

Analysis of genetic diversity and distances in Asian cattle breeds using microsatellite markers

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Abstract

This study defined the genetic diversity of five breeds of cattle in Asia by analyzing 6 microsatellite markers in 270 animals. Based on expected mean heterozygosity, the lowest genetic diversity was exhibited in Japanese black cattle (HE=0.5849), and the highest in Chinese yellow cattle (HE=0.8073). Average proportion of genetic variation due to interpopulation subdivision among these five cattle breeds varied between 11.7 and 12.5%. The genetic distances were roughly divided into three groups: Japanese black cattle, Holstein, and the three remaining breeds. This clustering agrees with the origin and geographical distributions of these five cattle breeds.

Keywords: Cattle breeds, genetic diversity, microsatellite marker.

1. Introduction

Most of the cattle in Asia are classified as *Bostaurus* and characterized by lack of humps. Earlier remains of domestic bovine from at least 8000 years ago were found in Anatolia. However, the earliest domestic cattle found in Asia dated to 5000 years ago, several thousand years after domestication by primary aurochs in west Asia (Payne and Hodges, 1997). With the introduction of rice cultivation, cattle migrated from north China via the Korean peninsula to Japan around the second century A.D. (Mannen *et al.*, 1998). Many indigenous breeds are facing extinction and genetic variability in small population because European breeds were often used as sire lines for crossbreeding with Chinese indigenous cattle breeds.

The existence of genetic polymorphism or diversity in a population is the basis of genetic improvement by selection and needs to be accurately estimated (Lee *et al.*, 2007; Lee *et al.*,

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2010; Lee, 2009; Yeo *et al.*, 2006). The current genetic composition of a species influences the capacity of its members to adapt to future physical and biotic environmental influences. However, selection for economically desirable traits can lead to genetic erosion of a breed, which likely limits genetic gain for production, as well as for future fitness traits. Therefore, an analysis of the genetic composition of breeds is an important tool. Microsatellites have proved to be useful polymorphic markers for analysis of genetic diversity. Microsatellite based studies in livestock have concentrated mainly on pigs, cattle, and sheep, and more than a thousand cattle microsatellite markers can now be chosen (Barendse *et al.*, 1994; Kappes *et al.*, 1997; Zhang *et al.*, 2007). North-east Asian cattle (Hanwoo, Chinese yanbian cattle, Japanese cattle and Holstein) were analyzed using microsatellite markers (Kim *et al.*, 2002). Until now, there has been little information about southern Chinese yellow cattle. This study regards 6 microsatellite markers in five cattle breeds of diverse geographical origin, in order to assess the level of genetic diversity and genetic distance among cattle breeds. Development of several microsatellites is very important as useful tools for genetic characterization of the five species of cattle and to provide a means to quantify genetic diversity and to determine if a relationship exists between genetic distance estimates and observed heterosis.

2. Materials and methods

2.1. Sample collection for DNA analysis

Tissue samples were taken from individuals located in distinct geographical areas and chosen at random without consideration among animals. Table 2.1 shows these five breeds' information.

Table 2.1 Details of sampling for five cattle populations.

| Breed | Location of sampling | Type of sample | No.of sample |
|-----------------------|----------------------|----------------|--------------|
| Hanwoo | Korea | Tissue | 100 |
| Korean brindle cattle | Korea | Tissue | 50 |
| Holstein | Korea | Tissue | 50 |
| Chinese yellow cattle | China | Tissue | 47 |
| Japanese black cattle | China | Tissue | 23 |
| Total | | | 270 |

2.2. PCR conditions and DNA genotyping

6 bovine microsatellite markers were selected from the CaDBase database on genetic diversity (<http://www.projects.roslin.ac.uk/cdiv/accessdb.html>). Approximately 20ng of genomic DNA per PCR was used. Samples were amplified in a reaction volume of 15uL containing 7.85uL of deionized, distilled water, 1.5uL of 10×buffer (including 25mM MgCl₂), 0.3uL of a mixture of 5mM each dNTP, 0.25uL of DMSO (SIGMA, France), 2.0uL of the labeled forward primer, 2.0uL of the reverse primer, and 0.1uL of F-Taq DNA polymerase. A standard thermocycling protocol of 5min at 94°C, followed by 30 cycles of 1min at 94°C by thermal cycler (Biometra, Goettiagen, Germany), 30sec at the annealing temperature, and 1min at 72°C, and ending with an extension phase of 10min at 72°C was used and repeated 30times.

ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems co., Foster City, USA) was used for analysis of the PCR product. PCR products were filled with Hi-Di™ Formamide (Geneticanalysisgrade, U.S.A). ABI PRISM Genemapper v4.0 software (Applied Biosystems co., Foster City, USA) was used for analysis of the result.

2.3. Statistical analysis

Genetic diversity can be evaluated according to the number of alleles per locus, average number of alleles for all loci, heterozygosity, PIC content, and coefficient of gene differentiation. The GENETIX (version 4.05) computer package computes several basic parameters of population genetics, such as Nei's D and H, Wright's F-statistics (the Weir-Cockerham's and Robertson-Hill's estimators, 1984), and linkage disequilibrium D according to Black & Krafur. And also two estimators (FST and GST) of genetic variation as a result of inter-population subdivision were estimated. Genetic distance between subpopulations according to Nei (1978) were calculated. Then a hierarchical cluster analysis was performed using the Allele frequencies were used to generate the DA distances for each pair of four cattle populations. A neighbour-joining tree based on the genetic distances (1) and TreeView 1.6.6 (Page, 1996).

$$I = (\sum P_{ix} \bullet P_{iy}) \div [(\sum P_{ix}^2) \cdot (\sum P_{iy}^2)]^{1/2}.$$

Where the P_{ix} is the proportion of allele i in population X, P_{iy} is the proportion of allele i in population Y.

3. Results

3.1. Genetic variability

Overall, 81 alleles were detected for the 6 microsatellite loci in 270 animals from the five cattle breeds which showed the number of alleles per locus ranging from 12 (BM1818) to 16 (BM2113) and with a mean of 13.5. Chinese yellow cattle generally have more alleles per locus than other cattle breeds. Japanese black cattle have fewer alleles than other cattle breeds. All the loci were polymorphic and several unique alleles were found in yellow cattle, but they may not use as breed markers because of their low frequencies in the small sample size.

Genetic diversity is measured by heterozygosity, polymorphic information content (PIC), and other factors. From the Table 2 below, for these five breeds, PIC was between 0.5061 (Japanese black cattle) and 0.7289 (yellow cattle), and mean expected heterozygosity (HE) varied from 0.5996 (Japanese black cattle) to 0.8158 (yellow cattle), while mean observed heterozygosity (HO) was from 0.5849 (Japanese black cattle) to 0.8073 (yellow cattle).

Gene diversity (HS and HT) and two estimators (FST and GST) of genetic differentiation of the five Asian cattle were approximately equivalent in each markers (Table 3.2), HS per locus varied from 0.6534 (BM1818) to 0.7488 (ETH104). HT results showed the range from 0.7839 (BM1818) to 0.8585 (ETH104), respectively. FST per locus varied from 0.0653 (BM2113) to 0.15 (BM1818) when analyzed across populations. According to Wright (1978), FST between 0.05 and 0.15 indicates moderate differentiation.

Table 3.1 Mean observed (HO) and expected (HE) heterozygosity and polymorphic information content (PIC) values

| Breed | No. of samples | $HO^{(a)}$ | $HE^{(b)}$ | $PIC^{(c)}$ |
|-----------------------|----------------|------------|------------|-------------|
| Hanwoo | 100 | 0.7402 | 0.7440 | 0.7030 |
| Korean brindle cattle | 50 | 0.7387 | 0.7285 | 0.6895 |
| Holstein | 50 | 0.7287 | 0.7361 | 0.6892 |
| Chinese yellow cattle | 47 | 0.8073 | 0.8158 | 0.7289 |
| Japanese black cattle | 23 | 0.5849 | 0.5996 | 0.5061 |

(a)HO=Heterozygosity observed

(b)HE=Heterozygosity expected

(c)PIC=Polymorphic information content

Table 3.2 Gene diversity and gene differentiation of microsatellites.

