chloroform

extraction method from the Molecular Cloning Laboratory Manual (Sambrook and Russell 2002). A bovine mtDNA D-loop hypervariable region sequence was used to design the primer (Forward 5′-CTGCAGTCTCACCATCAACC-3′, Reverse 5′-GTGTAGATGCTTGCATGTAAGT-3′), which amplified a 617-base-pair fragment of D-loop using polymerase chain reaction (PCR). The 50 μL PCR reaction mix system contained 22 μL ddH_2O, 1 μL forward primer (0.01 mmol.μL^{-1}), 1 μL reverse primer (0.01 mmol.μL^{-1}), 25 μL premixed polymerase (Takara, Dalian, Liaoning, PRC) and 1 μL DNA (50 ng.μL^{-1}). The PCR reaction procedure consisted of a first denaturation step at 94 °C for 3 min, followed by 35 cycles of 30 s at 94 °C, 1 min at 51 °C for annealing, 1 min at 72 °C with an elongation step of 10 min at 72 °C in the last cycle, and stored at 4 °C. PCR products were detected by 2% agarose gel electrophoresis, then purified and sequenced (Sangon Biological Engineering, Inc. Shanghai, PRC).

Data Analysis

Sequences of the mtDNA D-loop were manually edited using ChromasVersion2.33 (http://www.technelysium.com.au/chromas.html). All analyzed sequences were collected by MEGA 5.0 (http://www.megasoftware.net) and the database was established. All sequences were aligned using Clustal X (http://www.igbmc.ustrasbg.fr/pub/ClustalX) multiple alignment software. Sites representing a gap in any of the aligned sequences were excluded from the analysis. Dnasp 5.10.1 (http://www.ub.edu/dnasp) was used to analyze nucleotide variable sites, single nucleotide polymorphisms, number of haplotypes, and to conduct Tajima's neutrality test and compute diversity of haplotypes, average numbers of nucleotide difference, and nucleotide diversity.

RESULTS AND DISCUSSION

Variation in the mtDNA D-loop Sequences

According to the complete nucleotide sequence of mtDNA D-loop (15792–16338 bp, 1–363 bp) (Genbank accession number V00654/J01394), 617 bp segments containing the hypervariable region of the mtDNA D-loop in 542 cattle were analyzed and 140 polymorphic sites were found. The percentage variation of nucleotides was 22.69%. Analysis detected 47 singleton variable sites with two variants, one singleton variable site with three variants, 82 parsimony informative sites with two variants, and 10 parsimony informative sites with three variants (Table 1). Nucleotide composition analysis of the 542 cattle showed that the average percentage of A, T, C and G was 34.3%, 29.4%, 23.2% and 13.1%, respectively. The average percentage of A + T (63.7%) was much greater than that of G + C (36.3%). The percentage of G + C in the mtDNA fragments ranged from 21% to 50%. Jia et al. (2007) reported that the percentage of G + C ranged from 21% to 43% in invertebrates and from 37% to 50% in vertebrates, similar to the results from this study.

Genetic Diversity of mtDNA D-loop

Parameters of genetic diversity of mtDNA D-loop among Chinese Yellow Cattle populations in the Loess Plateau are shown in Table 2. The total haplotype diversity (Hd ± SD), average number of nucleotide differences (k) and nucleotide diversity (Pi) were 0.971 ± 0.004, 14.538, and 0.02379 among 542 individuals in the four populations, respectively. The Tajima's D values ranged from −0.99401 to 0.00806 (P>0.10), and are consistent with the neutral mutation hypothesis. Cai et al. (2007), in a study of 18 beef cattle populations in China, similarly reported that the haplotypes identified in their study did not demonstrate deviations from the neutral mutation hypothesis.

Table 1. Mitochondrial DNA (mtDNA) D-loop variable sites in four Chinese Yellow Cattle populations in the Loess Plateau.

Population [1]	Variable Sites	Singleton Variable Sites	Parsimony Informative Sites
QC	89	32	57
ZS	99	38	61
PL	82	31	51
GY	70	25	45

[1] QC – Qinchuan, ZS – Zaosheng, PL – Pingliang, GY – Guyuan

Haplotype Diversity of mtDNA D-loop

There were 244 haplotypes defined based on 140 variable sites among the 542 individuals in the four populations. The frequencies of the different haplotypes showed considerable variability. The haplotype with the greatest frequency was H211 (12.0%). Other haplotypes with greater frequencies were H101 (7.0%), H48 (5.7%), and H57 (4.6%). Other haplotypes ranged in frequency from 0.2% to 3.5%.

Table 2. Genetic diversity of mitochondrial DNA (mtDNA) D-loop variable sites in four Chinese Yellow Cattle populations in the Loess Plateau.

