*Journal of Life Science* 2009 Vol. 19. No. 2. 169~173 ©JLS / ISSN 1225-9918

# Assessment of Genetic Diversity of Horse Breeds Using Microsatellite Makers

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Received October 16, 2008 / Accepted January 30, 2009

To assist in selection schemes we estimate the genetic diversity of the horse breeds. Genetic diversity at 13 microsatellite loci was compared in six horse breeds : Jeju Native Horse, American Quarter, Jeju Racing Horse, Mongolian Horse, Japanese Horse and Thoroughbred. All of the equine microsatellite used in this study were amplified and were polymorphic. The expected total heterozygosity over all the populations varied between 0.669 and 0.869 and the expected heterozygosity within population range from 0.569 to 0.219 in this study. The low coefficient of gene differentiation value showed that only 0.118 of the diversity was between horses breeds. The constructed dendrogram from the genetic distance matrix showed little differentiation between horse breeds using DISPAN program. The genetic distance using 13 microsatellites ranged between 0.137 and 0.414 for the six horse breeds. These results confirm the potential use of equine microsatellite loci as a tool for genetic studies in horse populations. The genetic diversity of the six horse breeds to each other closed to their geographical distribution. Suggesting that the loci would be suitable for horse breeds parentage testing. Therefore, Microsatellite marker seems to be very useful for clarifying the evolutionary relationships of closely related populations.

**Key words** : Genotype, genetic diversity, microsatellite markers, horse breeds

## Introduction

Recently many investigators have used microsatellte DNA loci for studying the evolutionary relationships of closely related populations, and some authors proposed new genetic measures for this purpose. Allele frequency data are useful for studying evolutionary relationships of closely related species or populations. Microsatellite DNA has been used for studies of genetic variability and population structure in horse breeds [8]. Genetic characterization is the first step in the conservation of breeds and the determination of future breeding strategies. The term microsatellite, also short tandem repeats (STRs), refers to a class of codominant DNA markers which are inherited in a Mendelian fashion. Microsatellite are highly polymorphic and abundant sequences dispersed throughout most eukaryotic nuclear genomes [11,24]. A large number of microsatellite markers have been mapped for various species including humans, mice, cattle, sheep, pigs and poultry [6]. Domestic animals are products of selection, improvement and domestication processes. The selection pressure to which are submitted has forced races and domestic populations to adapt to specific ecological niches. These breeds have also undergone the effects of genetic drift, mutation and artificial selection. Accordingly, the preservation and conservation these breeds are of significant value for nature and for humans [5]. For a decade, concerns regarding many native breeds have increased, because many of them are disappearing by mixing and replacement [5]. In the present study, we performed a routine DNA typing with 13 microsatellite markers for genetic diversity in horse breeds.

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## Materials and Methods

### Animals and DNA extraction

We collected blood samples from Thorughbred horses (n=44), Jeju racing horses (n=56), Jeju native horses (n=41), Japanese horses (n=5), Mongolian horse (n=39), and American Quarter (n=11). A total number of 196 horses from six horse breeds were tested. No horses were injured in any way during the collecting of samples. Genomic DNAs were

prepared from whole blood samples. Genomic DNAs from six horse breeds were extrated using the Mag Extractor System MFX-2000 (Toyobo, Osaka, Japan). DNA was extracted from whole sample by the high salt extraction procedure, suspended in TE pH 8.0, and stored at 4°C.

### Microsatellite markers

In this study we used the following 13 microsatellite loci: AHT4, AHT5, ASB2 [1], HMS3, HMS6, HMS7 [7], HTG10 [10], VHL20 [23], TKY321 [19,20,22], CA425 [4], ASB17 [2] and LEX33 [3] (Table. 1).

### Multiplex PCR conditions

Multiplex PCRs were carried out in 10 μl reactions caintaining 50 ng of genomic DNA, 10 pmol of each primer, 200 μM of each dNTP, 0.5 units of Taq DNA polymerases (Promega), and reaction buffer with 1.5 mM $MgCl_2$ PCR was carried out in a 9700 GeneAmp PCR system by an initial denaturation at 95°C for 10 min, followed by 30 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 60 sec. The thermal profile ended with a final extansion at 72°C for 60 min. PCR products were detected by 8% polyacrylamide gel electrophoresis using an Applied Biosystems 377 DNA sequencer with Genescan analysis software (version 3.1) using Genotyper analysis software (version 2.0)

### Statistical analysis

Allele frequencies and mean heterozygosity values for each polymorphic locus were obtained using the MS toolkit S/W [14]. The average expected heterozygosity for each population (Hs), the gene diversity in the total population (Ht), and coefficient of gene differentiation Gst [11] were estimated using the computer programme DISPAN [13], and tested by permutation test. Genetic distance and phylogenetic trees among populations were obtained with the distance measure $D_A$ [12]. Takezaki and Nei [18] suggested the $D_A$ distance for making phylogenetic trees when the interest of the study mainly focused on the topology rather than evolutionary time. Distance data was analysed with the neighbour-joining (NJ) method of clustering [15]. The NJ method

