Analysis of Microsatellite DNA Polymorphisms in Five China Native Cattle Breeds and Application to Population Genetics Studies

Jin Hai-Guo, Zhao Yu-min and Zhou Guo-li*

Branch of Animal Husbandry Jilin Academy of Agricultural Science, Gongzhuling, Jilin 136100, P. R. China

ABSTRACT : Five China native cattle breeds have been characterized by using 10 microsatellite DNA markers. The studied populations can be divided into five groups: Luxi cattle, Nanyang cattle, Jinnan cattle, Qinchuan cattle and Yanbian cattle. Allele frequencies were calculated and used for the characterization of the breeds and the study of their genetic relationships. Heterozygosity, polymorphism information content, the effective number of alleles was calculated. Nei's standard genetic distance (1978) was calculated and used for a neighbor-joining tree construction. NJ tree showed that Luxi cattle, Nanyang cattle, Jinnan cattle and Qinchuan cattle are closely related, whereas Yanbian cattle are clearly distinct from other four populations. The genetic relationship of five breeds corresponds to their history and geographic origins. This work analyzes the recent origin of these populations and contributes to the knowledge and genetic characterization of China native breeds. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1696-1700)***

Key Words : China Native Cattle, Genetic Diversity, Microsatellite Markers, Population Genetics

INTRODUCTION

In the world of animal production, a good method to identify animals and their products unambiguously is of utmost importance in order to obtain accurate selection and high-quality products. In the light of the continuously growing demand for better products, faster and more reliable methods of identification of individuals are essential. Identification methods such as typing of blood groups and biochemical polymorphisms have proved their usefulness, but the discriminating power of these techniques is less than that of DNA markers. Moreover, the number of different tissues on which the typings can be done is very limited and represents a significant limitation of such methods. Several DNA-based technologies to type polymorphic loci have been developed in the last decade. These techniques include restriction fragment length polymorphism (RFLP), variable number of tandem repeats (VNTR), single-strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), random-amplified polymorphic DNA (RAPD) and also methods which make use of the polymorphism of short tandem repeats, i.e. the so-called microsatellites. These are now more than a thousand cattle microsatellites to choose from (Barendse et al., 1994; Kappes et al., 1997). Genotyping can be done on most tissues and cell types, and international comparison tests under the auspices of the International Society for Animal Genetics (ISAG) to establish international standards exist.

Luxi cattle, Nanyang cattle, Jinnan cattle, Qinchuan cattle, Yanbian cattle are five excellent yellow cattle breeds in China. Despite the introduction of several European cattle breeds into China cattle breeds for upgrading native cattle breeds, native people have endeavored to preserve local breeds as unique genetic sources. Awareness of the value of genetic resources has stimulated the study of the genetic diversity of native breeds. However, most of the researches have been accomplished on European cattle breeds and very little information is available concerning the genetic diversity of five China native cattle breeds. The purpose of the present study was to examine genetic diversity among five China native cattle populations by documenting microsatellite DNA polymorphisms. We also estimated the gene differentiation and the genetic relationship within and between five China native cattle breeds.

MATERIALS AND METHODS

Samples collection and DNA extraction

Fresh blood samples were collected from 50 animals from each of the five cattle breeds: Luxi cattle (LX), Nanyang cattle (NY), Jinnan cattle (JN), Qinchuan cattle (QC) and Yanbian cattle (YB) located at distinct geographical areas and chosen at random without consideration of the relationships among the animal. The main locations of these cattle breeds and their historic origin come from the northwest of Shangdong province, southeast of Henan province, Nanyang of Shanxi province, Qinchuan of Shanxi province and Yianbian of Jiling provice, respectively. LX cattle, NY cattle, JN cattle and QC cattle locate in middle east region of P. R. China. YB cattle locates in northeast region of P. R. China. Genomic DNA was isolated from blood samples by the phenol/chloroform extraction method according to Strauss (1991) and

* Corresponding Author: Zhou Guo-Li. College of life science, Liaocheng University, Liaocheng, Shandong 252059, P. R. China. Tel: +86-635-8239908, E-mail: glzhou@lctu.edu.cn

Received September 22, 2004; Accepted May 17, 2005

Tabl4e 1. The optimized conditions of PCR and distribution of the microsatellite on chromosome

Microsatellite loci	Chromosome	Annealing temperature (°C)	Concentration of Mg^{2+} (Mm)
ETH3	19	65	1.5
ETH10	5	65	1.5
ETH225	9	65	1.5
BM1824	1	58	1.2
BM2113	2	58	1.2
TGLA48	7	56	1.2
TGLA53	16	56	2.0
TGLA122	21	55	2.0
TGLA126	20	55	1.5
TGLA227	18	55	1.5

dissolved in TE solution. The DNA samples were stored at -20°C and/or at 4°C.