Population[1]	Diversity of Haplotypes (Hd ± SD)	Average No. of Nucleotide Differences (k)	Nucleotide Diversity (Pi)	Tajimati Test D
QC	0.946 ± 0.012	16.312	0.02661	0.00806
ZS	0.976 ± 0.005	13.685	0.02236	-0.76650
PL	0.966 ± 0.010	14.503	0.02370	-0.31445
GY	0.975 ± 0.009	13.778	0.02248	-0.99401

[1] QC – Qinchuan, ZS – Zaosheng, PL – Pingliang, GY – Guyuan

There were 202 haplotypes unique to one of the four populations and 42 haplotypes shared among the four populations. Cai et al. (2007) identified 47 haplotypes at 105 polymorphic sites among 136 individuals from 18 populations of Chinese beef cattle.

The haplotype diversities for the four populations are given in Table 2. The haplotype diversity of the ZS population was 0.976 ± 0.005, which was numerically greatest among the four populations. The haplotype diversities of GY population (0.975 ± 0.009) and of PL population (0.966 ± 0.010) were lower, and the haplotype diversity of QC population (0.946 ± 0.012) was the least. The haplotype diversities of ZS and GY populations were greater than the average haplotype diversity (0.971 ± 0.004) of the four populations, and those for the QC and PL populations were lower. The genetic diversity of QC, as evidenced by haplotype diversity, was low relative to that of Zaosheng cattle. Haplotype diversity of PL and GY cattle did not appear largely diminished compared with that of ZS. Brenneman et al. (2007) studied genetic diversity among Angus, American Brahman, Senepol, and Romosinuano cattle breeds and reported diversity estimates ranging from 0.64 in Romosinuano to 0.75 in Brahman. Cai et al. (2007) researched mtDNA diversity in the cytb gene and genetic lineages of 18 cattle breeds from *Bos taurus* and *Bos indicus* and reported estimates from populations in central China ranging from 0.71 in Jiaxian cattle to 0.93 in Nanyang cattle, with Qinchuan cattle reported as 0.86.

Nucleotide Diversity of mtDNA D-loop

Estimates of nucleotide diversity for the QC, ZS, PL, and GY populations are given in Table 2. Nucleotide diversity was greatest in QC cattle (0.02661) and least in ZS cattle (0.02236). These estimates for the hypervariable region of the mtDNA D-loop are greater than those of Cai et al. (2007) who reported nucleotide diversities for the cytb gene ranging from 0.00029 to 0.01048. Cai et al. (2007) also reported that cattle breeds from central China had the greatest nucleotide diversity with nucleotide diversity in Qinchuan cattle reported at 0.00823, which was less than the Qinchuan hypervariable D-loop estimate in the current study.

Unique Haplotypes of mtDNA D-loop

The number of unique haplotypes in Qinchuan, Zaosheng, Pingliang, and Guyuan cattle was 56, 71, 42, and 33, respectively, accounting for 22.95%, 29.10%, 17.21% and 13.52% of the total haplotypes (244) in each population (Table 3). Brenneman et al. (2007) reported a mean frequency of private alleles in Angus, Brahman, Senepol, and Romosinuano of 0.102. Although clearly the Qinchuan, Pingliang, and Guyuan cattle breeds are unique, a significant number of haplotypes were shared among those breeds as well as Zaosheng cattle. It appears reasonable to infer that Qinchuan, Pingliang, and Guyuan cattle were derived from Zaosheng cattle.

Table 3. Number of unique (diagonal) and shared (off-diagonal) haplotypes for four populations of Chinese Yellow Cattle.

Population[1]	QC	ZS	PL	GY
QC	56	21	17	13
ZS		71	13	11
PL			42	12
GY				33

[1]QC – Qinchuan, ZS – Zaosheng, PL – Pingliang, GY – Guyuan

CONCLUSION

Mitochondrial DNA has been a valuable tool for assessment of genetic diversity in cattle. The present study evaluated the genetic diversity and structure of native cattle breeds in the Loess Plateau of western China using the hypervariable D-loop region of mtDNA. Genetic diversities of Zaosheng, Pingliang, and Guyuan cattle were similar while that of Qinchuan cattle was lower than those of the other

populations. It appears that Qinchuan, Pingliang and Guyuan native cattle might have been derived from Zaosheng cattle. Additionally, preservation of Qinchuan, Pingliang, and Guyuan cattle may save a portion of the genetic diversity in the Zaosheng cattle if population numbers of Zaosheng are insufficient for effective preservation efforts.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the project funding provided by the National Scientific and Technological Plan in Rural Areas of "Twelfth Five-year" (2015BAD03B04-4) and the Scientific Research Project of the Colleges and Universities in Gansu Province (2013A-063).

REFERENCES CITED

BRADLEY DG, MACHUGH DE, CUNNINGHAM P, LOFTUS RT. 1996. Mitochondrial diversity and the origins of African and European cattle. Proc Natl Acad Sci USA 93: 5131–5135.