| Marker | No. of alleles | $HS^{(a)}$ | $HT^{(b)}$ | $FST^{(c)}$ | $GST^{(d)}$ |
|-------------|----------------|------------|------------|-------------|-------------|
| BM2113 | 16 | 0.7367 | 0.8089 | 0.0653 | 0.0893 |
| CSSM66 | 14 | 0.7332 | 0.8396 | 0.0959 | 0.1267 |
| ETH10 | 13 | 0.7488 | 0.7488 | 0.0936 | 0.1277 |
| ETH3 | 13 | 0.7040 | 0.8083 | 0.0978 | 0.1291 |
| ETH225 | 13 | 0.7084 | 0.7994 | 0.1248 | 0.1138 |
| BM1818 | 12 | 0.7084 | 0.7994 | 0.1500 | 0.1665 |
| Multi-locus | 13.5 | 0.7141 | 0.8156 | 0.1168 | 0.1254 |

(a)HS=Heterozygosity within subpopulation

(b)HT=Total heterozygosity

(c)FST=Differentiation between subpopulations by genetic drift

(d)GST=Coefficient of gene differentiation

Note. FST is significant at $p < 0.05$ (data not shown)

FST for this experiment was 0.1168, indicating moderate differentiation. Levels of apparent breed differentiation were considerable, and multilocus values indicate that about 11.68% ($p < 0.05$, data not shown) of the total genetic variation could be explained by breed differences, with the remaining 88.32% being due to differences among individuals.

3.2. Genetic distances

Genetic divergence among the populations based on allele frequencies was calculated according to DA genetic distance (Nei *et al.*, 1983) using the TreeView1.6.6 (Page, 1996) computer program. According to Nei (1978) and Luo *et al.* (2006), genetic distances correlate strongly with geographical distances. Table 4 showed the DA genetic distances ranged from 0.196 (between Hanwoo and Korean brindle cattle) to 0.56 (between Japanese black cattle and Korean brindle cattle). A neighbour joining slanted cladogram of five cattle breeds using Nei's standard distance is shown in Figure 4.1. The clustering of the five populations into three groups: Japanese black cattle, Holstein, and the three remaining breeds.

In addition, Hanwoo and Korean brindle cattle share the same origin, while Chinese yellow has closer relationship with the Hanwoo and Korean brindle cattle. The Japanese black cattle displayed a distinct cluster from other three Asian cattle breeds. Moreover, the origin of European Holstein cattle is farther from these other four cattle breeds.

Table 3.3 Nei's DAgenetic distance among five cattle breeds.

| | Holstein | Hanwoo | Chinese yellow | Korean brindle | Japanese black |
|----------------|----------|--------|----------------|----------------|----------------|
| Holstein | 0.000 | 0.341 | 0.329 | 0.389 | 0.453 |
| Hanwoo | 0.341 | 0.000 | 0.328 | 0.196 | 0.427 |
| Chinese yellow | 0.329 | 0.328 | 0.000 | 0.383 | 0.434 |
| Korean brindle | 0.389 | 0.196 | 0.383 | 0.000 | 0.560 |
| Japanese black | 0.453 | 0.427 | 0.434 | 0.560 | 0.000 |

4. Discussion

In order to assess the genetic variability and population structure of Asian cattle breeds, 6 microsatellite loci were analyzed for a total of 270 individuals, including Hanwoo, Korean brindle cattle, Chinese yellow cattle, Japanese black cattle and Holstein. The average number of alleles per locus, considering all five populations, was 13.5. It has been determined that microsatellite loci used to estimate genetic distances should have at least four alleles in order to reduce the standard errors of distance estimates (Barker *et al.*, 1994). Thus, all the microsatellite markers used in this study are suitable for population genetic diversity analyses.

Observed and expected heterozygosity, two estimators (F_{ST} and G_{ST}) of gene differentiation, and Nei's DA distance, were evaluated. Based on expected mean heterozygosity, the lowest genetic diversity was exhibited in Japanese black cattle ($HE=0.5996$) and the highest in Chinese yellow cattle ($HE=0.8037$). Except for Japanese black cattle, other three kinds of Asian cattle showed higher heterozygosity compared with Holstein. Chinese yellow cattle showed the highest diversity values, whereas Japanese Black cattle showed lower diversity than the other breeds. Diversity of Chinese cattle was higher than that of Korean cattle and Japanese black cattle, because extensive breeding programs have not been undertaken, providing a possible reason for the high degree of genetic diversity in these four populations. Improvements to the Chinese feeding system and post-mortem aging are urgently needed to improve beef production in China. Since the early 19th century, when the concept of a breed grew in currently, many European bovine breeds have become genetically isolated and in most cases their origins can be traced to a small pool of founder individuals. This is also the case for the Holstein cattle in Korea, which may have experienced a similar breeding practice. Thus, not only the narrow genetic background of this breed but also the inbreeding and the higher selection pressure, have contributed to the loss of genetic variation, which is revealed by reduced allele numbers and lower PIC values (Shan *et al.*, 2002). Japanese black cattle have experienced a similar breeding practice to the case of European cattle. At the same time, Japanese black 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.

