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Microsatellite Variation in Jeju and American horses and their Phylogenetic Relationship

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ABSTRACT

The aim of this study was to evaluate the microsatellite (Ms) variation in Jeju and American horses and their phylogenetic relationship, and to establish a lineage-based breeding plan for domestic American miniature horse (AMH) and Quarter horse (QH) in Korea. A total of 83 horse samples (27 AMHs, 32 QHs, and 24 Jeju horses [JHs]) were genotyped with 17 Ms markers. The observed number of alleles per locus ranged from 4 (HTG7) to 12 (ASB17), with an average value of 7.71, 7.41, and 6.11 in AMH, QH, and JH, respectively. Of the 17 Ms markers, HTG4, HTG6, HTG7, HMS1 markers had relatively low PIC values (<0.6) in AMH and HMS1 in QH, and ASB2, HMS7, HTG4, ASB23, HMS1, HTG10 in JH. An average level of genetic variation was as follows: AMH, H_E= 0.7300 and H_O= 0.7317; QH, H_E= 0.7505 and H_O= 0.7011; and JH, H_E = 0.6917 and H_O= 0.7011. QH and JH had a genetic distance of 0.3831, and JH and AMH had a genetic distance of 0.2883. Of the three breeds, QH and JH formed clearly near groups and the AMH formed clearly different groups. This study is judged to be suitable for individual identification and parentage testing for registration of the lineage of AMH and QH. Therefore, the results of this study are an effective tool for genetic research and preservation of these horse breeds.

Key words: American Miniature Horse, Microsatellite Marker, Parentage Verification, Quarter Horse.

INTRODUCTION

The domestication of horses is believed to have been domesticated by humans for meat or milk between 4,000 and 3,000 BC in northern Ukraine or Kazakhstan. Therefore, it is estimated that humans started raising horses from the Neolithic Age to the Bronze Age. The origin of the Quarter horse (QH) is believed to be a cross between a native American horse originating in Spain in the 1600s and an English horse imported to Virginia from west of the Mississippi (Tryon et al. 2007).

The QH also has more than 2 million horses registered by the American Quarter Horse Association, one of the most popular horses in the world (AQHA 2019). QH has a thick neck, long, sloping shoulders, thick chest, short back, and long hips. These features are impeccably straight, coupled with structurally correct legs and feet, and are equally well-developed and well-balanced. These characteristics, based on agility and speed (Avila et al. 2018), demonstrate the best abilities seen in professional rodeos and Wrangler National Finals rodeos in rodeo time events team roping, barrel racing, tie-down roping and steer wrestling, and are the only western additions to the Fédération Équestre Internationale (FEI) World Equestrian Games. It also has an edge in racing, a sport. In addition to racing, ranching and western events, quarter horses are also used for a variety of events from jumping to dressage. Its best ability is by far the sprint compared to other breeds (Roth et al. 2021). Some horses have been measured at speeds of 88.5 km/h. In addition, the amazing character (gentleness, calmness) and trustworthiness of the QH are used in treatment and rehabilitation riding classes by horse auxiliary activities (Watson et al. 2020). In Korea, it is often used for horseback riding for adults who are experiencing horseback riding for the first time. Since 2011, Gyeongsang buk-do has imported 50 horses to the United States every year for 5 years for the purpose of increasing farm household income and distributing riding horses and supplying them to farm households.

American miniature horses (AMH) are ‘miniature’ versions of well-balanced horses that are no larger than

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larger dogs and have the morphological characteristics found in most horse breeds (Brooks et al. 2010). AMH come in a variety of colors and types, and their gentle nature makes it easy for children and toddlers who are new to horses to become familiar with horses without hesitation. Despite their small size, they are very versatile, so they excel in various fields such as driving, halter, jumping, and obstacles. With a height of less than 34 inches, it is mainly used for light driving rather than horseback riding, but it is widely used for children's horseback riding in Korea.

Currently, about 27,000 animals are raised in Korea, including 300 QHs, 100 AMHs, and 7,000 Jeju horses (JHs), a Korean native horse (Shin et al. 2020). Each breed has its own characteristics and strengths, and despite the need to be managed and maintained, systematic personal and lineage management has not been carried out yet.