Microsatellite markers

A total of 10 bovine microsatellite markers were studied in the different cattle breeds (Table 1), including 5 microsatellite markers approved for diversity studies by the EU AIRE 2066 Concerted Action Group, and recommended by the MoDAD program (FAO) (ETH3, ETH10, ETH225, BM1824, BM2113). The other 10 microsatellites are included in the international Cattle DNA polymorphism comparison tests held under the auspices of ISAG (TGLA48, TGLA53, TGLA122, TGLA126, TGLA227) (Georges et al., 1992; Fries et al., 1993; Barendse et al., 1994; Bishop et al., 1994). Primers and map position of markers can be found in The Domestic Animal Diversity Information System (http://www.fao.org/dad-is).

Microsatellite analysis

10 microsatellite markers for the genetic diversity study in cattle were used for the analysis of five China native cattle breeds. The polymerase chain reaction (PCR) was accomplished in a total volume of 25 μl containing 50 ng of genomic DNA, $MgCl_2$ (concentration shown in Table 1), 200 μM of each dNTP, 4 pmol of each primer, and 1 unit of Taq polymerase. The PCR cycle was accomplished by denaturation for 1 min at 94°C, primer annealing for 1 min at the desired temperature (Table 1), and an extension for 1 min at 72°C and repeated 30 times. The PCR products were separated on a 10-12% polyacrylamide gel according to size of products and gels were stained with silver nitrate (silver staining) (Lu, 1993) after electrophoresis to read fragment sizes by AlphaImager software (Alpha Innotech Co., USA, Version 5.1).

Statistical analysis

Allele frequencies were determined by direct counting. Gene heterozygosity (h) and effective number of alleles (No.E.A.), Polymorphism information content (PIC) of microsatellite loci were calculated on the basis of allele frequencies.

Gene heterozygosity was calculated according to Nei et al. (1978).

$$h = 1 - \sum_{i=1}^{n} P_i^2$$

The effective number of alleles, a reciprocal of the degree of homozygosity, was calculated in accordance with Hines et al. (1981).

$$n_e = 1 \Big/ \sum_{i}^{n} P_i^2$$

where P_i is the frequency of i^{th} allele from a certain locus; n is the number of alleles from a certain locus.

PIC values were calculated by using the method described by Botstein et al. (1980).

$$PIC = 1 - (\sum_{i=1}^{n} P_i^2) - (\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 P_i^2 P_j^2)$$

where P_i and P_j stand for frequency of band i and band j respectively in one population; n is the number of alleles from a certain locus.

Nei's standard genetic distance between the breeds were calculated according to Nei et al. (1978). Phylogenetic trees derived form Neighbor-joining (NJ) method were generated with programs in the phylip software package (Felsenstein, 1995).

$$D_s = -Ln(I) \qquad I = J_{XY} \Big/ \sqrt{J_X J_Y}$$

$$J_X = \sum_{j=1}^{r} \sum_{i=1}^{m_j} x_{ij}^2 \Big/ r \quad J_y = \sum_{j=1}^{r} \sum_{i=1}^{m_j} y_{ij}^2 \Big/ r \quad J_{XY} = \sum_{j=1}^{r} \sum_{i=1}^{m_j} x_{ij} y_{ij} \Big/ r$$

where I stand for similarity coefficients of between breeds; x_{ij} and y_{ij} is the frequency of i^{th} allele from j^{th} locus in the x and y population respectively; m_j is the number of alleles from j^{th} locus; r is the number of alleles from a certain locus.

