



Letter

Genetic Polymorphism of Jeju Horses by Microsatellite DNA Markers in Korea

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We determined the genetic diversity and distance between Jeju and Thoroughbred horses by genotyping for 20 microsatellite loci consisting of (TG)_n repetitive sequence. The expected heterozygosity ranged from 0.1 to 0.789 in the Jeju horses and from 0.505 to 0.824 in the Thoroughbred horses. Polymorphic information content (PIC) values ranged from 0.09 to 0.709 in the Jeju horses and 0.365 to 0.730 in Thoroughbred horses. There were no significant differences in heterozygosity and PIC values between Jeju and Thoroughbred horses. However, LEX035 was estimated relatively high heterozygosity (0.789) and PIC value 0.709 in Jeju horses and LEX050 was respectively 0.824, and 0.730 in the Thoroughbred horses. We may conclude that the genetic differentiation was low between Jeju and Thoroughbred horses. LEX 050, LEX055, LEX059 and LEX 063 could be used as genetic markers for differentiating Jeju from Thoroughbred horses.

Key words: Jeju horses, genetic polymorphism, microsatellite DNA

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Microsatellites are short tandem repeat sequences consisting of repeat unit of 2-6 base pair (bp) in length. Most repeat sequences of 2-6 bp have been identified in vertebrates and microsatellite DNA seems to be relatively abundant in genomes of many taxa. Microsatellite loci have proved to be powerful tools for gene mapping, paternity test and genetic population structure because of their high heterozygosity. Microsatellites have been suggested to play a role in gene regulation and recombination but there was very little knowledge concerning their function (FitzSimmons *et al.*, 1995; Forbes *et al.*, 1995).

Recently, microsatellite polymorphisms have been used to determine allele frequencies in interspecific population and intrapopulation (Buchanan *et al.*, 1994).

Korean native horses, Jeju horses called, as Jorangs are 120-130 cm in height and indigenous to Jeju islands. Based on mitochondrial D-loop sequences analysis, Jeju horses were mixed origin in their maternal lineage, and may have inhabited the island before the introduction of Mongolian horses in 1276 (Kim *et al.*, 1999). However, genetic markers, phylogenetic relationships and species conservation have not been extensively studied in Jeju horses using molecular technique. Microsatellite mutation rates are approximately 10^{-3} - 10^{-4} (Dietrich *et al.*, 1992; Weissenbach *et al.*, 1992). According to divergence time of ancestral *Equus* species, microsatellite loci were suitable marker for studying genetic polymorphism of Jeju horses population in Korea.

In this paper, we described allele size, allele number, heterozygosity, and polymorphic information content (PIC) values, which are useful for a measure of polymorphism for a marker locus used in linkage analysis, to determine the

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genetic diversity of Jeju horses in Korea compared with Thoroughbred horses using microsatellite DNA markers consisting of (TG)_n repetitive sequences (Shete *et al.*, 2000).

Materials and Methods

Genomic DNA was extracted from peripheral blood obtained from each 20 sample in both Jeju and Thoroughbred horses. Jeju horses samples were provided from Jeju National University and Thoroughbred samples were from KRA (Korea Racing Authority). The microsatellite loci were amplified by 30-35 cycles, each cycle consisting of denaturation for 30 sec at 95°C, annealing for 30 sec at 55°C, and extension for 30 sec at 72°C. We used 20 primers consisting of (TG)_n repetitive sequence based on the data (LEX 034-063) (Coogee and Bailey, 1997; Coogee *et al.*, 1997). The amplified PCR fragments were identified via 3% agarose gel electrophoresis and run on 6% denatured polyacrylamide gel. The gel was identified using general silver staining method. To identify or confirm exact base pair of microsatellite loci, 1-2 samples per microsatellite loci were selected for sequencing. Statistical analysis was utilized for CERVUS program (Version 1.0, The University of Edinburgh, 1988; <http://helios.bto.ed.ac.uk/evolgen/cervus/>). Allele size, number of alleles, PIC, and heterozygosity were calculated for each microsatellite loci.

Results

The number of alleles ranged from 2 to 5 per locus. Not all samples were amplified for all loci. Total 64 alleles in Jeju horses and 73 alleles in Thoroughbred horses were detected across the 20 loci analyzed. Allele size, number of allele, PIC values and heterozygosity per loci were summarized in Table 1. The variation of allele size among Thoroughbred horses was higher than Jeju horses. The differences of allele size at microsatellite loci are due to the variation in the numbers of repeats (Buchanan *et al.*, 1994; Forbes *et al.*, 1995).

All microsatellite loci were polymorphic in this study. Expected heterozygosity was ranged from 0.100 to 0.789 in the Jeju horses, and 0.505 to 0.824 in the Thoroughbred racehorses. The mean value of the PIC of dinucleotide microsatellites was 0.482 in Jeju horses and 0.562 in Thoroughbred racehorses. The highest heterozygosity and PIC were estimated at LEX035 (0.789, 0.709) in Jeju horses and LEX050 (0.824, 0.730) in Thoroughbred racehorses. The lowest heterozygosity and PIC was observed at LEX050 (0.100, 0.090) in Jeju horses and LEX043 (0.505, 0.365) in Thoroughbred racehorses. According to the values of PIC and heterozygosity, there were no significant differences among those microsatellite loci between Jeju and Thoroughbred horses in LEX050, LEX05, LEX059 and LEX063. In