Genetic markers have been used to determine the ancestry of animals for decades. There are various methods for identification and paternity in horses. Currently, parentage verification of horses is performed using a short tandem repeater (STR), and microsatellite (Ms) refers to a class of codominant DNA markers inherited in Mendelian fashion. In horses, Ms DNA marker was first reported by Ellegren et al. (1992) and Marklund et al. (1994). At present, Ms is analyzing using the StockMarks® for Horse Equine 17-plex genotyping kit (Applied Biosystems, Foster City, CA, USA) and the Equine genotype TM Panel 1.1 (Finnzymes Diagnostics, Espoo, Finland) in horse. Based on these results, it is applied to the parentage testing. And to get more accurate results, each laboratory is using TKY additional markers. But, recently, single nucleotide polymorphisms (SNPs) have been trying to introduce a horse's parentage verification. The SNPs are biallelic sequences that are abundantly distributed across most eukaryotic genomes (Salisbury et al. 2003; McKay et al. 2008). SNPs have a very low mutation rate (10^{-8} vs 10^{-3}) compared to STRs, and are short in length, making them ideal for analysis using automated high-throughput techniques, and can be analyzed with multiple techniques, such as denaturing high-performance liquid chromatography (DHPLC) (Liu et al. 1998), invader assays (Mein et al. 2000), and TaqMan® assays (Hui et al. 2008). Recently, interest in SNPs research has been increasing for forensic science (Lee et al. 2005; Lessig et al. 2005) and parentage testing of domestic animals (Werner et al. 2004; Rohrer et al. 2007; Hirota et al. 2010; Lopes et al. 2018; Browning et al. 2018; Pook 2019; Randhawa et al. 2020; Pook et al. 2020. Přibáňová et al. 2020; Long 2021; Hu et al. 2021; Gebrehiwot et al. 2021; Nolte et al. 2022).

The aim of this study was to evaluate the Ms variation in Jeju and American horses and their phylogenetic relationship, and to secure basic data for accurate pedigree management of QH and AMH in Korea.

MATERIALS AND METHODS

Genomic DNA extraction and Microsatellite loci analysis

Genomic DNA from 83 horses (27 AMHs, 32 QHs, and 24 JHs) hair roots was extracted using MFX-2000 (Toyobo, Osaka, Japan) according to the manufacturer's protocols (Tozaki et al. 2001). A total of 17 Ms loci were used for analysis in this study (Table 1). Analysis of this study was performed according to the manufacturer's protocols (Stockmarks®, Applied Biosystems, USA). Each DNA marker was tested using an ABI 3130 xl Genetic Analyzer and GeneMapper Software ver. 4.0 (Applied Biosystems, USA), the size of the alleles (base pairs) for each marker was determined according to the International Society for Animal Genetics (ISAG) standard.

Statistical Analysis

Statistical analyses of 17 Ms loci were performed using R software (Jombart and Ahmed 2011) to analyze basic population-level statistics and genetic diversity. The total number of alleles, polymorphism information content (PIC), observed heterozygosity (H_O), expected heterozygosity (H_E) were calculated. And the gene distance between breeds was calculated based on the standard gene distance value of Nei obtained using the R package (Kamvar et al. 2014).

RESULTS

Analysis of Genetic Diversity

In this study, 17 Ms loci were used to identify genetic diversity and relationships among horse breeds. The observed number of alleles per locus ranged from 4 (HTG7) to 12 (ASB17), with an average value of 7.71, 7.41, and 6.11 in AMH, QH, and JH, respectively. Of the 17 Ms loci, HTG4, HTG6, HTG7, HMS1 loci had relatively low PIC values (<0.6) in AMH and HMS1 in QH, and ASB2, HMS7, HTG4, ASB23, HMS1, HTG10 in JH (Table 2). An average level of genetic variation was as follows: AMH, H_E=0.7300 and H_O=0.7317; QH, H_E=0.7505 and H_O=0.7011; and JH, H_E =0.6917 and H_O=0.7011 (Table 2).

Parentage Testing

The results of parentage testing in 2 foals are shown in Table 3. Foal 1 did not inherited alleles from sire or dam, and excluded by the incompatibility of 11 markers (AHT5, HMS3, HMS6, HMS7, HTG10, ASB17, ASB23, LEX3, CA425, HMS2, and HTG6). But foal 2 was identified as paternity in accordance with Mendel's genetic law.