RESULTS

Genetic diversity of microsatellites

Number of alleles, size range of alleles, PIC, heterozygosity and effective number of alleles are given in Table 2. All the loci were polymorphic and the number of alleles varied between four (BM1824 and TGLA48) and 13 (TGLA122), with generally little difference between the

Table 2. Genetic characterisitics of 10 microsatellite loci in five China native cattle breeds

Locus	Breed	No. allele	Size range (bp)	PIC	h	No. E.A.*
ETH3	LX	7	117-129	0.73	0.76	4.13
	NY	6	117-127	0.72	0.73	4.10
	JN	6	117-129	0.69	0.70	3.99
	QC	7	109-127	0.71	0.72	4.08
	YB	5	117-127	0.67	0.63	3.50
ETH10	LX	8	209-223	0.70	0.75	4.11
	NY	7	209-225	0.72	0.76	4.15
	JN	8	209-227	0.70	0.73	4.08
	QC	7	209-225	0.67	0.71	3.98
	YB	6	209-221	0.65	0.70	3.26
ETH225	LX	8	140-152	0.74	0.75	4.32
	NY	6	140-150	0.72	0.74	4.30
	JN	7	140-152	0.70	0.72	4.19
	QC	7	140-152	0.71	0.74	4.26
	YB	6	140-150	0.69	0.70	3.43
BM1824	LX	5	178-190	0.65	0.74	4.01
	NY	5	178-190	0.64	0.71	3.99
	JN	5	178-190	0.60	0.69	3.53
	QC	6	178-188	0.62	0.71	3.95
	YB	4	178-188	0.59	0.64	3.18
BM2113	LX	7	121-139	0.76	0.79	5.20
	NY	8	125-141	0.78	0.80	5.38
	JN	7	121-139	0.75	0.77	5.09
	QC	6	125-139	0.70	0.72	3.98
	YB	7	125-141	0.73	0.78	4.99
TGLA48	LX	5	73-79	0.47	0.60	2.68
	NY	4	73-77	0.41	0.56	2.63
	JN	4	73-77	0.39	0.55	2.49
	QC	5	73-77	0.50	0.63	3.09
	YB	4	73-77	0.37	0.51	2.01
TGLA53	LX	12	152-178	0.85	0.86	5.22
	NY	12	152-180	0.84	0.83	4.83
	JN	11	152-178	0.82	0.81	4.48
	QC	11	152-178	0.80	0.80	4.39
	YB	10	152-178	0.80	0.79	3.91
TGLA122	LX	12	139-179	0.79	0.83	5.41
	NY	13	137-181	0.75	0.81	5.22
	JN	12	139-177	0.73	0.79	4.46
	QC	12	139-177	0.74	0.80	5.08
	YB	11	137-177	0.70	0.74	4.14
TGLA126	LX	5	117-125	0.63	0.69	3.11
	NY	6	115-125	0.64	0.70	3.28
	JN	6	115-125	0.61	0.67	2.98
	QC	5	117-123	0.60	0.69	2.91
	YB	5	117-123	0.59	0.61	2.48
TGLA227	LX	9	79-105	0.84	0.84	5.05
	NY	7	83-99	0.81	0.80	4.68
	JN	8	83-103	0.80	0.80	4.42
	QC	8	79-105	0.78	0.79	4.39
	YB	7	79-103	0.76	0.77	3.58

* No. E.A.= Effective number of alleles.

cattle breeds. The effective number of alleles range from 2.68 (TGLA48) to 5.41 (TGLA122) in Luxi cattle, from 2.63 (TGLA48) to 5.38 (BM2113) in Nanyang cattle, from 2.49 (TGLA48) to 5.09 (BM2113) in Jinnan cattle, from 2.91 (TGLA126) to 5.08 (TGLA122) in Qinchuan cattle and from 2.01 (TGLA48) to 4.99 (BM2113) in Yianbian cattle. The mean effective number of alleles is highest in Luxi cattle (4.32) and lowest in Yanbian cattle (3.45), and average in Nanyang cattle (4.26) and Jinnan cattle (4.00) and Qinchuan cattle (4.01). These numbers are also reflected in the mean heterozygosity, being 0.76, 0.74, 0.72, 0.73 and 0.69, for Luxi cattle, NanYan cattle, Jinnan cattle, Qinchuan cattle and Yanbian cattle, respectively.

