

## Genetic Relationship among the Korean Native and Alien Horses Estimated by Microsatellite Polymorphism

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**ABSTRACT :** Microsatellite polymorphism and the genetic relationship were estimated using genotype information of 305 horses from 11 microsatellite loci. The breeds include the indigenous Korean breeds, Korean native horse (102) and Jeju racing horse (56) together with Japan Hokkaido horse (5), Mongolian horse (19), Thoroughbred horse (108), Quarter horse (11) and Przewalskii horse (4). Allelic frequencies, the number of alleles per locus were estimated by direct counting from observed genotype, and genetic variability was computed using the CERVUX software and DISPAN. The number of alleles per locus varied from 6 (HMS6) to 18 (ASB17) with an average value of 10.45 in horse breeds. The expected total heterozygosity ( $H_T$ ) and coefficient of gene differentiation ( $G_{ST}$ ) ranged 0.764-0.921 (the average value was 0.830) and 0.102-0.266 (the average value was 0.180) in horse breeds, respectively. Four populations (Przewalskii horse, Japan Hokkaido horse, Quarter horse, Thoroughbred horse) showed lower heterozygosity than the average value (the average value was 0.710). The expected heterozygosity within breed ( $H_S$ ) and mean no. of observed alleles ranged from 0.636±0.064 (Japan Hokkaido horse) to 0.809±0.019 (Mongolian horse), and from 2.73 (Przewalskii horse) to 8.27 (Korean native horse), respectively. The polymorphic information content (PIC) ranged from 0.490 (Przewalskii horse) to 0.761 (Mongolian horse) with an average value of 0.637 in horse breeds. The results showed three distinct clusters with high bootstrap support: the Korean native horse cluster (Korean native horse, Mongolian horse), the European cluster (Przewalskii horse, Thoroughbred horse), and other horse cluster (Jeju racing horse, Japan Hokkaido horse, and Quarter horse). A relatively high bootstrap value was observed for the Korean native horse cluster and European cluster (87%), and the Korean native horse and Mongolian horse (82%). Microsatellite polymorphism data were shown to be useful for estimating the genetic relationship between Korean native horse and other horse breeds, and also be applied for parentage testing in those horse breeds. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 6 : 784-788)

**Key Words :** Genetic Relationship, Korean Native Horse, Microsatellite Polymorphism

### INTRODUCTION

The Korean native horse is one of the Korean native animals which was designated as a natural monument No. 347 on February 1985, by government (Cho et al., 2001). At the present, Korean native horses are raised about 450 herds which were finished the pedigree registration. It has been insulated from other horse populations, and partly has been used racing horse in Jeju race course of Korea Racing Association. Jeju island is the major province to produce Thoroughbred horse in Korea, and has a potential to reduce the genetic resources of Korean native horse through cross breed horses (Korean native horse×Thoroughbred horse: Jeju racing horse). Jeju racing horses including the Korean native horse are raised about 8,000 herds in Korea, and used for racing, horseback riding and meat. So, it is necessary to detect and preserve genetic sources of the Korean native horse. Detailed information on levels of genetic diversity and patterns of gene structure of the Korean native horse is very important for meeting the demands of future breeding programs and for formulating effective conservation strategies of indigenous breeds.

Microsatellites have been commonly utilized for the assessment of genetic diversity, construction of genetic

maps, quantitative trait loci mapping and parentage testing (Li et al., 2004; Zhang et al., 2005). Tozaki et al. (2003) conducted a phylogenetic study of Thoroughbreds, seven Japanese horse populations, and four mainland-Asian horse populations using the microsatellite loci. Tozaki et al. (2003) in their study suggested that Japanese native horses including Japan Hokkaido horse originated from Mongolian horses migrating through the Korean peninsula.

In recent years, several studies have been undertaken to investigate the genetic characteristics of Korean native horses using either DNA markers (Cho et al., 2001; Cho and Cho, 2003) or biochemical markers (Cho et al., 1999; Cho et al., 2000). The genetic relationship of many horse populations in Europe has been investigated recently using microsatellites (Canon et al., 2000; Vila et al., 2001). There is, however, no further information is available for genetic diversity and phylogenetic relationship of Korean native breeds using microsatellites in Korea.

The present work was carried out to characterize genetic diversity and phylogenetic relationship among horse breeds including the Korean native horse based on allelic frequencies for eleven microsatellite loci.

