Genetic diversity analysis of Mongolian native cattle and other cattle breeds based on microsatellite marker

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ABSTRACT. In this study, we used genetic diversity analysis with Microsatellite (MS) markers to determine the relationships between two Mongolian native breeds (MNB) (n=78) which were compared with three foreign breeds (FB) (n=110) in Mongolia. For the analysis of genetic diversity, 11 recommended MS markers from a diversity panel conducted by the ISAG / FAO or previously reported MS markers were used. A total of 392 MS alleles were identified. The mean number of alleles (MNA) per locus across population was 7.126 and the mean observed heterozygosity (HObs), expected heterozygosity (HExp), and Polymorphism information content (PIC) were 0.753, 0.745, and 0.697, respectively. The five breeds were classified into two groups (FB in the first group, MNB in the second group), according to the neighbor - joining (NJ) tree, which was based on Nei's standart genetic distances. Both Factorial correspondence analysis (FCA), principal coordinates analysis (PCoA) showed that the Mongol (M) breed is highly distinct from the other breeds analysed. In conclusion, these results will provide useful information for the development of native Mongolian cattle breeding and individual traceability systems as well as aid in generating

strategies for conservation and breeding programs for the Mongolian cattle population.

Keywords: Microsatellite; Genetic diversity; Phylogenetic tree; Mongolian cattle

INTRODUCTION

In developing countries, genetic resources for livestock greatly contribute to the sustainable development of livelihood, security, and food safety by reducing hunger and poverty (Ben Sassi-Zaidy et al., 2015). Cattle are one of the most economically important livestock in Mongolia as they are main source of major export products such as meat and milk. Out of 1.57 million Mongolian cattle, 1.55 million supposedly belong to three indigenous Bos taurus cattle breeds which are Mongol (M), Selenge (S) and Khalkhun Golun. These breeds herd under broad pastoral systems. Mongolian native breed (MNB) generally is strong and sturdy, but have small body size. By far the M breed is the most common, with 1.53 million animals, and it is found throughout the most parts of the country (Lkhagvaa et al., 2003). According to the 2017 census, Mongolia supported approximately 3.4 million heads of cattle and 6.6% of all livestock.

In 1949, Mongolian indigenous cattle began to improve by crossbreeding with European breeds. Thus, the current breeds are numerous, but they are not known to be genetically distinct. The S breed numbering 19.2 thousand heads, was developed by crossing the Kazakh white headed breed with indigenous Mongolian cattle. The Kazakh white head cattle are a beef cattle breed from Kazakhstan and Russia. The breed was developed by crossing Hereford (H) cattle with Kazakh and Kalmyk cattle (Dankvert et al., 2007; Cherekaev and Cherekaeva, 1973) and was used as a base improver since 1950 to improve meat productivity of Indigenous Mongolian cattle. By 2015, 41.5 thousand Kazakh white head pure (KP) and Kazakh white head cross (KC) breeds were counted in Mongolia. Although the census of cattle breeds was done on yearly basis, there are no information is available on the genetic relationship between the M breeds (National Statistical office., 2001).

Microsatellite (MS) are widely used as genetic markers for genetic diversity analysis and breeding programs in developing countries as they are low cost and relatively simple to use in such analyses (Rege et al., 2011); MS markers using of genetic analysis this has yet to be conducted among Mongolian cattle; therefore, our research would be the first of its kind, especially when considering the use of a crossbreed cattle hybrid since 1949. As cattle herders are concerned about the quantity of livestock rather than the quality, genetic distance analysis is crucial to determine varying genetic distances. Hence, we used MS markers to study the genetic diversity of MNB and Foreign Breeds (FB). These molecular genetic markers can be used to assess genetic diversity as well as track the species origin and identification. In this study, we have determined genetic distance and polymorphisms of MNB and FB. Study on genetic diversity of MNB and FB is expected to provide scientific information for designing breeding strategies.