Table 3. Nei's (1978) standard distance among five China native cattle breeds

	Luxi	Nanyang	Jinnan	Qinchuan	Yanbian
Luxi	0				
Nanyang	0.0250	0			
Jinnan	0.1367	0.1035	0		
Qinchuan	0.1063	0.1266	0.0967	0	
Yanbian	0. 2346	0.2013	0.2938	0.3519	0

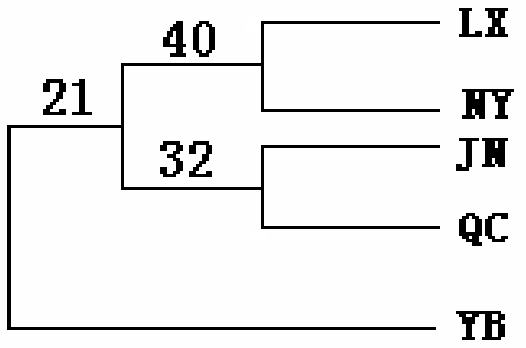

Figure 1. The NJ dendrogram of five China native cattle breeds using Ds, numbers on the nodes are percentage bootstrap values from 1,000 replications of resampled loci.

Genetic distances

Using Nei (1978) standard genetic distances (Table 3) and the NJ method of clustering, the dendrogram (Figure 1) of relationships among five China native cattle breeds was obtained. The LX and NY breeds were grouped together. The JN and QC breeds were grouped together. The YB breed has unique branch. That is, the closet breeds were found to be LX and NY cattle, JN and QC cattle. The most divergent was found to be the YB cattle.

DISCUSSIONS

The polymorphism information content (Botstein, 1980) is a parameter indicative of the degree of informativeness of a marker. Following this author and according to our data, we can consider: Except for microsatellite locus TGJG48 as moderately informative (0.25<PIC<0.5), other mirosatellite DNA loci display highly polymorphic (PIC>0.5). According to the selective standard of microsatellite DNA loci (Barker,

1994), microsatellite DNA loci ought to have four alleles at least. Therefore, ten microsatellite DNA loci in this study can be used for evaluation of genetic diversity. The effective number of alleles is an estimate of the number of alleles with equal frequencies corresponding to a particular PIC value. It is an inverse function of the theoretical homozygosity and it allows comparison of populations with different distributions of allele frequencies, reducing the effect of infrequent alleles. The mean effective number of alleles is highest in Luxi cattle (4.32) and lowest in Yanbian cattle (3.45) in this present study. Heterozygosity is an optimum parameter that reflects genetic variation of the population. In this present study, the mean heterozygosity is 0.76, 0.74, 0.72, 0.73 and 0.69, for Luxi cattle, Nanyan cattle, Jinnan cattle, Qinchuan cattle and Yanbian cattle, respectively.

Genotype data from 10 microsatellites typed in 250 animals were used here to assess the genetic diversity of five China native cattle breeds. Except for YB cattle, other cattle breeds (LX, NY, JN and QC) display a relatively high heteroaygosity compared with European cattle breeds (MacHugh, 1998; Martin-Burriel, 1999; Hanslik, 2000). On the other hand, YB cattle show the lowest genetic diversity from all analyses. Since the early 19th century, when the concept of a breed grew in currency (Felius, 1995), many European cattle breeds have become genetically isolated and in most cases their origins could be traced to a small pool of founder individuals. YB cattle have experienced a similar breeding practice to the case of European breeds. YB cattle could be also considered as a typical island population in which a founder effect and genetic drift could contribute to the loss of variation. Therefore, considering their recent founding population size, it is assumed that YB cattle would display a low level of gene diversity. Whereas, a small effort for breeding has been performed in other cattle breeds (LX, NY, JN and QC), providing a possible reason for the high degree of genetic diversity in these four populations.

Our tree (Figure 1) shows that LX cattle, NY cattle, JN cattle and QC cattle are closely related, whereas YB cattle are clearly distinct from the other four populations. A similar relationship between LX cattle, NY cattle, JN cattle and QC cattle may be a result of the massive introgression between these four populations due to adjacent to each other in these four populations. On the other hand, YB cattle exhibits other four populations outgroup, probably due to their smaller size or reproductive isolation showed in distinct geographical areas. The genetic relationship of five China native cattle breeds corresponds of their history and geographic origins.

