RESEARCH NOTE

Genetic diversity analysis of five cattle breeds native to China using microsatellites

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Introduction

Microsatellites have proved to be useful polymorphic markers for the analysis of genetic diversity. Microsatellite based studies in livestock have mainly concentrated on pig, cattle and sheep, and there are now more than a thousand cattle microsatellite markers to choose from (Barendse *et al*. 1994; Kappes *et al*. 1997). Genotyping of cattle 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. Awareness of the value of genetic resources in livestock has stimulated the study of the genetic diversity of native breeds. However most of such studies have been done on European cattle breeds and very little information is available concerning the genetic diversity of cattle breeds native to China. The purpose of the present study was to examine genetic diversity among five native Chinese cattle breeds through examining microsatellite DNA polymorphisms. We also estimated the genetic differentiation and the genetic relationship within and between the five native Chinese cattle breeds.

Materials and methods

Sample collection and DNA extraction

Fresh blood samples were collected from 50 animals from each of the five cattle breeds: Luxi (LX), Nanyang (NY), Jinnan (JN), Qinchuan (QC) and Yanbian (YB) located at distinct geographical areas in China. Individual animals of each breed were chosen at random without consideration of the relationships among the animals. Genomic DNA was isolated using whole blood lysis (Li *et al*. 1997) and dissolved in TE solution. The DNA samples were stored at – 20°C and/or at 4°C.

Microsatellite markers

A total of ten bovine microsatellite markers were studied in the different cattle breeds, including five 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 five microsatellites are included in the international Cattle DNA polymorphism comparison tests held under the auspices of ISAG (TGLA48, TGLA53, TGLA122, TGLA126, TGLA227) (Fries *et al*. 1993; Barendse *et al*. 1994; Bishop *et al*. 1994). Primers and map position of these markers can be found in The Domestic Animal Diversity Information System (*http://www.fao.org/dad-is*).

Microsatellite analysis

Ten microsatellite markers for the genetic diversity study in cattle were used for the analysis of five native Chinese cattle breeds. The polymerase chain reaction (PCR) was accomplished in a total volume of 25 μl containing 50 ng of genomic DNA, $MgCl_2$ (Mg^{2+} concentration for markers BM1824, BM2113 and TGLA48 was 1.2 mM; Mg^{2+} concentration for markers ETH3, ETH10, ETH225, TGLA126 and TGLA227 was 1.5 mM; Mg^{2+} concentration for markers TGLA53 and TGLA122 was 2.0 mM), 200 μM of each dNTP, 4 pmol of each primer, and 1 unit of *Taq* polymerase. The PCR cycle was accomplished by denatura-

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tion for 1 min at 94°C, primer annealing for 1 min at the desired temperature, an extension for 1 min at 72°C, with the whole cycle and repeated 30 times. The PCR products were separated on a 10–12% polyacrylamide gel, according to the size of the products, and the gels were stained with silver nitrate (silver staining) after electrophoresis to read fragment sizes using the AlphaImager software (Alpha Innotech Co., USA, Version 5.1).

Allele frequencies were determined by direct counting. Gene heterozygosity (H), effective number of alleles (n_e), polymorphism information content (PIC) of microsatellite loci, and Nei's standard genetic distance between the breeds were calculated, following Nei (1978), Hines *et al*. (1981) and Botstein *et al*. (1980). A phylogenetic tree of the five breeds was generated with programs in the Phylip software package, using neighbour-joining method.

Results and discussion

Genetic diversity of microsatellites

Number of alleles, size range of alleles, PIC, heterozygosity and effective number of alleles for the five breeds are given in table 1. 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 cattle breeds. The effective number of alleles ranged 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 was highest in Luxi cattle (4.32) and lowest in Yanbian cattle (3.45), and intermediate 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.

The polymorphism information content (Botstein *et al*. 1980) is a parameter indicative of the degree of informativeness of a marker. Following the criteria of Botstein *et al*. (1980), in the present study, microsatellite locus TGLA48 appeared to be only moderately informative ($0.25 < PIC < 0.5$), whereas the other mirosatellite loci studied were highly informative ($PIC > 0.5$). According to the selective standard of microsatellite loci (Barker 1994), microsatellite loci ought to have at least four alleles to be considered useful for the evaluation of genetic diversity. Based on this criterion, too, the ten microsatellite loci used in the present study can be considered useful for the evaluation of genetic diversity in cattle breeds.

Genotype data from ten microsatellites typed in 250 animals were used here to assess the genetic diversity of five native Chinese cattle breeds. Except for YB cattle, other

Table 1. Genetic characterisitics of 10 microsatellite loci in five China native cattle breeds.

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

Table 2. Nei's (1978) standard distance among five China native cattle breeds.

Cattle breed	Luxi	Nanyang	Jinnan	Qinchuan	Yanbian
Luxi	****				
Nanyang	0.0250	****			
Jinnan	0.1367	0.1035	****		
Qinchuan	0.1063	0.1266	0.0967	****	
Yanbian	0.2346	0.2013	0.2938	0.3519	****

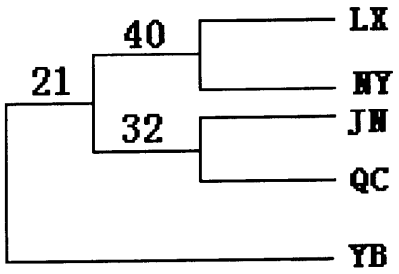

Figure 1. The neighbour joining dendrogram of five China native cattle breeds using Nei's (1978) standard distance. Numbers on the nodes are percentage bootstrap values from 1000 replications of resampled loci.

cattle breeds (LX, NY, JN and QC) displayed a relatively high heterozygosity compared with European cattle breeds (MacHugh *et al*. 1998; Martin-Burriel *et al*. 1999; Hanslik *et al*. 2000). On the other hand, YB cattle showed the lowest genetic diversity from all analyses. Since the early 19th century, when the concept of a breed grew in currency, 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 also experienced a similar breeding practice. 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 genetic variation. Therefore, considering their recent founding population size, it is assumed that YB cattle would display a low level of gene diversity. In the case of the other cattle breeds (LX, NY, JN and QC), however, extensive breeding programmes have not been undertaken, providing a possible reason for the high degree of genetic diversity in these four populations.

Using Nei's standard genetic distances (table 2) and the neighbour joining method of clustering, the dendrogram (figure 1) of relationships among the five 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. Thus, our data indicate that LX cattle, NY cattle, JN cattle and QC cattle are closely related, whereas YB cattle are clearly distinct from the other four populations (figure 1). 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 being geographically adjacent to each other. On the other hand, YB cattle are from a geographical area relatively far from the locations of the other four breeds. Thus, the genetic relationship of these five native Chinese cattle breeds corresponds to their breeding history and geographic origins.

This study is the first using microsatellite DNA markers to understand genetic diversity of native cattle breeds in China. Very little information is currently available to compare different cattle populations from China. Thus, although we have used only five representative breeds, the present study may be regarded as the beginning of attempts to understand the genetic diversity of local cattle breeds in China. Further investigations including more native Chinese cattle breeds would be useful to clarify their recent origin and relationships between them.

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