Genetic Distance and Genetic Relationships of Horse Populations

The results of analyzing the genetic relationships of the 3 horse breeds are as shown in Table 4 and Fig. 1. QH and JH had a genetic distance of 0.3831, and JH and AMH had a genetic distance of 0.2883. Of the three breeds, QH and JH formed clearly near groups and the AMH formed clearly different groups.

DISCUSSION

Ms are beneficial because they have a high rate of polymorphism and are useful for parentage testing in animals (Bowling et al. 1997; Cho et al. 2002; Kim and

Table 1: The primer sequences of the Ms loci used in this study

Locus	Primer sequence (5′→3′)	Allele size (bp)	References
AHT4	(FAM)-AACCGCCTGAGCAAGGAAGT, GCTCCCAGAGATTTACCCT	138-170	Binns et al. (1995)
AHT5	(VIC)-ACGGACACATCCCTGCCTGC, GCAGGCTAAGGGGGCTCAGC	128-152	Binns et al. (1995)
ASB2	(VIC)-CCACTAAGTGTCGTTTCAGAAGG, CACAACTGAGTTCTCTGATAGG	222-266	Breen et al. (1997)
ASB17	(PET)-GAGGGCGGTACCTTTGTACC, ACCAGTCAGGATCTCCACCG	89-131	Breen et al. (1997)
ASB23	(VIC)-GCAAGGATGAAGAGGGCAGC, CTGGTGGGTTAGATGAGAAGTC	176-212	Irvin et al. (1998)
CA425	(PET)-AGCTGCCTCGTTAATTCA, CTCATGTCCGCTTGTCTC	230-250	Eggleston-Stott et al. (1997)
HMS1	(PET)-CATCACTCTTCATGTCTGCTTGG, TTGACATAAATGCTTATCCTATGGC	166-178	Guerin et al. (1994)
HMS2	(NED)-CTTGCAGTCGAATGTGTATTAAAT, ACGGTGGCAACTGCCAAGGAAG	218-238	Guerin et al. (1994)
HMS3	(NED)-CCAACTCTTTGTCACATAACAAGA, CCATCCTCACTTTTTCACTTTGTT	150-174	Guerin et al. (1994)
HMS6	(VIC)-GAAGCTGCCAGTATTCAACCATTG, CTCCATCTTGTGAAGTGTAACTCA	153-171	Guerin et al. (1994)
HMS7	(FAM)-CAGGAAACTCATGTTGATACCATC, TGTTGTTGAAACATACCTTGACTGT	167-189	Guerin et al. (1994)
HTG4	(FAM)-CTATCTCAGTCTTGATTGCAGGAC, CTCCCTCCCTCCCTCTGTTCTC	127-141	Ellegren et al. (1992)
HTG6	(VIC)-GAAGCTGCCAGTATTCAACCATTG, CTCCATCTTGTGAAGTGTAACTCA	153-171	Ellegren et al. (1992)
HTG7	(NED)-CCTGAAGCAGAACATCCCTCCTTG, ATAAAGTGTCTGGGCAGAGCTGCT	118-130	Marklund et al. (1994)
HTG10	(NED)-CAATTCCCGCCCCACCCCCGGCA, TTTTTATTCTGATCTGTCACATTT	89-171	Marklund et al. (1994)
LEX3	(PET)-ACACTCTAACCAGTGCTGAGACT, GAAGGAAAAAAAGGAGGAAGAC	137-160	Coogle et al. (1996)
VHL20	(FAM)-CAAGTCCTCTTACTTGAAGACTAG, AACTCAGGGAGAATCTTCCTGAG	89-107	Van Haeringen et al. (1994)

Table 2: Number of alleles, heterozygosity, and PIC of the 17 Ms markers in 3 horse breeds