Since 1989, microsatellite DNA has been used in location of quantitative trait loci of livestock (Ashwell, 1999; Crawford, 1991; Davis, 1998). A saturated microsatellite based linkage map for cattle provide the foundation for identification of loci contributing to the genetic variance for economic traits (ETL) and the exploitation of marker assisted selection (MAS) for phenotypes of interest (Fries, 1993). In accordance with other investigations (Arranz, 1996), our results indicated that, in general, variability of microsatellite DNA loci is much larger than that of traditional genetic markers based on unique-sequence DNA mutations. Microsatellite DNA can be a useful tool in genetic studies such as parentage determination, population studies, linkage analysis and genome mapping.

This study is the first using microsatellite DNA markers to understand genetic diversity of five China native cattle breeds. Only little information is currently available to compare different cattle populations from China. Although we have used only five representatives to understand genetic backgrounds of five China native cattle breeds. The present study contributes to the knowledge of genetic diversity of local cattle breeds in China. Further investigations including more China native cattle breeds would be useful to clarify their recent origin and relationships between these local breeds.

REFERENCES

Arranz, J. J., Y. Bayon and F. San Primitivo. 1996. Comparison of protein markers and microsatellites in differentiation of cattle population. Anim. Genet. 27:415-419.

Ashwell, M. S. and C. P. Van Tassell. 1999. Detection of putative loci affecting milk, health, and type traits in a US Holstein population using 70 microsatellite markers in a genome scan. J. Dairy Sci. 82:2497-2502.

Barendse, W., S. M. Armitage and L. M. Kossarek. 1994. A genetic linkage map of the bovine genome. Nat. Genet. 6:227-235.

Barker, J. S. F. 1994. A global protocol for determining genetic distances among domestic livestock breeds. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, and Ontario, Canada. 21:501-508.

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, J. Yoo and C. W. Beattie. 1994. A genetic linkage map for cattle. Genet. 136:619-639.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis. 1980. Construction of a genetic likage map in human using restriction fragment length polymorphisms. Anim. J. Hum. Genet. 32:314-331.

Crawford, A. M., F. C. Buchanan and P. A. Swarbrick. 1991. The use of dinucleotide repeats or microsatellites as genetic markers in domestic animals. Proceedings of the New Zealand Society of Animal Production 51:79-83.

Davis, G. P., D. J. S. Hetzel and N. J. Corbet. 1998. The mapping of quantitative trait loci for birth weight in a tropical beef herd. In: Proceedings of 6th World Congress on Genetics Applied to Livestock Production, Armidale and NSW, Australia. 26:441-

444.

Felius, M. 1995. Cattle breeds-An Encyclopedia. Misset, Doetinchem, The Netherlands.

Felsenstein, J. 1995. Phylip (Phylogeny Inference Package), Version 3.57c, Department of Genetics, University of Washington. Saettle, WA.

Fries, R., A. Eggen and J. E. Womack. 1993. The bovine genome map. Mamm. Genome. 4:405-428.

Georges, M. and J. M. Massey. 1992. Polymorphic DNA Markers in Bovidae. WO Publ. no. 92/13102. World Intellectual Property Organization, Geneva.

Hanslik, S., B. Harr, G. Brem and C. Schlotterer. 2000. Microsatellite analysis reveals substantial genetic differentiation between contemporary New World and Old World Holstein Friesian populations. Anim. Genet. 31:31-38.

Hines, H. C., J. P. Zikakis and G. F. Haenlein. 1981. Linkage relationships among loci of polymorphisms in blood and milk of cattle. J. Dairy Sci. 64(1):71-76.

Kappes, S. M., J. W. Keele and R. T. Stone. A second-generation linkage map of the bovine genome. Genome Res. 7:235-249.

Lu, S. D. 1993. An experiment technology of molecular biology. Higher Education Publishing Company, Beijing, China.

MacHugh, D. E., R. T. Loftus, P. Cunningham and D. G. Bradley. 1998. Genetic structure of seven European cattle breeds assessed using 20 microsatellites markers. Anim. Genet. 29:333-340.

Martin-Burriel, I., E. Garcia-Muro and P. Zaragoza. 1999. Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. Anim. Genet. 30:177-182.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89:583-590.

Strauss, W. M. 1991. Preparation of genomic DNA from mammalian tissue. In: Current Protocols in molecular Biology (Ed. F. M. Ausubel, R. Brent and R. E. Kingston) Vol. 1, pp. 2.2.1-2.2.3. John Wiley and Sons. New York, NY., USA.