### MATERIALS AND METHODS

#### Sample collection and DNA extraction

Genomic DNAs were prepared from whole blood and

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**Table 1.** Number of allele, observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), polymorphic information content (PIC) and exclusion probability (PE) of microsatellite loci in 102 Korean native horses

Loci	No. of allele	$H_O$	$H_E$	PIC	PE
AHT4	9	0.853	0.833	0.806	0.658
AHT5	7	0.824	0.782	0.743	0.568
ASB2	10	0.706	0.715	0.680	0.503
ASB17	13	0.873	0.849	0.827	0.694
CA425	9	0.843	0.780	0.745	0.577
HMS3	9	0.755	0.811	0.780	0.619
HMS6	6	0.706	0.700	0.658	0.471
HMS7	5	0.471	0.477	0.448	0.283
HTG4	6	0.549	0.556	0.506	0.323
HTG10	10	0.804	0.788	0.756	0.592
VHL20	7	0.765	0.786	0.747	0.569

hair roots samples, which were collected from 305 individuals of seven horse populations: Thoroughbred horse ( $n = 108$ ), Korean native horse ( $n = 102$ ), Jeju racing horse ( $n = 56$ ), Quarter horse ( $n = 11$ ), Mongolian horse ( $n = 19$ ), Przewalskii horse ( $n = 4$ ) and Japan Hokkaido horse ( $n = 6$ ). Jeju racing horse raised in Jeju island is a crossbreed horse between Korean native horse and Thoroughbred horse. Genomic DNAs from samples were extracted using MagExtractor System MFX-2000 (Toyobo, Japan) according to the manufacturer's protocol (Tozaki et al., 2001).

#### PCR and microsatellite analysis

Eleven microsatellite loci, AHT4, AHT5, ASB2, ASB17, CA425, HMS3, HMS6, HMS7, HTG4, HTG10 and VHL20 were used for the analysis of the horse breeds. PCR was accomplished in a total volume of 15  $\mu$ l of the following mixture: 40 ng of genomic DNA, each primer, 1.25 mM of dNTPs, 2.5  $\mu$ l of 10x reaction buffer, and 5 U of *Taq* polymerase (Applied Biosystems, USA). PCR amplification was as follows: first step was performed by initial denaturation for 10 min at 95°C, followed by 30 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 1 min. An extension step at 72°C for 60 min was added after the final cycle (Dimsoski, 2003). Multiplex PCR systems were performed in a GeneAmp PCR System 9700 (Applied Biosystems, USA). PCR products were denatured with formamide and electrophoresis was carried out on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) using the recommended protocol. Fragment size analysis was performed with genotype software Ver.3.7 (Applied Biosystems, USA).

#### Statistical analysis

Allelic frequencies, the number of alleles per locus were estimated by direct counting from observed genotype, and polymorphic information content (PIC) was computed

using the CERVUX software (Marshall et al., 1998). Genetic variability estimates of average observed heterozygosity, expected total heterozygosity ( $H_T$ ), expected within population heterozygosity ( $H_S$ ), coefficient of gene differentiation ( $G_{ST}$ ), total and mean number of alleles per population were calculated using DISPAN (Ota, 1993). Genetic differences among populations were estimated by calculating the  $D_a$  genetic distance (Nei et al., 1983). Phylogenetic trees were constructed from  $D_a$  genetic distance matrix according to the neighbor-joining (NJ) method (Saitou and Nei, 1987) implemented by DISPAN (Ota, 1993).

## RESULTS

#### Microsatellite polymorphism

Number of allele, observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), polymorphic information content and exclusion probability (PE) in the Korean native horse were shown in Table 1. The number of alleles per locus varied from 5 (HMS7) to 13 (ASB17) with an average value of 8.27 in the Korean native horse. The observed heterozygosity and the expected heterozygosity ranged 0.471-0.873 (the average value was 0.741) and 0.477-0.849 (the average value was 0.734) in the Korean native horse, respectively. The PIC value and exclusion probability ranged 0.448-0.827 (the average value was 0.700) and 0.283-0.694, respectively. Of the 11 markers, AHT4, AHT5, ASB17, CA425, HMS3, HTG10 and VHL20 loci have a relatively high PIC value (over 0.7) in the Korean native horse.

#### Heterozygosities and the number of alleles

A total of 115 alleles were observed among the 305 animals assayed and demonstrated that they were highly polymorphic in all horse populations. Measures of genetic variability were shown in Table 2 and 3. The number of alleles per locus varied from 6 (HMS6) to 18 (ASB17) with an average value of 10.45 in horse breeds. The expected total heterozygosity ( $H_T$ ) and coefficient of gene differentiation ranged 0.764-0.921 (the average value was 0.830) and 0.102-0.266 (the average value was 0.180) in horse breeds, respectively. Four populations (Przewalskii horse, Japan Hokkaido horse, Quarter horse, Thoroughbred horse) showed lower heterozygosity than the average value (the average value was 0.710). The expected heterozygosity within breed and mean no. of observed alleles ranged from  $0.636 \pm 0.064$  (Japan Hokkaido horse) to  $0.809 \pm 0.019$  (Mongolian horse), and from 2.73 (Przewalskii horse) to 8.27 (Korean native horse), respectively. The polymorphic information content ranged from 0.490 (Przewalskii horse) to 0.761 (Mongolian horse) with an average value of 0.637 in horse breeds studied.