MATERIALS AND METHODS

Blood sample and DNA collection

In the present study, whole blood samples were collected from two native Mongolian breeds (S, M) and three foreign breeds (H, KP, KC) from Selenge Aimag, Zuunburen Sum; Dundgovi Aimag, Adaatsag Sum; and Tov Aimag, Onjuul Sum in Mongolia, as shown in Table 1. DNA was extracted from the blood samples using QuickGene 810 following the manufacturer's instruction method. The extracted genomic DNA was used for experiments after measuring the concentration and purity using the ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) (Figures 1 and 2).

Table 1. Description of the 5 cattle breeds in the present study.

Population	The number of Sample
Selenge breed [S]	38
Mongol native breed [M]	40
Kazakh white head purebred [KP]	40
Kazakh white head crossbreed [KC]	30
Hereford breed [H]	40
Total	188

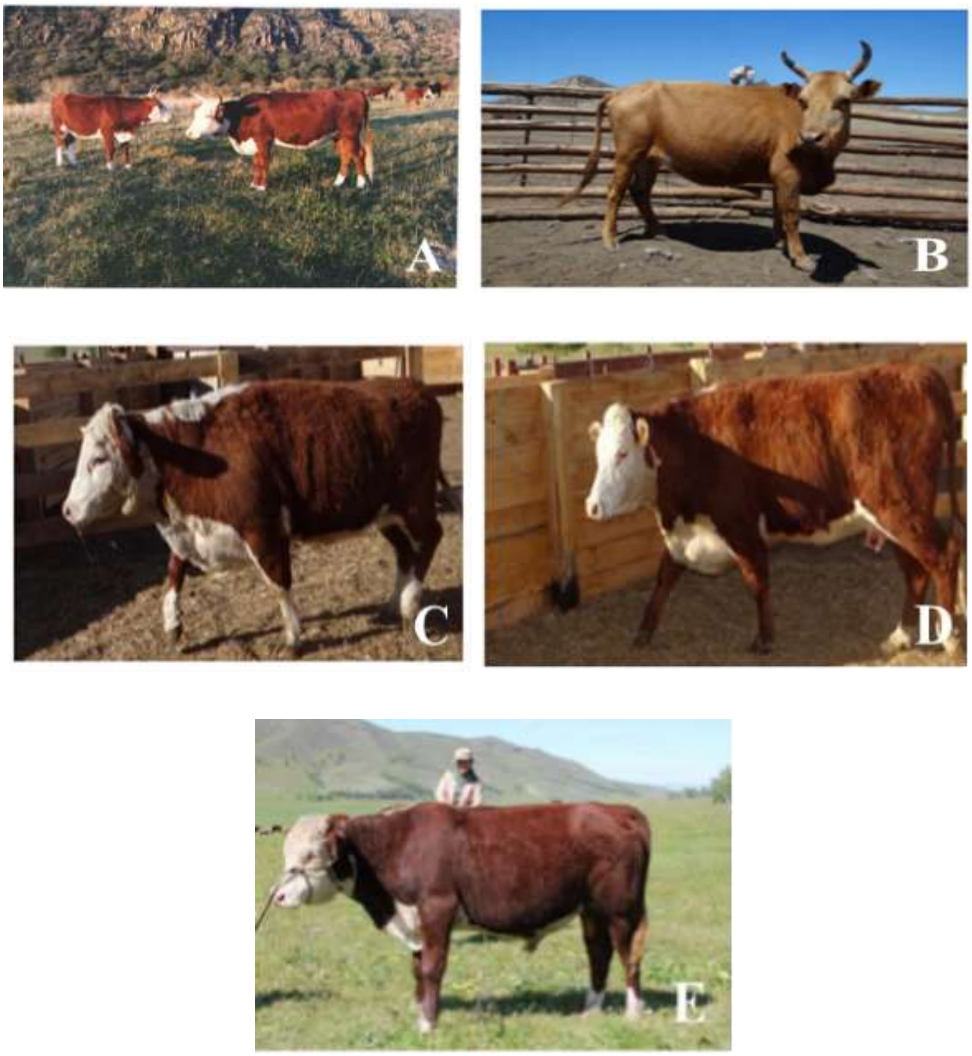

Figure 1. Breeds of cattle examined in this study. S breed (A), M breed (B), KP breed (C), H breed (D), KC breed (E)

