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Original Article

Genetic diversity and relationship of Halla horse based on polymorphisms in microsatellites

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ABSTRACT Halla horse is crossbreed between Jeju and Thoroughbred horses and is used for riding, racing and meat production. Thus, molecular genetic studies are needed to establish and preserve the industrially valuable Halla horses. This study aimed to analyses the genetic diversity and population structure through 12 microsatellite (MS) markers for Halla and putatively related 3 breeds (Jeju, Mongolian and Thoroughbred horses). On average, the number of alleles, observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}), and polymorphic information content (PIC) among all horses were 10, 0.767, 0.799, and 0.771, respectively. Neighbor-joining tree and STRUCTURE analysis showed that Halla horses were between Thoroughbred and Jeju horses, tend to more influenced by Thoroughbred horses. Therefore, these results could be considered for use as the basic genetic breed relationships resource among the horse breeds (Jeju, Mongolian, and Thoroughbred horses) related to the origins of the Halla horse.

Keywords: Halla horse, horse, genetic diversity, relationship, Microsatellite marker

INTRODUCTION

In the past, horses were used to the development of means of transport and communication and spread of agricultural machinery. However, the quality of human life improved, the range of uses of horses changed for purposes related to cultural life, such as leisure activities and sightseeing (Seo et al., 2016).

According to 2016 report, about 26,000 horses are breeding in Korea and 57.2% of them are breeding in Jeju island (Seo et al., 2016). The horses breeding in Jeju island are subdivided into Halla horses (75.1%), Jeju horses (6.1%) and Thoroughbred horses (18.8%) (Seo et al., 2016). To improve the ability of the Jeju horse in racing, crossbreeding with the Thoroughbred horse was initiated. This led to the emergence of hybrid breeds, such as the Halla horse.

Halla horse is crossbreed between Jeju and Thoroughbred horses and is used for riding, racing and meat production. But, a little research has been conducted on Halla horses because of the perception of crossbreed and people's weighted interest toward Jeju horses.

Therefore, molecular genetic studies are needed to establish and preserve the industrially valuable Halla horses. And it is essential to do genetic characterization to compared different horse populations for a knowledge about their genetic specificity can help us to do the best plan about their management and conservation (Berber et al., 2014).

This study was conducted to analyze the genetic background and polymorphism in various horse breeds (Jeju, Mongolian, and Thoroughbred horses) related to the origins of the Halla horse in Korea using 12 microsatellite (MS) markers.

MATERIALS AND METHODS

Animals and DNA isolation

For DNA analysis using microsatellites, 200 Halla horses and 84 Jeju horses in Korea, 36 Mongolian horses in Mongolia and 40 Thoroughbred horses (International Society for Animal Genetics, ISAG Comparison test DNA sample) were used. Excluding the Thoroughbred horses, genomic DNA was extracted from hair roots of 320 horses using the methods described for QuickGene DNA tissue kits (FUJIFILM, Tokyo, Japan), and the concentration and purity of the isolated genomic DNA were evaluated using an ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA), and used for analysis.

Information on microsatellite (MS) markers

The genetic diversity of the horse breeds was analyzed using 12 MS markers recommended by the ISAG: AHT4, AHT5, ASB2, ASB17 (Binns et al., 1995); ASB23 (Irvin et al., 1998); HMS2, HMS3, HMS6, HMS7 (Guékin et al., 1994); HTG4 (Ellegren et al., 1992); HTG10 (Marklund et al., 1994) and VHL20 (van Haeringen et al., 1994).

Short Tandom Repeat (STR) Genotyping

Multiplex PCR amplification was conducted using Equine Genotypes Panel 1.1 Kit (Thermo Fisher Scientific, USA) and PCR was performed using GeneAmp PCR System 9700 (Applied Biosystems, CA, USA). STR genotyping was performed using an Genetic Analyzer 3130xl (Applied Biosystems, CA, USA) following previously described methods (Seo et al., 2016).