**Table 2.** Characterization of 11 microsatellite loci analyzed with 7 horse breeds

Loci	No. of alleles	H <sub>T</sub>	H <sub>S</sub>	G <sub>ST</sub> *
AHT4	10	0.842	0.756	0.102
AHT5	8	0.823	0.720	0.125
ASB2	15	0.881	0.681	0.227
ASB17	18	0.921	0.755	0.181
CA425	11	0.807	0.656	0.187
HMS3	11	0.846	0.688	0.187
HMS6	6	0.771	0.613	0.205
HMS7	7	0.764	0.658	0.140
HTG4	8	0.794	0.583	0.266
HTG10	11	0.831	0.699	0.159
VHL20	10	0.848	0.683	0.195
All loci	115	0.830	0.681	0.180

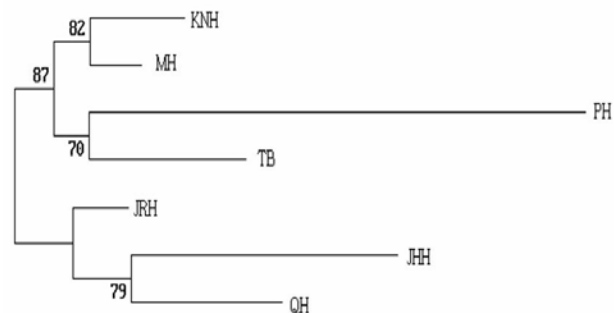
\* All breeds examined, H<sub>T</sub>: expected total heterozygosity, H<sub>S</sub>: expected within total heterozygosity, G<sub>ST</sub>: Gene differentiation.

### Genetic distances and population relationship

Estimates of the Da genetic distances and standard genetic distances among the 7 populations based on the allele frequency data on 11 microsatellite loci were presented in Table 4 and 5. The largest Da (0.8234) and D<sub>S</sub> (1.9215) distances were observed between Przewalskii horse and Japan Hokkaido horse, and the smallest Da (0.1255) and D<sub>S</sub> (0.1566) distances between Korean native horse and Mongolian horse. Dendrogram, based on the calculated genetic distances and constructed by the use of the N-J method, was presented in Figure 1. The data clearly showed three distinct clusters with high bootstrap support: the Korean native horse cluster (Korean native horse, Mongolian horse), the European cluster (Przewalskii horse, Thoroughbred horse), and other horse cluster (Jeju racing horse, Japan Hokkaido horse, and Quarter horse). A relatively high bootstrap value was observed for the Korean native horse cluster and European cluster (87%), and the Korean native horse and Mongolian horse (82%).

### DISCUSSION

Recently, many microsatellites were isolated from the horse genome (Tozaki et al., 2000), and the microsatellites

**Figure 1.** Neighbor-joining trees by DA genetic distances. Numbers indicate bootstrap values in percentage after 1,000 resampling.

showed multiple alleles as well as high heterozygosity among European horse breeds such as Thoroughbred horse. Microsatellites may be a useful approach for resolving the relationship of the Korean native and alien horse populations. Many kinds of microsatellites are informative due to their high polymorphism and they are useful in paternity testing of animals (Bowling et al., 1997; Cho et al., 2002; Kim and Choi, 2002; Cho and Cho, 2004; Li et al., 2004; Sun et al., 2004; Yoon et al., 2005). The method was used extensively to construct structure of the closely related populations and breed allocating of animals (Kim and Choi, 2002; Li et al., 2004; Sun et al., 2004; Cho, 2005). In cattle, pig, horse and dog populations, pedigree control has been performed on routine basis in most countries. These controls rely on microsatellite typing that has been standardized through regular comparison tests under the auspices of the International Society for Animal Genetics (ISAG).