Table 1. Characteristics of microsatellite loci of Jeju and Thoroughbred horses

| Primer name | Repeat sequence | Jeju Horses | | | | Thoroughbred horses | | | |
|-------------|--------------------|------------------|----------------|-------|----------------|---------------------|----------------|-------|----------------|
| | | Product size(bp) | No. of alleles | PIC | Heterozygosity | Product size(bp) | No. of alleles | PIC | Heterozygosity |
| LEX034 | (TG) ₁₇ | 244-252 | 4 | 0.627 | 0.721 | 244-252 | 3 | 0.548 | 0.653 |
| LEX035 | (TG) ₁₆ | 247-261 | 5 | 0.709 | 0.789 | 247-265 | 5 | 0.642 | 0.732 |
| LEX037 | (TG) ₁₄ | 190-194 | 3 | 0.586 | 0.695 | 188-194 | 4 | 0.627 | 0.721 |
| LEX038 | (TG) ₁₃ | 140-146 | 4 | 0.632 | 0.721 | 142-148 | 4 | 0.613 | 0.712 |
| LEX040 | (TG) ₁₃ | 148-154 | 4 | 0.653 | 0.742 | 148-154 | 4 | 0.584 | 0.684 |
| LEX041 | (TG) ₁₂ | 148-154 | 4 | 0.623 | 0.721 | 146-152 | 4 | 0.633 | 0.732 |
| LEX043 | (TG) ₁₈ | 238-240 | 2 | 0.351 | 0.479 | 238-240 | 2 | 0.365 | 0.505 |
| LEX046 | (TG) ₁₈ | 114-120 | 4 | 0.602 | 0.706 | 114-120 | 4 | 0.665 | 0.753 |
| LEX047 | (TG) ₁₄ | 247-251 | 3 | 0.436 | 0.563 | 249-253 | | 0.424 | 0.542 |
| LEX048 | (TG) ₁₂ | 169-171 | 2 | 0.365 | 0.505 | 169-173 | 3 | 0.424 | 0.542 |
| LEX050 | (TG) ₁₅ | 118-120 | 2 | 0.090 | 0.100 | 118-128 | 5 | 0.730 | 0.824 |
| LEX052 | (TG) ₁₃ | 197-203 | 3 | 0.448 | 0.582 | 195-199 | 3 | 0.442 | 0.532 |
| LEX053 | (TG) ₁₅ | 127-133 | 4 | 0.685 | 0.774 | 127-133 | 4 | 0.640 | 0.726 |
| LEX055 | (TG) ₁₈ | 199-209 | 3 | 0.303 | 0.353 | 199-209 | 3 | 0.513 | 0.616 |
| LEX057 | (TG) ₁₅ | 162-166 | 3 | 0.513 | 0.616 | 162-166 | 3 | 0.442 | 0.532 |
| LEX058 | (TG) ₂₁ | 228-232 | 3 | 0.555 | 0.658 | 224-230 | 3 | 0.534 | 0.647 |
| LEX059 | (TG) ₁₃ | 224-228 | 2 | 0.164 | 0.189 | 224-232 | 3 | 0.466 | 0.568 |
| LEX061 | (TG) ₁₇ | 130-136 | 3 | 0.492 | 0.611 | 126-136 | 5 | 0.627 | 0.716 |
| LEX062 | (TG) ₁₅ | 190-196 | 4 | 0.583 | 0.680 | 190-196 | 4 | 0.675 | 0.763 |
| LEX063 | (TG) ₁₆ | 238-244 | 2 | 0.222 | 0.268 | 222-244 | 4 | 0.645 | 0.737 |

comparison with allele distribution, the same number and the same allele loci were found only in 7 (LEX040, 043, 046, 052, 055, 057, 062) of 20 loci in both horses.

Discussion

The utility of microsatellites testing has been examined in limited trials of human, cattle and dog parentage. Microsatellite markers of horses have been studied in pedigree assignment, the genetic structure of specific level and the linkage analysis of the gene for family disease (Alford *et al.*, 1994; Glowatzki-Mullis *et al.*, 1995; Bailey *et al.*, 1997). Bowling *et al.* (Bowling *et al.*, 1997) reported that microsatellite testing is a more suitable marker than blood typing for the expanding field of genome mapping of horses. Differences in allele size and polymorphism among taxa may be explained by characterizing microsatellite loci (FitzSimmons *et al.*, 1995; Pepin *et al.*, 1995). The range of sizes found for simple repeat sequences depends on the method used to detect them and sample size.

In this study, it was shown that Jeju horses have lower polymorphic loci than thoroughbred horses (Table 1) even though there were no significant differences between Jeju horses (mean of heterozygosity=0.574) and Thoroughbred racehorses (mean of heterozygosity=0.662) based on heterozygosity. We assume that Jeju horses have been maintaining their conserved genetic uniformity because they have inhabited a limited area, Jeju island. Although the genetic polymorphism between Jeju and Thoroughbred horses could not be exactly clarified due to the small sample size and microsatellite loci, our results will provide stronger evidence to support the genetic difference between Jeju and Thoroughbred horses. We propose that the microsatellite marker will be a powerful tool for pedigree assignment of Jeju horses and will contribute to conservation of Korean native species. In addition, LEX050, LEX055, LEX059 and LEX063 will be candidate markers for differentiating Jeju horses from Thoroughbred horses. Further study is required for much more precise understanding of the genetic polymorphism and the genetic diversity of Jeju horses comparing with other horses.

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