Statistical analysis of data

Data from genotyping were analyzed using Cervus V 3.0 (Marchall et al., 1998) and MS Excel toolkit version 3.1 (Park, 2001) to calculate allele frequencies at each locus for each population, H_{exp}, H_{obs}, and PIC values. Inbreeding-like effects within subpopulations (genetic distance [F_{st}]), among subpopulations (within inbreeding [F_{is}]), and within the entire population (total inbreeding [F_{it}]) were analyzed by F-statistics (Wright, 1965). PCoA (Principal Coordinates Analysis) was conducted for the 12 MS markers using GenAlEx 6.4. Factorial correspondence analysis (FCA), which is a weighted PCoA method, was performed using the allele frequency data for the individuals of all 4 breeds and 12 MS markers using the GENETIX software (Belkhir, 2003; Tantia et al., 2006).

The genetic diversity among the populations was evaluated based on allele frequencies according to genetic distance D_A (Nei et al., 1983) using the POPTREE2 (Takezaki et al., 2010). A phylogenetic tree was constructed from the distances using neighbor - joining method (Saitou and Nei 1987). Population structure was analyzed using STRUCTURE version 2.3.4 (Pritchard et al., 2000), the length of burn-in was set to 500 and the number of Markov chain Monte Carlo (MCMC) iteration was set to 1,000. On the basis of the value of ΔK (Evanno et al., 2005), determined using Structure Harvester (Earl, 2001), we estimated the optimal K value.

RESULTS

The H_{exp}, H_{obs}, PIC, and F-statistics values for the 4 breeds are summarized in Table 1. For the 12 MS markers, the allele number was confirmed to range from 6 (HMS6) to 17 (ASB17). The total allele number for the 12 markers was 120, and the mean allele number was 10. The H_{obs} values ranged from 0.619 (HTG4) to 0.827 (HTG10). AHT4 showed the highest H_{exp} and PIC values of 0.858 and 0.840, respectively, while HTG4 showed the lowest H_{exp} and PIC values of 0.652 and 0.609, respectively.

F-statistics were estimated in fixation indices as genetic differentiation (F_{st}), global heterozygote deficit among the 4 horse breeds (F_{it}), and heterozygote deficit within the breed/line (F_{is}) among the 12 MS markers (Table 1). Among these markers, F_{st} ranged from 0.022 (ASB23) to 0.074(AHT5); the global heterozygote deficit among the 4 horse breeds (F_{it}) ranged from 0.010 (ASB17) to 0.086

Table 1.The statistical analysis of heterozygosity (H_{obs} and H_{exp}), Polymorphism Information Content (PIC), and F-statistics value using selected 12 microsatellite markers

Locus	No. of allele	H_{obs}	H_{exp}	PIC	F_{st} (θ)	F_{it} (F)	F_{is} (f)
AHT4	9	0.816	0.858	0.840	0.050	0.078	0.029
AHT5	7	0.807	0.829	0.804	0.074	0.086	0.013
ASB2	13	0.819	0.826	0.803	0.052	0.014	−0.040
ASB17	17	0.794	0.804	0.783	0.032	0.010	−0.022
ASB23	12	0.769	0.818	0.794	0.022	0.075	0.054
HMS2	9	0.730	0.793	0.762	0.072	0.119	0.051
HMS3	10	0.725	0.814	0.787	0.060	0.128	0.072
HMS6	6	0.750	0.763	0.724	0.037	0.023	−0.014
HMS7	9	0.739	0.755	0.720	0.052	0.028	−0.025
HTG4	7	0.619	0.652	0.609	0.050	0.060	0.011
HTG10	12	0.827	0.839	0.819	0.039	0.055	0.017
VHL20	9	0.808	0.831	0.810	0.046	0.028	−0.019
Mean	10	0.767	0.799	0.771	0.049	0.059	0.011

H_{obs}: Observed heterozygosity, H_{exp}: Expected heterozygosity, PIC: Polymorphic information content, F_{st}: Genetic distance, F_{it}: Total inbreeding, F_{is}: Within inbreeding.