In the present study, the allele frequencies and genotype distributions of the Korean native and alien horses were analyzed to investigate a phylogenetic relationship among the populations including Mongolian horse to consider the ancestral breed for the Korean native horse. The allele frequency was distinctive for each breed and was the main feature of the population genetic structure. In this study, the studied loci were more polymorphic in Mongolian horse than in other horse breeds, demonstrating that Mongolian

**Table 3.** Expected heterozygosity (H<sub>E</sub>), observed heterozygosity (H<sub>O</sub>), mean number of allele, polymorphic information content (PIC) of microsatellite loci for each breed

Population	Sample size	Mean no. of allele	H <sub>E</sub>	H <sub>O</sub>	PIC
Korean native horse (KNH)	102	8.27	0.734±0.035	0.741	0.700
Japan Hokkaido horse (JHH)	5	3.55	0.636±0.064	0.691	0.518
Jeju racing horse (JRH)	56	8.09	0.782±0.024	0.789	0.749
Mongolian horse (MH)	19	8.18	0.809±0.019	0.833	0.761
Przewalskii horse (PH)	4	2.73	0.649±0.040	0.591	0.490
Quarter horse (QH)	11	5.27	0.692±0.030	0.752	0.619
Thoroughbred horse (TB)	108	5.45	0.674±0.041	0.700	0.625
Pooled sample	305	5.93	0.710±0.036	0.728	0.637

**Table 4.** Matrix of  $D_a$  genetic distances observed among the horse breeds

	KNH	PH	JHH	QH	MH	TB
PH	0.5651					
JHH	0.4293	0.8234				
QH	0.4116	0.7456	0.3615			
MH	0.1255	0.5499	0.4215	0.3865		
TB	0.2941	0.5645	0.5760	0.3669	0.2212	
JRH	0.2400	0.5791	0.3610	0.1985	0.2141	0.3162

horse have retained the largest amount of genetic variation of all the populations studied. In addition, Mongolian horse possessed all the alleles found in the Korean native horse, with the exception of ASB2, HMS3 and HMS7 marker. Also, the Korean native horse possessed all the alleles found in Japan Hokkaido horse. These results supports the hypothesis that Korean native horse is descended from the ancestral populations of Mongolian horse. This assumption is supported by the historical fact that native horses on the Asian continent were frequently transported to Japan through the Korean peninsula (Tozaki et al., 2002). These results were almost consistent with results of this study.

Evaluation of microsatellite polymorphism can be used for differentiation of closely related species. Usually, data on frequency of microsatellite alleles are used to estimate a genetic distance between populations (Klukowska et al., 2003). In this study, the largest  $D_a$  (0.8234) and  $D_s$  (1.9215) distances were observed between Przewalskii horse and Japan Hokkaido horse, and the smallest  $D_a$  (0.1255) and  $D_s$  (0.1566) distances between Korean native horse and Mongolian horse. Takezaki and Nei (1996) proved that for reconstruction of phylogenetic trees it is important to study a large number of loci. The relatively small number of analysed microsatellite did not influence the dendrogram topology, as only five branches were distinguished. The population size also has effects on assignment accuracy. In this study, the phylogenetic tree showed three distinct clusters with high bootstrap support: the Korean horse cluster (Korean native horse, Mongolian horse), the European cluster (Przewalskii horse, Thoroughbred horse), and other horse cluster (Jeju racing horse, Japan Hokkaido horse, and Quarter horse). A relatively high bootstrap value was observed for the Korean native horse cluster and European cluster (87%), and the Korean native horse and

Mongolian horse (82%).

The results clearly show that Korean native horse and Mongolian horse are closely related to each other when compared with other horse breeds. This information can be used as fundamental data for meeting the demands of future breeding program to be based on greater knowledge of genetic structure and relationship between horse breeds. On the other hand, Japan Hokkaido horse exhibits the Korean native horse and Mongolian horse outgroup, probably due to their smaller size. Further investigation is required to explain the relationship of the populations using more microsatellite markers or other markers such as SNP and the segregation of genes. Also, to have a firm conclusion for resolving an origin and pedigree in the Korean native horse, more extensive investigation might be required. As these data show, microsatellites are a useful tool for studying the genetic relationship among closely related horse breeds. Since the microsatellites in this study are highly polymorphic, they can also be applied for parentage testing in the Korean native horse, and more efforts should be devoted to making an efficient and reliable evaluation of genetic variations by means of microsatellite for the Korean native horse.

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**Table 5.** Matrix of standard genetic distances ( $D_s$ ) and standard errors observed among the horse breeds

	KNH	PH	JHH	QH	MH	TB	JRH
KNH	-	0.2375	0.2265	0.1627	0.0443	0.0696	0.1620
PH	0.9610	-	0.3658	0.3422	0.2087	0.2552	0.1655
JHH	0.6751	1.9215	-	0.1838	0.1796	0.2484	0.1617
QH	0.7478	1.6843	0.4142	-	0.1667	0.2018	0.0410
MH	0.1566	0.8391	0.6313	0.5627	-	0.0770	0.1534
TB	0.5535	0.8859	1.0908	0.5673	0.3095	-	0.2167
JRH	0.4855	1.0096	0.4687	0.1815	0.3854	0.5309	-

Genetic distances are below the diagonal, and standard errors are above the diagonal.

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