Marker	No. of allele			H_O			H_E			PIC		
	AMH	QH	JH	AMH	QH	JH	AMH	QH	JH	AMH	QH	JH
AHT4	7	6	9	0.7800	0.5900	0.8800	0.7900	0.6900	0.8300	0.7596	0.6559	0.8116
AHT5	7	7	7	0.7800	0.7800	0.7900	0.7600	0.7500	0.7400	0.7333	0.7131	0.7058
ASB2	6	9	6	0.7400	0.8400	0.6700	0.8100	0.8200	0.6400	0.7779	0.7942	0.5982
HMS3	7	7	6	0.6700	0.9400	0.7500	0.7400	0.8100	0.7800	0.7040	0.7864	0.7469
HMS6	6	7	5	0.8900	0.7200	0.6700	0.8000	0.6800	0.7100	0.7667	0.6282	0.6583
HMS7	7	7	6	0.8100	0.7800	0.5000	0.8100	0.8100	0.5200	0.7833	0.7852	0.4878
HTG4	4	7	6	0.3000	0.5900	0.7100	0.3200	0.6900	0.6500	0.3088	0.6503	0.5899
HTG10	9	10	8	0.8100	0.9100	0.9200	0.8000	0.8400	0.7800	0.7772	0.8202	0.7481
VHL20	9	8	5	0.8900	0.8800	0.9200	0.8300	0.8400	0.7200	0.8107	0.8151	0.6753
ASB17	12	10	8	0.8500	0.7500	0.8300	0.8200	0.8200	0.7800	0.7970	0.7995	0.7560
ASB23	7	6	6	0.8100	0.8100	0.6200	0.8100	0.8300	0.6100	0.7827	0.8023	0.5825
HMS1	7	5	4	0.6300	0.5900	0.5800	0.6400	0.5600	0.5800	0.5944	0.5166	0.5098
LEX3	9	10	7	0.7800	0.7800	0.4600	0.8300	0.8600	0.7800	0.8144	0.8450	0.7472
CA425	9	7	7	0.8900	0.8400	0.8300	0.7700	0.6800	0.7600	0.7439	0.6416	0.7217
HMS2	6	8	6	0.7000	0.7800	0.5800	0.7900	0.7300	0.7100	0.7547	0.7025	0.6636
HTG6	6	7	4	0.4800	0.6600	0.3800	0.4500	0.6800	0.4400	0.4254	0.6327	0.3884
HTG7	4	5	4	0.6300	0.6900	0.8300	0.6400	0.6700	0.7300	0.5911	0.6109	0.6820
Mean	7.71	741	6.11	0.7317	0.7605	0.7011	0.7300	0.7505	0.6917	0.7014	0.7176	0.6513

*AMH: American miniature horse, QH: Quarter horse, JH: Jeju horse, H_O: Observed heterozygosity, H_E: Expected heterozygosity, PIC: Polymorphism Information Content.

Choi 2002; Cho and Cho 2004; Li et al. 2004; Sun et al. 2004; Sugiura et al. 2020); they have been used extensively to examine the structure of closely related populations and breed allocation of animals (Kim and Choi 2002; Li et al. 2004; Sun et al. 2004; Yoon et al. 2005; Cho 2005). In the case of cows, pigs, horses, donkeys, and dogs, pedigree control has been routinely carried out by paternity in most countries, which conforms to the standards of the International Society for Animal Genetics (ISAG).

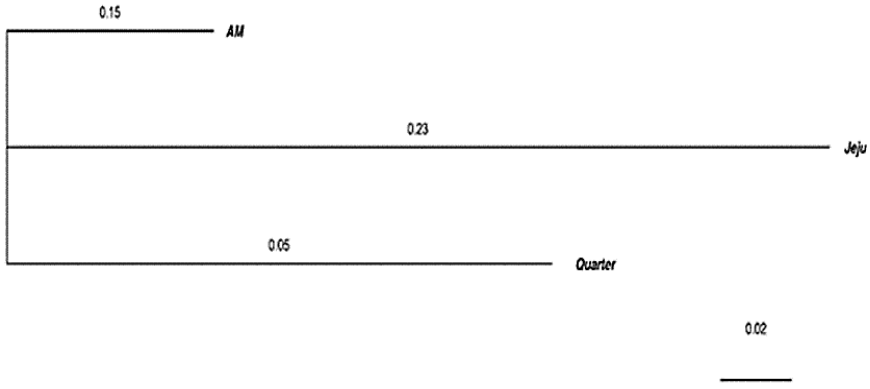

Fig. 1: The phylogenetic tree analysis in 3 horse populations. *AM: American miniature horse, Quarter: Quarter horse, Jeju: Jeju horse.