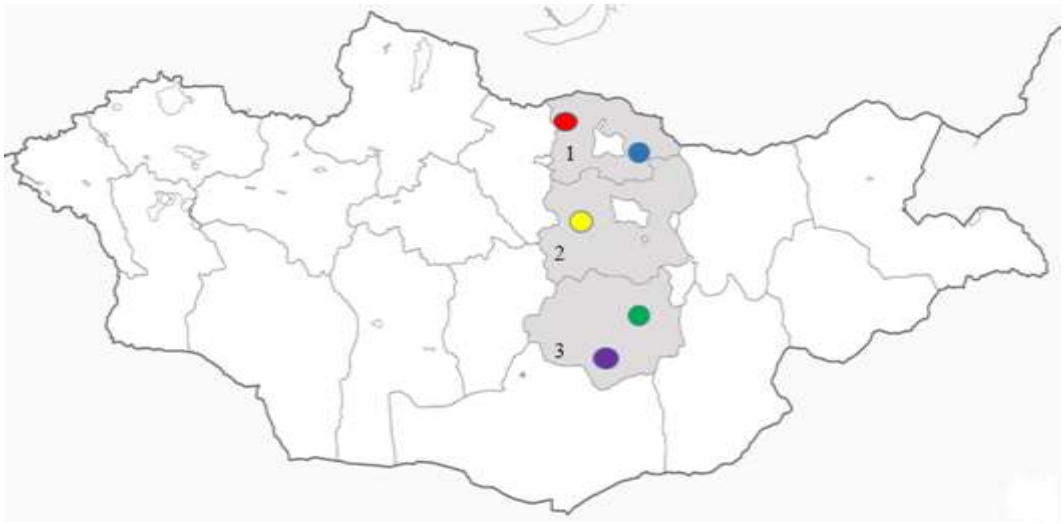

Figure 2. Locations of two MNB and three FB from central Mongolia. 1. Selenge aimag 2. Tov aimag 3. Dundgovi aimag. red, S breed; blue, KC breed; yellow, M breed; green, H breed; purple, KC breed.

Information on the microsatellite marker

In this study, we chose 11 MS markers based on the guidelines of the International Society of Animal Genetics (ISAG) and United Nations Food and Agriculture Organization (FAO) whereas the previously reported (Choy et., al 2015) (Table 2).

Multiplex PCR composition and methods

The total reaction volume of 15 µL containing 1 µL genomic DNA (20–50 ng/µL), 0.2 µL Hot Start Taq DNA Polymerase (Genetbio, Nonsan, Korea), 0.5 µL of each primer (forward and reverse), 1.5 µL dNTPs, and 1.8 µL 10x buffer was used to perform PCR using the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The PCR amplification was conducted using following conditions: pre-denaturation at 95°C for 10 minutes followed by 30 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. After 30 minutes of extension at 72°C, the reaction was terminated at 4°C.

Table 2. Primers of the 11 microsatellite markers.