Table 2.The statistical analysis of heterozygosity (H_{obs} and H_{exp}) and polymorphism information content (PIC) observed across 12 microsatellite loci for each breeds.

Breeds	H_{obs}	H_{exp}	PIC
Halla horses	0.782	0.786	0.756
Jeju horses	0.749	0.738	0.698
Mongolian horses	0.771	0.801	0.764
Thoroughbred horses	0.722	0.758	0.712

H_{exp}:Expected heterozygosity, H_{obs}:Observed heterozygosity, PIC: Polymorphic information content.

(AHT5), and the heterozygote deficit within the breed/line (F_{is}) ranged from-0.040 (ASB2) to 0.072 (HMS3). The breed statistics generated by the 12 MS markers in 4 horse breeds are shown in Table 2. The mean H_{obs} ranged from 0.722 in the Thoroughbred horse to 0.782 in Halla horse. Average H_{exp} ranged from 0.738 in the Jeju horse to 0.801 in the Mongolian horse.

Fig. 1 illustrates the population relationships based on PCoA using individual multilocus genotypes of the 12 MS markers. The contribution to the variance of the principal components exceeded 60%, including the three components. The first and second components contributed 27.72% and 17.30%, respectively, and the third component contributed 16.29%. Clearly, based on the first component, Thoroughbred and Jeju horses were confirmed to be separated. Furthermore, Halla horse was confirmed to be between Thoroughbred and Jeju horses. The neighbor-network analysis of the 4 breeds confirmed the Factorial correspondence analysis (FCA) results as the horse breeds

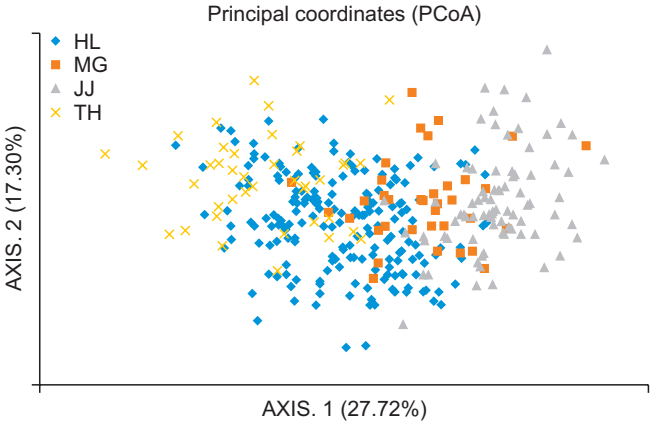

Fig. 1. PCoA of allele frequencies from 12 microsatellite markers typed in 4 breeds using GenAlEx. HL, Halla horse; MG, Mongolian horse; JJ, Jeju horse; TH, Thoroughbred horse.

segregated in a similar pattern of Fig. 1 (Fig. 2). Based on the results of neighbor - joining analysis, Halla horse was between Thoroughbred and Jeju horses and separated the Thoroughbred and Jeju horses (Fig. 3).

We applied the STRUCTURE program (Pritchard et al., 2000) to estimate the relationship between the Halla horse and putatively related breeds (Fig. 4, Supplementaty Table 1). On the basis of the ΔK value of 11.764 obtained using Structure Harvester, we estimated the optimal K value to be 3, corresponding to 4 breeds. These populations resulted in the separation of the 3 breeds and as shown above, Thoroughbred and Jeju horses were confirmed to be separated.

