

## Microsatellite Polymorphism and Genetic Relationship in Dog Breeds in Korea

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**ABSTRACT** : Microsatellite polymorphism and their genetic relationships were estimated using genotype information of 183 dogs from 11 microsatellite loci. The breeds include the indigenous Korean breeds Jindo dog (30), Poongsan dog (20) and Miryang dog (44) together with Chihauhau dog (31) and German Shepherd dog (58). Jindo dogs showed the highest expected heterozygosity ( $0.796 \pm 0.030$ ) and polymorphic information contents (0.755) in all populations. The phylogenetic analysis showed the existence of two distinct clusters supported by high bootstrap values: the Korean native dogs and other dogs. They clearly show that Poongsan dog and Miryang dog are closely related to each other when compared with Jindo dog. Microsatellite polymorphism data was shown to be useful for estimating the genetic relationship between Korean native dogs and other dog breeds, and also can be applied for parentage testing in those dog breeds. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 8 : 1071-1074)

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

### INTRODUCTION

The dog is one of the companion animals which is almost certainly the first species to have been domesticated by man since about 40,000 years ago. The modern domestic dog (*Canis familiaris*) originated from an ancestral wild wolf (*Canis lupus*). Recently, dogs are raised as a pet or companion animal, and for the various purposes of rescuing, hunting, guarding and military activities about 2 million herds in Korea. Therefore, accurate determination of relatedness and efficient control of pedigree registration is of great importance in dog breeding (Cho and Cho, 2003). In Korea, Jindo dog, Poongsan dog, and Sapsaree are one of the Korean native animals which were designated by the government as special natural monument, No. 53, No. 128, and No. 368, respectively (Park, 2002). Among them, Jindo dogs are favored by Koreans often because they have a strong obedience and instinct of return. The record of its origin from Mongolian dogs or, genetic isolation in offshore Jindo islet, which lies within a few kilometers away from the southwestern part of Korean peninsula, is often referred. Jindo islet has been designated as the sanctuary for Jindo dogs, and the area is under the strict control of local government (Youn et al., 2001). Poongsan dogs are a representative indigenous dog breed in North Korea and shows calm, but enduring personality as a hunting dog. However, original bloodline of many Poongsan dogs currently raised in South Korea is being questioned.

In recent years, several studies have undertaken to investigate the genetic characteristics of dog using either DNA markers (Chae et al., 1998; Youn et al., 2001; Cho and Cho, 2003) or biochemical markers (Ha et al., 1998). There

is however, no further information is available genetic diversity and phylogenetic relationship of dog breeds in Korea.

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

### MATERIALS AND METHODS

#### Sample collection and DNA extraction

Genomic DNAs were prepared from whole blood samples, which were collected from 183 individuals of five dog breeds (sample sizes are shown in parenthesis): Chihauhau dog (31), Poongsan dog (20), Miryang dog (44), Jindo dog (30) and German Shepherd dog (58). Miryang dog raised in southern counties is similar to Jindo dog. Genomic DNAs from samples were extracted using MagExtractor System MFX-2000 (Toyobo, Japan) according to the manufacturer's protocols (Tozaki et al., 2001).

#### PCR and microsatellite analysis

Eleven microsatellite markers, FHC2010, FHC2054, FHC2079, PEZ1, PEZ6, PEZ8, PEZ10, PEZ11, PEZ12, PEZ15 and PEZ17 were used for the analysis of the dog breeds, and the primers are given in Table 1. PCR was accomplished in a total volume of 10  $\mu$ l of the following mixture: 40 ng of genomic DNA, primer mix, 25 mM of  $MgCl_2$ , 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 mins at 95°C, followed by 20 cycles at 95°C for 30 sec, 58°C for 30 sec, 72°C for 1 min, and 15 cycles at 95°C for 30 sec, 56°C for 30 sec, 72°C for

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**Table 1.** Characteristics of 11 microsatellite markers used in this study

Loci	Fluorescent dye	Primer sequences (5'→3')	Allele size range (bp)
FHC2010	(FAM)-AAATGGAACAGTTGAGCATGC	CCCCTTACAGCTTCATTTTCC	208-260
GHC2054	(FAM)-GCCTTATTCATTGCAGTTAGGG	ATGCTGAGTTTTGAACTTTCCC	141-181
FHC2079	(NED)-CAGCCGAGCACATGGTTT	ATTGATTCTGATATGCCCAGC	266-294
PEZ1	(FAM)-GGCTGTCACCTTTCCCTTTT	CACCACAATCTCTCTCATAAATAC	92-136
PEZ6	(NED)-ATGAGCACTGGGTGTTATAC	ACACAATTGCATTGTCAAAC	164-212
PEZ8	(NED)-TATCGACTTTATCACTGTGG	ATGGAGCCTCATGTCTCATC	221-257
PEZ10	(NED)-CTTCATTGAAGTATCTATCC	CCTGCCTTTGTAAATGTAAG	236-336
PEZ11	(JOE)-ATTCTCTGCCTCTCCCTTTG	TGTGGATAATCTCTTCTGTC	122-158
PEZ12	(JOE)-GTAGATTAGATCTCAGGCAG	TAGTCTCGGTAGGGTGTGG	245-313
PEZ15	(JOE)-CTGGGGCTTAACCCAAGTTC	CAGTACAGAGTCTGCTTATC	188-296
PEZ17	(NED)-CTAAGGGACTGAACTTCTCC	GTGGAACCTGCTTAAGATTC	194-226