MS Marker	Ch.	Size range	Primer Sequence
TGLA227	18	76-104	F: CGAATTCCAAATCTGTTAATTTGCT R:ACAGACAGAAACTCAATGAAAGCA
BM2113	2	123-143	F: GCTGCCTTCTACCAAATACCC R: CTTCCTGAGAGAAGCAACAC
TGLA53	16	154-188	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA
ETH10	5	212-224	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC
SPS115	15	246-260	F: AAAGTGACACAACAGCTTCTGGAG R: AACGAGTGTCCTAGTTTGGCTGTG
TGLA126	20	116-122	F:CTAAATTTAGAATGAGAGAGGCTTCT R: TTGGTCTCTATTCTCTGAATATTCC
TGLA122	21	137-181	F: CCCTCCTCCAGGTAAATCAGC

			R: AATCACATGGCAAATAAGTACATAC
			F: GAGTAGAGCTACAAGATAAACTTC
INRA23	3	196-222	R: TAACTACAGGGTGTTAGATGAACT
			F: GAACCTGCCTCTCCTGATTGG
ETH3	19	105-125	R: ACTCTGCCTGTGGCCAAGTAGG
			F: GATCACCTTGCCACTATTTCCT
ETH225	9	141-159	R: ACATGACAGCCAGCTGCTACT
			F: GAGCAAGGTGTTTTTCCAATC
BM1824	1	178-192	R: CATTCTCCAACTGTTCCTTG

MS, Microsatellite; Ch, Chromosome; R, Reverse; F, Forward.

Microsatellite genotype analysis

The genotyping mixture contained 1 μL PCR product, 8.9 μL Hi-Di formamide (Applied Biosystems, Foster City, CA, USA), and 0.1 μL GeneScan ™ 500LIZ size standard (Applied Biosystems, Foster City, CA, USA). Fragment analysis was performed using the Genetic Analyzer 3730xl (Applied Biosystems, Foster City, CA, USA) by subjecting fragments to capillary electrophoresis, and allele sizes were used to determine each MS marker using GeneMapper version 4.1 (Applied Biosystems, Foster City, CA, USA). The determined alleles were collected via Microsoft Excel (Microsoft, Redmond, WA, USA) and then applied for statistical analysis.

Statistical analysis

MS Toolkit software (Park SDE et al., 2001) was used to calculate the number of alleles, HExp, HObs, and PIC. The homozygosity (H0) and heterozygosity (Hi) are expressed according to Eq. 1.

$$H_o = \sum P_I^2 H_i = 1 - H_o \qquad \text{Eq. 1}$$

Pi is the frequency of each allele in the Microsatellite Marker. In addition, the mean heterozygosity of all MS markers analyzed and the heterozygosity of the ith MS marker H was calculated with Eq. 2.

$$H = \sum H_{i/r} \qquad \text{Eq. 2}$$

$$PIC = \left(\sum_{I=1}^{K} P_I^2 \right) - \sum_{I=1}^{K-1} - \sum_{I-1}^{K} P_I^2 P_j^2 \qquad \text{Eq. 3}$$

PIC values of each group were calculated according to Eq. 3. In this equation, PI and PJ represent the probability that the ith and jth allele appear. Principal coordinate analysis (PCoA) and factorial component analysis (FCA) were performed by GenAlEx 6.4 (Peakall et al., 2006) and Genetix (Belkhir et al., 2004) using each marker-specific allele frequency to identify genetic correlations between groups. The Nei's standart genetic distance (Nei et al., 1983) was calculated and Neighbor - joined trees were estimated using with DISPAN program (Ota, 1993).

RESULTS

In this study, genetic distance was examined among MNB and FB in Mongolian regions using genetic diversity analysis on MS markers. Analyses of the genetic diversity and genetic relationships of 188 cattle were carried out using 11 MS markers.

Polymorphism of microsatellite marker, within and between populations

The evaluated by the number of alleles, HObs, HExp, and PIC for 11 MS markers were calculated and are reported in Table 3. Polymorphisms of MS markers are determined using the following criteria: if the sum of HExp is ≥ 0.6 and PIC is ≥ 0.5, then the marker was determined to be highly polymorphic (Seo et al., 2017).