Compared with Kim's study on genetic diversity of north-east Asian cattle using microsatellites in 2002, the result of diversity in this study is very similar. Chinese yanbian cattle have the highest genetic diversity whereas the Japanese black cattle have the lowest genetic diversity. However, the heterozygosity results of Hanwoo and Holstein area little different, which is probably due to differences in sampling location, genotyping methods, and so on.

F_{ST} depends on total genetic differentiation among breeds. The result of this study which estimated F_{ST} 11.7%, G_{ST} 12.5% of genetic variation is a little higher compared to the

study of genetic diversity (FST 10.9%, GST 9.9%) of north-east Asian cattle (Kim, *et al.*, 2002). Moreover, (similar values) with this (FST 11.2%, GST 10.4%) found in European cattle breeds (MacHugh *et al.*, 1998), but also higher than 10.7% in north European breeds (Kantanen *et al.*, 2000), 6.8% in south European beef cattle breeds (Jordana *et al.*, 2003), 6% in twelve *Bos taurus* and zebu breeds (Ibeagha-Awemu and Erhardt, 2005), and seven yak breeds in Russia, Mongolia, and China (Guo *et al.*, 2005). Considering the history of the origin and geographic distribution of the breeds studied, a higher level of differentiation among the five populations was expected.

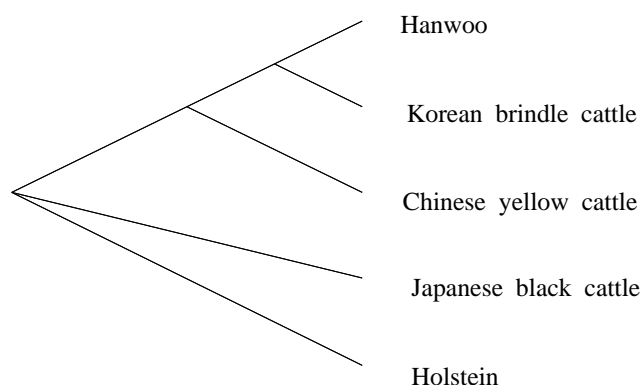


Figure 4.1 The neighbour joining slanted cladogram of five cattle breeds using Nei's standard distances.

N-J tree based on Nei's DA genetic distance (Figure 4.1) showed that Hanwoo and Korean brindle cattle are grounded together, which means they have the close relationship than the other three cattle populations. The Chinese yellow cattle is clustered within the Korean population. Japanese black cattle are clearly not clustered with the other Asian cattle breeds which are the same results with Kim *et al.* (2002). Holstein has a different branch with the other four Asian cattle populations. In Korea, there are three Hanwoo breeds that divided into brown, black and brindle cattle. Korean brindle cattle are coat colored and muzzle pigmentation variants of Hanwoo. On the other hand, Chinese yellow cattle are from the southern part of China, which means a slight difference of yellow cattle from the northern part of China originated from *Bos taurus indicus*. Hanwoo originated from *Bos indicus* & *Bos primigenius* is a little farther from the genetic relationship.

In Kim's study (2002), the Yanbian breed, present in the north of China, is humpless cattle and has been shown to be genetically close to European breeds based on blood polymorphisms and karyotype analyses (Sun *et al.*, 2008). Yanbian cattle may have greater relationship compared to those from the southern part of China. Japanese black cattle exhibit other Asian cattle breeds out group, probably due to their smaller sample size and reproductive isolation. Results showed that high genetic distances are related to geographical distances, rather than differences between morphological groups, to which some cattle breeds were affiliated. Other factors, such as different selection programs and level of performance traits, can also cause this problem. The increase in the number of Hanwoo samples may have caused the decrease in heterozygosity. This can also cause the difference between

these cattle breeds. Thus, the genetic relationship of these five cattle breeds corresponds to their breeding history and geographic origin.

This study defined the genetic diversity of five Asian cattle breeds by analysis of 6 microsatellite markers in 270 animals. Also, the result of this study can be used to determine the level of genetic variation within and among populations, as well as the population structure.

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