**Table 2.** Characterization of 11 microsatellite loci analyzed with five dog breeds

Loci	Dog map location	No. of alleles	HT	HS	GST*
FHC2010	S5	11	0.702	0.515	0.266
FHC2054	CFA12	11	0.852	0.749	0.121
FHC2079	S5	7	0.480	0.435	0.093
PEZ 1	CFA7	9	0.787	0.627	0.204
PEZ 6	CFA16	12	0.883	0.784	0.112
PEZ 8	S16	9	0.716	0.643	0.101
PEZ10	S9	24	0.906	0.797	0.120
PEZ11	CFA8, 9	13	0.844	0.661	0.216
PEZ12	CFA3	17	0.819	0.642	0.216
PEZ15	CFA16	18	0.760	0.572	0.247
PEZ17	S1	9	0.840	0.722	0.141
All loci		140	0.781	0.650	0.168

\* All breeds examined, H<sub>T</sub>: expected total heterozygosity.

H<sub>S</sub>: expected within total heterozygosity, G<sub>ST</sub>: Gene differentiation.

1 min. An extension step of 72°C for 30 min was added after the final cycle (Denise et al., 2004). 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 protocols. Fragment size analysis was performed with genotype software Ver.3.7 (Applied Biosystems, USA). The internal size standard Genescan-ROX 400 (Applied Biosystems, USA) was used for sizing alleles. In addition, sample No. 1 from the International Society for Animal Genetics (ISAG) 2004 comparison test was used as reference to standardize allele sizes.

### Statistical analysis

Allelic frequencies, the number of alleles per locus were estimated by direct counting from observed genotype, and polymorphic information contents (PIC) was computed using the CERVUS software (Marshall et al., 1998). Genetic variability estimates of average observed heterozygosity, expected total heterozygosity (H<sub>T</sub>), expected within population heterozygosity (H<sub>S</sub>), coefficient of gene

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

Population	Sample size	Mean no. of allele	H <sub>E</sub>	H <sub>O</sub>	PIC
Chihuahua	31	6.27	0.719±0.033	0.765	0.665
Jindo	30	8.55	0.796±0.030	0.748	0.755
Miryang	44	6.18	0.663±0.039	0.618	0.600
Poongsan	20	4.73	0.555±0.062	0.595	0.502
Shepherd	58	8.09	0.567±0.071	0.470	0.538
Pooled sample	183	12.73	0.660±0.047	0.639	0.612

differentiation (G<sub>ST</sub>), total and mean number of alleles per population were calculated using DISPAN (Ota, 1993). Genetic differences among populations were estimated by calculating the D<sub>A</sub> genetic distance (Nei et al., 1983). Phylogenetic trees were constructed from DA genetic distance matrix according to the neighbor-joining (NJ) method (Saitou and Nei, 1987) implemented by DISPAN (Ota, 1993). Also, each breed genetic distances based on allele sharing was analyzed using Microsat (Minch et al., 1995).

## RESULTS

### Microsatellite polymorphism

A total of 140 alleles were observed among the 183 animals assayed and demonstrated that they were highly polymorphic in all dog breeds. Measures of genetic variability are shown in Table 2 and 3. The number of alleles per locus varied from 7 (FHC2079) to 24 (PEZ10) with an average value of 12.73 in dog breeds. The expected total heterozygosity (H<sub>T</sub>) and coefficient of gene differentiation (G<sub>ST</sub>) ranged 0.480-0.906 (the average value was 0.781) and 0.093-0.266 (the average value was 0.168) in dog breeds, respectively.

The expected heterozygosity within breed (H<sub>S</sub>) and mean no. of observed alleles ranged from 0.555±0.062 (Poongsan dog) to 0.796±0.030 (Jindo dog), and from 4.73 (Poongsan dog) to 8.55 (Jindo dog), respectively. The polymorphic information contents (PIC) ranged from 0.502

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

	Chihauhau	Jindo	Miryang	Poongsan
Jindo	0.1622	-	-	-
Miryang	0.3923	0.2591	-	-
Poongsan	0.3280	0.2488	0.3497	-
Shepherd	0.3417	0.3244	0.5448	0.4932

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

	Chihauhau	Jindo	Miryang	Poongsan	Shepherd
Chihauhau	-	0.0488	0.1412	0.2015	0.1701
Jindo	0.1535	-	0.0932	0.0882	0.1530
Miryang	0.6365	0.3281	-	0.1357	0.3140
Poongsan	0.4929	0.3073	0.4723	-	0.3400
Shepherd	0.5820	0.5385	1.2443	1.0575	-

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

(Poongsan dog) to 0.755 (Jindo dog) with an average value of 0.612 in dog breeds.