In the present study, the average HExp value of 11 microsatellite markers was found to be 0.745 with values ranging from 0.726 to 0.777. The highest value, 0.777, was found for the HExp of the M breed, while the lowest value of 0.726 corresponded to the KP breed. HObs ranged from 0.706 to 0.793 with an average of 0.753; the highest HObs were found in KC at 0.793, and the lowest was detected at 0.706 in the KP breed. The M and KC breeds showed the most probable heterozygosity, and the KP breed showed the lowest known heterozygosity among each of the five breeds. PIC ranged from 0.674 to 0.736 with an average of 0.697; the value was highest in the M breed at 0.736 and lowest in the KP breed at 0.674. The M breed showed the highest polymorphism among the 5 breeds, and KP exhibited the lowest. The 11 MS loci from the entire population were analysed and 392 alleles consisting of 72 alleles detected in the S breed, 94 alleles in the M breed, 70 alleles in the KP, 78 alleles in the KC, and 78 alleles in the H breed. The number of alleles per locus ranged from 6 (BM1824) to (TGLA112), with a mean of 10.5 in all.

The result genetic distance analysis result showed that the genetic distance between H breed and KP breed was the lowest. In contrast, the distance between the KP and M breed was the highest (Table 3). This suggests that the specific heterozygous genotypes are distributed at a high frequency in this breed and may be attributed to them. Altogether, these results suggest that 11 microsatellite markers are sufficient for use as polymorphic markers in polymorphism analysis of MNB.

Table 3. Genetic Diversity analysis in Mongolian native and foreign breeds.

Name of breeds	NA	MNA	H_{Exp}	H_{Obs}	PIC
Selenge breed	72	6.545	0.732	0.760	0.687
Mongol breed	94	8.545	0.777	0.775	0.736
Kazakh white head purebreed	70	6.363	0.726	0.706	0.674
Kazakh white head crossbreed	78	7.090	0.758	0.793	0.710
Hereford breed	78	7.090	0.734	0.734	0.680
Mean	**392**	**7.126**	**0.745**	**0.753**	**0.697**

Genetic differentiation among populations

The Neighbor - joining tree (NJ tree) showing the genetic distances between populations (Figure 3). Three FB and two MNB cluster into two groups. The H, KP and KC breed in the first group; M and S breed in the second group. KC breed was established by crossing H and KP breeds. The clustering of breeds into 2 groups by Nei's DA genetic distance analysis highlights the presence of clear genetic separation between breeds. As phylogenetic tree may not take into account the effects of admixture between breeds, we therefore performed PCoA as an alternative approach to understand the genetic relationship among breeds.

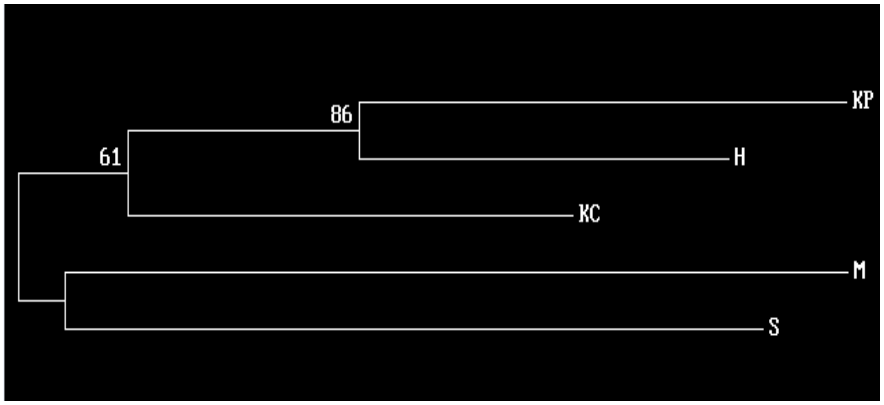

Figure 3. Neighbour – Joined tree showing the genetic distances among the five breeds using Nei's DA genetic distance on the basis of alleles frequencies from the 11 microsatellite loci. The number in the branch indicates the percentage of occurrence in 1000 bootstrap replicates. S- Selenge, M-Mongol, KP – Kazakh white head purebred, KC – Kazakh white head crossbreed and H-Hereford