BRENNEMAN RA, CHASE JR CC, OLSON TA, RILEY DG, COLEMAN SW. 2007. Genetic diversity among Angus, American Brahman, Senepol and Romosinuano cattle breeds. Anim Genet 38: 50–53.

CAI X, CHEN H, LEI CZ, WANG S, XUE K, ZHANG B. 2007. mtDNA diversity and genetic lineages of eighteen cattle breeds from *Bos taurus* and *Bos indicus* in China. Genetica 131: 175–183.

CHEN YC, CAO HH. 2000. Diversity of Chinese yellow cattle breeds and their conservation. Chinese Biodiversity 9(3): 275–283.

CHOI TJ, LEE SS, YOON DH, KANG HS, KIM CD, HWANG IH, KIM CY, JIN X, YANG CG, SEO KS. 2012. Determination of genetic diversity among Korean Hanwoo cattle based on physical characteristics. Asian-Australasian J Anim Sci 25: 1205.

DELGADO JV, MARTÍNEZ AM, ACOSTA A, ÁLVAREZ LA, ARMSTRONG E, CAMACHO E, CAÑÓN J, CORTÉS O, DUNNER S, LANDI V, MARQUES JR, MARTÍN-BURRIEL I, MARTÍNEZ OR, MARTÍNEZ RD, MELUCCI L, MUÑOZ JE, PENEDO MCT, POSTIGLIONI A, QUIRÓZ J, RODELLAR C, SPONENBERG P, UFFO O, ULLOA-ARVIZU R, VEGA-PLA JL, VILLALOBOS A, ZAMBRANO D, ZARAGOZA P, GAMA LT, GINJA C. 2012. Genetic characterization of Latin-American Creole cattle using microsatellite markers. Anim Genet 43: 2–10.

DI LORENZO P, CECCOBELLI S, PANELLA F, ATTARD G, LASAGNA E. 2015. The role of mitochondrial DNA to determine the origin of domestic chicken. World's Poult Sci J 71: 311–317.

FELIUS M. 1995. Cattle Breeds: An Encyclopedia. (No. Ed. 1) Netherlands: C Misset, bv. 799 p.

GINJA C, PENEDO MCT, MELUCCI L, QUIROZ J, MARTÍNEZ-LÓPEZ OR, REVIDATTI A, MARTÍNEZ-MARTÍNEZ A, DELGADO JV, GAMA LT. 2010. Origins and genetic diversity of New World Creole cattle: inferences from mitochondrial and Y chromosome polymorphisms. Anim Genet 41: 128–141.

JIA SG, CHEN H, ZHANG GX, WANG ZG, LEI CZ, YAO R, HAN X. 2007. Genetic variation of mitochondrial D-loop region and evolution analysis in some Chinese cattle breeds. J Genet Genom 34: 510–518.

KANTANEN J, EDWARDS CJ, BRADLEY DG, VIINALASS H, THESSLER S, IVANOVA Z, KISELYOVA T, CINKULOV M. 2009. Maternal and paternal genealogy of Eurasian taurine cattle (*Bos taurus*). Heredity 103: 404–415.

KIKKAWA Y, TAKADA T, SUTOPO NK, NAMIKAWA T, YONEKAWA H, AMANO T. 2003. Phylogenies using mtDNA and SRY provide evidence for male-mediated introgression in Asian domestic cattle. Anim Genet 34 (2): 96–101.

KIM KI, LEE JH, LEE SS, YANG YH. 2003. Phylogenetic relationships of Northeast Asian cattle to other cattle populations determined using mitochondrial DNA D-Loop sequence polymorphism. Biochem Genet 41(3–4): 91–98.

MIRETTI MM, DUNNER S, NAVES M, CONTEL EP, FERRO JA. 2004. Predominant African-derived mtDNA in Caribbean and Brazilian Creole cattle is also found in Spanish cattle (*Bos taurus*). J Hered 95: 450–453.

NOTTER DR. 1999. The importance of genetic diversity in livestock populations of the future. J Anim Sci 77: 61–69.

OUBURG NJ, ANGELONI FP, VERGEER P. 2010. An essay on the necessity and feasibility of conservation genomics. Conserv Genet 11(2): 643–653.

SAMBROOK J, RUSSELL DW. 2002. Molecular Cloning: A Laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press.

SPIELMAN D, BROOK BW, BRISCOE DA, FRANKHAM R. 2004. Does inbreeding and loss of genetic diversity

decrease disease resistance? Conserv Genet 5(4): 439–448.

TROY CS, MACHUGH DE, BAILEY JF, MAGEE DA, LOFTUS RT, CUNNINGHAM P, CHAMBERLAIN AT, SYKES BC, BRADLEY DG. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature 410: 1088–1091.

WINDIG JJ, ENGELSMA KA. 2010. Perspectives of genomics for genetic conservation of livestock. Conserv Genet 11(2): 635–641.