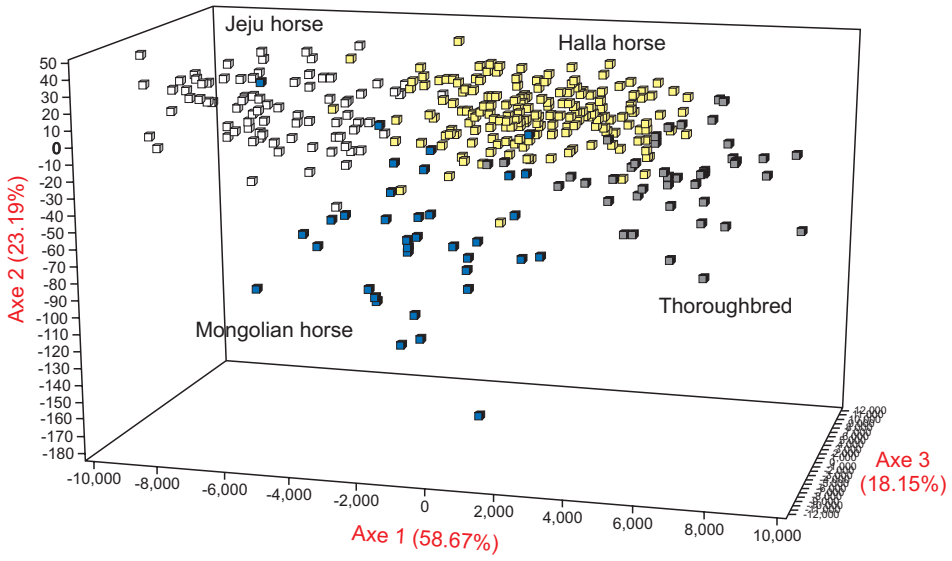

Fig. 2. Correspondence analysis of allele frequencies from 12 microsatellite markers typed in 4 breeds using Genetix405. Yellow, Halla horse; White, Jeju horse; Blue, Mongolian horse; Gray, Thoroughbred horse.

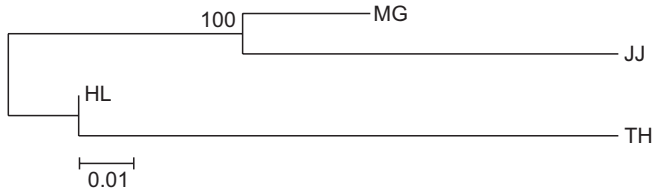

Fig. 3. Phylogenetic tree for 4 breeds on 12 microsatellite markers using neighbor - joining method. HL, Halla horse; MG, Mongolian horse; JJ, Jeju horse; TH, Thoroughbred horse.

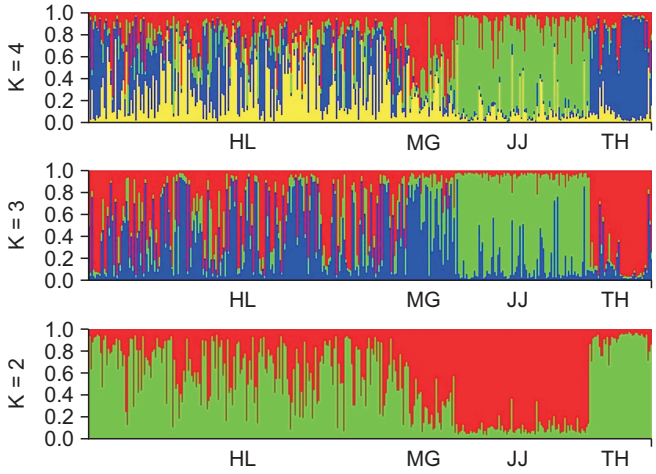

Fig. 4. Population structure of the analyzed 4 breeds using model-based clustering method implemented in STRUCTURE at K = 2, 3 and 4. Each column represents the proportion in which an individual belongs to a different coloured cluster. The optimal K value was estimated to be 3 (ΔK = 11.764), as determined by Structure Harvester. HL, Halla horse; MG, Mongolian horse; JJ, Jeju horse; TH, Thoroughbred horse.