Table 1. Characteristics of 13 microsatellite loci used in this study

| Locus | Primer sequence (5′-3′) | Allele size (bp) | References |
|---|---|---|---|
| ATH4 | (FAM)- AACCGCCTGAGCAAGGAAGT<br>GCTCCCAGAGAGTTTACCCT | 138-170 | Binns et al. (1995) |
| ATH5 | (VIC)- ACGGACACATCCCTGCCTGC<br>GCAGGCTAAGGGGGCTCAGC | 128-152 | Binns et al. (1995) |
| ASB2 | (VIC)- CCACTAAGTGTCGTTTCAGAAGGCA<br>CAACTGAGTTCTCTGATAGG | 222-256 | Breen et al. (1997) |
| HMS3 | (NED)- CCAACTCTTTGTCACATAACAAGA<br>CCATCCTCACTTTTTCACTTTGTT | 150-174 | Guerin et al. (1994) |
| HMS6 | (JOE)- GAAGCTGCCAGTATTCAACCATTGC<br>TCCATCTTGTGAAGTGTAACTCA | 153-171 | Guerin et al. (1994) |
| HMS7 | (FAM)- CAGGAAACTCATGTTGATACCATC<br>TGTTGTTGAAACATACCTTGACTGT | 167-189 | Guerin et al. (1994) |
| HTG4 | (FAM)- CTATCTCAGTCTTGATTGCAGGAC<br>CTCCCTCCCTCCCTCTGTTCTC | 127-141 | Ellegren et al. (1992) |
| HTG10 | (NED)- CAATTCCCGCCCCACCCCCGGCATT<br>TTTATTCTGATCTGTCACATTT | 89-171 | Marklund et al. (1994) |
| VHL20 | (FAM)- CAAGTCCTCTTACTTGAAGACTAG<br>AACTCAGGGAGAATCTTCCTGG | 89-107 | Van Haeringen et al. (1994) |
| TKY321 | (FAM)- TTGTTGGGTTAGGTATGAAGGGTG<br>TCAATGTGAGCTTCAAGAAC | 180-220 | Tozaki et al. (2001) |
| CA425 | (PET)- AGCTGCCTCGTTAATTCA<br>CTCATGTCCGCTTGTCTA | 230-250 | Eggleston-Stott et al. (1997) |
| ASB17 | (PET)- GACGGCGGTACCTTTGTACC<br>ACCAGTCAGGATCTCCACCG | 89-131 | Breen et al. (1997) |
| LEX33 | (PET)- ACACTCTAACCAGTGCTGAGACTGA<br>AGGAAAAAAAGGAGGAAGAC | 137-160 | Coogle et al. (1996) |

procedure only unrooted trees. All these calculation were carried out using the DISPAN package [13].

## Results

The equine microsatellite were all well-amplified in the horses. The average gene diversity based on the expected total heterozygosity over all loci was 0.796, while, it ranged from 0.669 (HTG4) to 0.869 (TKY321) for individual loci. The average gene diversity based on the Expected heterozygosity within populaiton across all loci in the total sample was 0.691 and ranged from 0.569 (HTG4) to 0.769 (TKY321). The average coefficient of gene differentiation (Gst) over the 13 loci was 0.061 for CA425 to 0.219 for LEX33 (Table 2). The mean number of alleles per locus of six horses ranged from 3.46 to 8.62 in the Mongolian horse (Table 3). The mean observed heterozygosity (Ho) ranged from 0.649 in the Mongolian horse to 0.804 in Jeju racing horse. Average expected heterozygosities ($H_E$) ranged from 0.639 in the Japanese horse breed to 0.797 in the Mongolian horse. The $D_A$ distance, using 13 microsatellites, ranged between 0.137 and 0.4146 for the six horse breeds (Table 4). A neighbour-joining tree was constructed, and the reliability of the obtained tree was examined by 1000 bootstrap replicates (Fig. 1). The most robust features of the topology were the Jeju racing horse-Mongolian horse cluster (84% support) and the cluster (96% support) formed by American Quarter and Thoroughbred which are all from the Japanese horse.

Table 2. Total number and range of observed alleles, average heterozygosity Hs and Ht, coefficient of differentiation Gst. in horse breeds

| Locus | Chromosome location | Size range (bp) | No. of alleles | Ht | Hs | Gst |
|---|---|---|---|---|---|---|
| ATH4 | 24 | 100-160 | 10 | 0.816 | 0.745 | 0.088 |
| ATH5 | 6 | 130-148 | 8 | 0.814 | 0.714 | 0.123 |
| ASB2 | 15 | 221-259 | 14 | 0.838 | 0.664 | 0.208 |
| HMS3 | 9 | 133-171 | 10 | 0.798 | 0.716 | 0.103 |
| HMS6 | 4 | 157-169 | 7 | 0.726 | 0.621 | 0.145 |
| HMS7 | 1 | 171-185 | 8 | 0.733 | 0.659 | 0.101 |
| HTG4 | 9 | 128-142 | 8 | 0.669 | 0.569 | 0.109 |
| HTG10 | 21 | 90-112 | 12 | 0.771 | 0.652 | 0.105 |
| VHL20 | 30 | 84-104 | 10 | 0.828 | 0.675 | 0.184 |
| TKY321 | 3 | 208-232 | 13 | 0.869 | 0.769 | 0.114 |
| CA425 | 2 | 232-250 | 11 | 0.762 | 0.715 | 0.061 |
| ASB17 | 2 | 91-121 | 16 | 0.871 | 0.769 | 0.117 |
| LEX33 | 1 | 198-216 | 11 | 0.853 | 0.667 | 0.219 |
| All loci | | | | 0.796 | 0.691 | 0.129 |