Table 3: One case of parentage testing by 17 Ms markers in Quarter horse

Horse	AHT4	AHT5	ASB2	HMS3	HMS6	HMS7	HTG4	HTG10	VHL20	ASB17	ASB23	HMS1	LEX3	CA 425	HMS2	HTG6	HTG7
Sire	J/O	K/N	N/O	I/R	P/P	N/O	K/M	L/Q	I/M	N/O	J/K	J/M	S/S	M/N	L/L	G/J	N/O
Dam	H/O	N/N	K/K	I/I	L/O	O/O	K/P	R/S	I/Q	N/Q	L/U	J/M	L/O	J/N	J/O	K/O	K/O
Foal 1	O/O	J/N	K/N	M/R	P/P	J/J	K/M	L/O	M/Q	N/R	S/U	J/M	K/L	N/O	L/L	K/O	K/O
Foal 2	O/O	N/N	K/N	I/I	L/P	O/O	K/K	L/R	I/M	N/N	J/L	J/J	L/S	N/N	L/O	K/J	K/O

*Alphabetical allele codes for all loci are identical to the assignments from the 2000 ISAG Horse Comparison Test.

Table 4: Matrix of Da genetic distances observed among the three horse breeds

Breeds	AMH	JH	QH
AMH	0		
JH	0.2883	0	
QH	0.2105	0.3831	0

*****AMH: American miniature horse, QH: Quarter horse, JH: Jeju horse.

To meet the demand of the domestic QH market and secure better QH quality in Korea, it is necessary to select QHs with excellent pedigree to start and strengthen QH breeding. Currently, however, there is insufficient research on the reproduction and inheritance of QH compared to other breeds such as JH and other horses in Korea. Studies have shown that the validity and reliability of markers are estimated based on the polymorphism information content (PIC) values of each marker. Therefore, it is judged that markers with relatively high PIC values (PIC >0.5) will be useful for paternity verification of QH and AMH.

An indicator of diversity in a single gene pool is heterozygosity. In association analysis or connection imbalance analysis, high heterogeneous bonding is more desirable (Kang et al. 2009; Goor et al. 2010). Although heterozygosity increases when multiple groups are mixed, heterozygosity is generally associated with mutations in the population without interbreeding of groups (Oberbauer et al. 2010). In the case of genetic characteristic analysis using a Ms marker, heterogeneity may be determined from the degree of mixing of the target group and the other group. If pure lineage is preserved through strong selection without species mixing, the value of heterozygosity is low. If other varieties are mixed, heterozygosity is high. However, heterozygosity is higher when more individuals are used in the study, so it can be difficult to judge the hybridization of species by heterozygosity alone.

It was found that the average number of alleles was 7.71, 7.41, and 6.11 in AMH, QH, and JH, respectively, and an average genetic variation levels in AMH (H_E = 0.7300 and H_O = 0.7317) and QH (H_E = 0.7505 and H_O = 0.7011), and JH (H_E = 0.6917 and H_O= 0.7011). These results suggest that the genetic diversity of QH or AMH is more abundant than that of JH. Also, it is considered useful for individual identification or paternity test of these horse breeds, except for markers that show relatively low PIC values (<0.6) for HTG4, HTG6, HTG7, HMS1 markers in AMH and HMS1 marker in QH. As a result of examining the relationships of the AMH and the QH through the genetic distance, it was found to be close to QH and JH. It was confirmed that this supports rumors that QH has been introduced to Jeju Island and raised.

While discussions are currently underway on whether to use SNPs instead of Ms DNA markers for parentage testing and individual identification of horses, the findings suggest that Ms is also effective as a tool for genetic research and preservation of QH and AMH.

Conclusion

This study is judged to be suitable for individual identification and parentage testing for registration of the lineage of AMH and QH. Therefore, the results of this study are an effective tool for genetic research and preservation of these horse breeds.

Author's Contribution

All research protocols in this study were designed, and conducted by C Jang, who also contributed to data acquisition. GJ Cho and O Baatartsogt contributed to the interpretation of the experimental results and the writing the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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