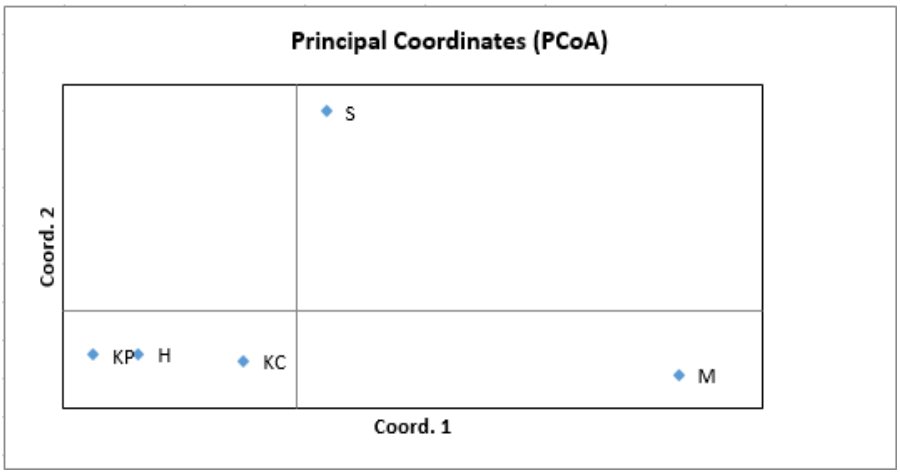

Figure 4. Principal Coordinates Analysis (PCoA) of allele frequencies from 11 microsatellite loci genotypes in five populations using the GenAIEx. S- Selenge, M-Mongol, KP – Kazakh white head purebred, KC – Kazakh white head crossbreed and H-Hereford.

PCoA was performed including all populations and loci using allele frequencies to summarize relationship between breeds (Figure 4). The first, second, third Principal coordinate (PCo) represented 42.34%, 32.23% and 17.22% of the total variation, respectively. The first three PCo account for 91.09% of the total variation and the incorporation of five PCo explains all the variation (data not shown). The analysis indicated a grouping at the three foreign breeds (H, KP and KC).

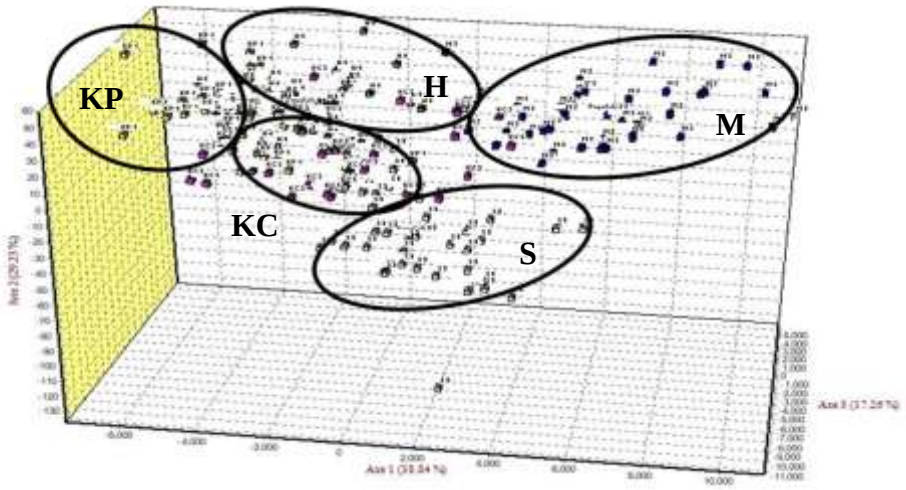

Figure 5. Factoral Components Analysis (FCA) of allele frequencies from cattle microsatellite loci genotypes calculated using Genetics S- Selenge, M-Mongol, KP – Kazakh white head purebreed, KC – Kazakh white head crossbreed, H-Hereford

The FCA analysis revealed the very clear separation between the MNB and FB (Figure 5). About 85.33% of the variance was accounted for the first to three dimensions of the FCA Axis 1 (38.84% of total variance explained), which separated S and M from the MNB. Axis 2 (29.23%) further separated KP, KC and H from foreign breeds. Axis 3 (17.26%) distinctly separated M and S breed.