DISCUSSION

This study aimed to analyses the genetic diversity and population structure through 12 microsatellite (MS) markers for Halla and putatively related 3 breeds. The allele number was confirmed to range from 6 (HMS6) to 17 (ASB17) and the H_{obs} values ranged from 0.619 (HTG4) to 0.827 (HTG10). AHT4 showed the highest H_{exp} and PIC values of 0.858 and 0.840, respectively, while HTG4 showed the lowest H_{exp} and PIC values of 0.652 and 0.609, respectively. In a previous study by Seo et al. (2016) the genetic diversity of 3,880 Halla horses were evaluated using MS markers (Seo et al., 2016). The allele number was reported to be the same or higher for all markers than in this study, excluding the HMS7 marker. These results were considered to be due to the population size. However, in this study, the observation that the HMS7 marker showed a higher allele frequency than in the previous study was considered to be due to the other populations (Jeju, Mongolian, and Thoroughbred horses). Botstein et al. (1980) reported that for animal traceability, PIC > 0.5 and H_{exp} > 0.6 are the most reasonable informative loci for application in genetics. These 12 MS markers used in this study showed high polymorphism in the 4 breeds.

Fig. 1 illustrates the population relationships based on PCoA using individual multilocus genotypes of the 12 MS markers. Clearly, based on the first component, Thoroughbred and Jeju horses were confirmed to be separated. Furthermore, Halla horse was confirmed to be between Thoroughbred and Jeju horses. The neighbor-network analysis of the 4 breeds confirmed the Factorial

correspondence analysis (FCA) results as the horse breeds segregated in a similar pattern of Fig. 1 (Fig. 2). Based on the results of neighbor - joining analysis, Halla horse was between Thoroughbred and Jeju horses and separated the Thoroughbred and Jeju horses (Fig. 3). These results are attributed to the fact that Halla horse is crossbreeding between Thoroughbred and Jeju horses. It is also supposed that Halla horse is an original breed and with a specific genetic structure comfort its industrial interest. Furthermore, the phylogenetic tree showed that Mongolian horse and Jeju horse were closely related species (Fig. 3). It is supported by the historical fact that Jeju horses were mixed origin in their maternal lineage (Lee et al., 2010).

To further investigate the relationship between the Halla horse and putatively related breeds, we analyzed the STRUCTURE program (Fig. 4). These populations resulted in the separation of the 3 breeds and as shown above, Thoroughbred and Jeju horses were confirmed to be separated. Halla horses were between Thoroughbred and Jeju horses, tend to more influenced by Thoroughbred horses and similar to Mongolian horses.

In this study, we analyzed the genetic variation and relationship of Halla and putatively related 3 breeds (Jeju, Mongolian and Thoroughbred horses) using 12 MS marker, and we support the view that Halla horses originated between Thoroughbred and Jeju horses.

Therefore, these results could be considered for use as the basic genetic breed relationships resource among the horse breeds (Jeju, Mongolian, and Thoroughbred horses) related to the origins of the Halla horse. And it also obtained in the present study contribute to establishing the cross combination of the Halla horse and will provide important to support the future monitoring of population diversity. Futhermore, since the MS markers in this study are highly polymorphic, they can also be applied for paternity testing.

CONCLUSION

In conclusion, we analyses the genetic diversity and population structure through 12 microsatellite (MS) markers for Halla and putatively related 3 breeds (Jeju, Mongolian and Thoroughbred horses). As a results, Neighbor-joining tree and STRUCTURE analysis showed that Halla horses were between Thoroughbred and Jeju horses, tend to more influenced by Thoroughbred horses. Therefore, we support the view that Halla horses originated between Thoroughbred and Jeju horses.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ETHICS APPROVAL

The study was approved by the Hankyong National University Animal Ethics Committee (No.2015-4).

AUTHOR CONTRIBUTIONS

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Formal analysis: Ji Su Jung, Jiyeon Seong
Funding acquisition: Hong Sik Kong
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Project administration: Hong Sik Kong
Resources: Hong Sik Kong
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Supervision: Hong Sik Kong
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SUPPLEMENTARY MATERIALS

Supplementary material can be found via https://doi.12750/JARB.36.2.76.

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