Ht: Expected total heterozygosity, Hs: Expected heterozygosity within population, Gst: Coefficient of gene differentiation

Table 3. Number of alleles per locus and heterozygosity in average over 13 microsatellites in 6 horse populations

| Population | Mean sample size per locus | Mean No. of alleles per locus | Mean heterozygosity | |
|---|---|---|---|---|
| | | | Obseved | Expected |
| JNH | 41 | 6.23 | 0.675 | 0.700 |
| Jap | 5 | 3.46 | 0.708 | 0.639 |
| Qua | 11 | 5.46 | 0.755 | 0.705 |
| JRH | 56 | 8.38 | 0.804 | 0.794 |
| Mong | 39 | 8.62 | 0.649 | 0.797 |
| Tho | 44 | 5.85 | 0.691 | 0.728 |
| Means | 196 | 6.31 | 0.713 | 0.605 |

Table 4. Matrix of $D_A$ genetic distance among six horse breeds

| | JNH | Jap | Qur | JRH | Mong |
|---|---|---|---|---|---|
| Jap | 0.4146 | | | | |
| Qua | 0.3725 | 0.3944 | | | |
| JRH | 0.2013 | 0.3431 | 0.2242 | | |
| Mong | 0.1414 | 0.3520 | 0.2776 | 0.1373 | |
| Tho | 0.3930 | 0.4103 | 0.1701 | 0.1841 | 0.2668 |

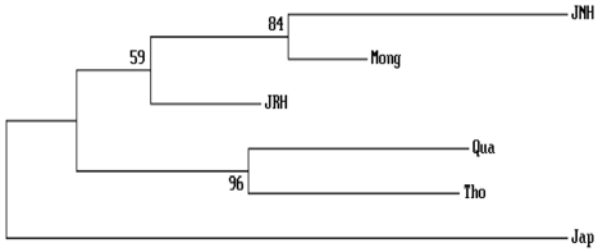


Fig 1. Neighbor-joining tree constructed using $D_A$ genetic distance matrix, showing the genetic relationships among the horse breeds. Numbers indicates the bootstrap values in percentage (1,000).

## Discussion

Recently, many microsatellites were isolated from the horse genome [22]. Microsatellite may be a useful approach for resolving the relationships of Horse populations. In this study we analyzed the allele frequencies and genotype distributions among American Quarter and Thoroughbred to consider the ancestral populations for Japanese horse populations. The average heterozygosity with each population of Mongolian horse range from 0.75 to 0.77. Mongolian horse had the highest values in all populations. In contrast, the average heterozygosity within each population of Japanese horse ranged between 0.34 and 0.66. In addition, Mongolian horse possessed all the alleles found in Japanese horse. These assumptions are supported by the his-

torical fact that native horses on the Asian continent were frequently transported to Japan through the Korean peninsula. Thus, the phylogenetic reconstructions using $D_A$ distance gave similar clustering results. In conclusion, we analyzed the genetic variation and phylogenetic relationship of six horse breeds using microsatellites. In the future, an investigation of the pedigree of population and the genetic relationships in those populations will be necessary. We confirmed the potential use of equine microsatellite loci as a tool for genetic study and parentage testing from these results.

## Acknowledgement

This work was supported by PNU Grants (2008) to PNU-Special Animal Biotechnology Center.

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## 초록 : Microsatellite makers를 이용한 마품종 간의 평가 및 유전적 다양성

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본 연구는 마품종 간의 유전적 배경과 다양성을 분석하기 위해 제주마, 경주마, 더러브렛, 몽고마, 쿼터호스, 일본마를 기초 축으로 생물체 집단 간의 유전적 배경 및 관련성을 연구하는데 도움 되는 microsatellite marker를 사용하여 13종의 좌위에 대한 개체별 유전자형을 분석하여 대립 유전자별 빈도에 있어 품종별 차이를 보이는 microsatellite loci들을 나타냈다. 전체 개체들의 유전적 다양성을 나타내는 유전자 좌위별 품종별 기대 이형질성은 0.669-0.869를 나타냈고, 전체 집단 간의 유전자 분화정도는 0.061-0.219를 나타냈다. 이러한 대립 유전자 빈도를 근거로 하여 DISPAN 프로그램을 이용하여 집단 간 표준 유전적 거리를 도식하여 유전자 좌위별 유전적 거리는 0.137-0.414를 나타내어 마품종간의 유전적 다양성과 관련성을 고찰할 수 있게 되었다. 따라서 초위성체 유전자 표지를 이용하여 집단 내의의 혈통분석 및 분자 진화학을 연구하는데 도움이 될 것으로 사료된다.