DISCUSSION

In Asia, totally 258 cattle breeds were reported, 11% of these breeds are recognized as being at the risk of extinction (Scherf, 2000). Currently, much attention is focused on the issue of preserving biological diversity in livestock (Zeng et al., 2010). The purpose of this study is to determine the genetic diversity and relationships of five cattle population using 11 MS marker. The results of genetic diversity analysis (Table 3), the NJ tree (Figure 3), PCoA (Figure 4), and FCA (Figure 5) provide genetic evidence for the differentiation of the five breeds, and polymorphism was observed in all regions. The level of variation depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within a species (Buchanan et al., 1994). KP breed exhibited a lower degree of genetic diversity (mean number of alleles [MNA] = 6.363, HExp = 0.726, HObs = 0.706, PIC = 0.674) than all other breeds in all measures of genetic diversity whereas a high degree of genetic diversity was observed in M breed (MNA = 8.545, HExp = 0.777, HObs = 0.775, PIC = 0.736). The M breeds are reportedly similar or higher than those of Mongolian, British and Spanish cattle breeds. (HExp = 0.658 to 0.69, HObs = 0.660 to 0.726) (Lkhagvaa et al., 2003; Wiener et al., 2004; Martin-Burriel et al., 2007; Padilla et al., 2009). Heterozygosity was observed for KP breed as quite low compared to other breeds which may be high genetic relationship between foreign breeds. High diversity in M breed may be attributed to breeding among large number of individuals in throughout most of the country.

The PCoA analysis indicated a grouping at the three foreign breeds (H, KP and KC). The MNB formed two groups. MNB were distant from the FB in the scatter diagram of first and third principal coordinates. Both the NJ tree and the PCoA analysis showed that the M breed is highly distinct from the other breeds analysed. The FCA results showed that individuals from H, KP and KC were slightly mixed, suggesting a closer relationship between them. This may be due to the improvement of the Kazakh white head cattle from Hereford cattle in the 1950 (Dankvert et al., 2007; Cherekaev and Cherekaeva, 1973).

The results indicated that MNB show a high level of genetic diversity. In analysis of genetic relationship and clustering, MNB were genetically differentiated from the FB. These analyses were important to preserve the genetic differentiation and purity of other cultivars as well as to utilize the basic data to improve the ability of each cultivar. Genetic diversity information is needed for establishing protection programs, especially for local Mongolian cattle resources, and to provide a suitable method for farmers to develop animal breeding programs.

CONCLUSION

In conclusion, our results indicated M breed has high genetic diversity and genetically distinct from other breeds. These findings may be used to develop individual traceability systems as well as aid generating strategies for conservation and breeding programs for the Mongolian cattle population. In the present study, we have used MS markers to clarify genetic diversity and relationship between 5 breeds. Despite the fact that the MS markers are high polymorphic, the MS typing technique is becoming limited use. Especially, because of the technical limit, the labor intensive and the cost of the experiment (Choi et al., 2018). In comparison, the single nucleotide polymorphism (SNP) analysis method has many advantages in terms of cost, accuracy of analyzing, time-consuming. Also, array-based full automated analysis system is used to identify allele genotypes (Vignal et al., 2002). The Mitochondrial DNA (mtDNA) has no recombination and lack of rapid nucleotide substitutions, making it suitable for genetic diversity studies and evolutionary studies (Hoque et al., 2013). So for further studies SNP and mtDNA markers are needed to be done.

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ETHICS APPROVAL

The study was approved by the Hankyong National University Animal Ethics Committee (No.2019-2)

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