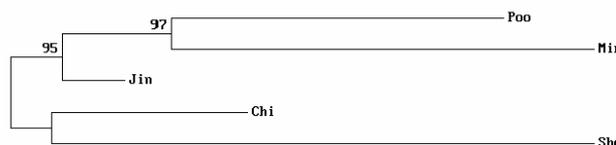
**Genetic distances and population relationship**

Estimates of the  $D_a$  genetic distances and standard genetic distances among the 5 populations based on the allele frequency data on 11 microsatellite loci are presented in Table 4 and 5. The largest  $D_a$  (0.5448) and  $D_s$  (1.2443) distances were observed between Miryang dog and German Shepherd, and the smallest  $D_a$  (0.1622) and  $D_s$  (0.1535) distances between Chihauhau dog and Jindo dog. Dendrogram, based on the calculated genetic distances and constructed by the use of the N-J method, is presented in Figure 1. They clearly showed two distinct clusters with high bootstrap support: Korean native dogs (Poongsan, Miryang, and Jindo) and other dog breeds (Chihauhau and German Shepherd). A relatively high bootstrap value (97%) was observed for Poongsan and Miryang cluster.

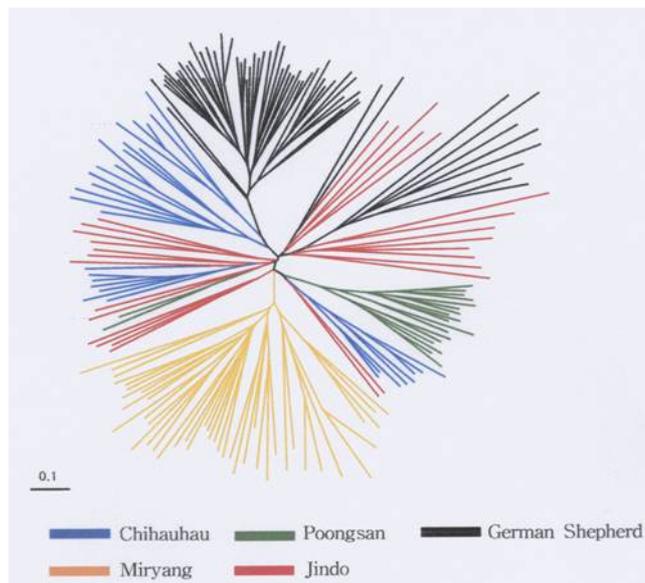
Radial presentation of the N-J dendrogram constructed from simple allele sharing statistics among 183 individuals representing five dog breeds are shown in Figure 2. Poongsan and Miryang were distinctly grouped as well as Figure 1. Jindo dogs divided into two groups containing German Shepherd and Chihauhau.

**DISCUSSION**

The dog marker genome map has been extensively developed during the recent years. At present almost 2,000 microsatellite markers has been described and many of them have been assigned to linkage groups or/and specific chromosomes (Klukowska et al., 2003). Many kinds of microsatellites are informative due to their high polymorphisms and they are useful in paternity testing of animals (Bowling et al., 1997; Han et al., 2000; Cho et al., 2002; Kim and Choi, 2002; Cho and Cho, 2004), and the method was used extensively to construct structure of the



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



**Figure 2.** Radial presentation of the N-J dendrogram constructed from simple allele sharing statistics among 183 individuals representing five dog breeds.

closely related populations and breed allocating of animals (Kim and Choi, 2002; Li et al., 2004; Sun et al., 2004). In cattle, pig, horse and dog populations, pedigree control has been performed on routine basis in most countries. These controls rely on a DNA typing that has been standardized through regular comparison tests under the auspices of the International Society for Animal Genetics (ISAG).

In this study, the studied loci were more polymorphic in Jindo dogs than in other dog breeds, demonstrating that Jindo dogs have retained the largest amount of genetic variation of all the populations studied. In addition, Jindo dogs possessed all the alleles found in Miryang dogs and Poongsan dogs, with the exception of PEZ6 and PEZ10, PEZ12 and PEZ15 marker, respectively. These results supports the hypothesis that Miryang dogs and Poongsan dogs are descended from the ancestral populations of Jindo dogs (Park, 2002).

Evaluation of microsatellite polymorphism can be used for differentiation of closely related species. Usually, data on frequency of microsatellite alleles is used to estimate a genetic distance between populations (Klukowska et al., 2003). In this study, the largest  $D_a$  (0.5448) and  $D_s$  (1.2443) distances were observed between Miryang dog and German Shepherd, and the smallest  $D_a$  (0.1622) and  $D_s$  (0.1535)

distances between Chihauhau dog and Jindo dog. Takezaki and Nei (1996) proved that for reconstruction of phylogenetic trees it is important to study a large number of loci. The relatively low number of analysed microsatellite did not influence the dendrogram topology, as only five branches were distinguished. In this study, the phylogenetic tree showed two distinct clusters, Korean native dogs (Poongsan, Miryang, and Jindo) and other dog breeds (Chihauhau and German Shepherd). They clearly show that Poongsan dog and Miryang dog are closely related to each other when compared with Jindo dog. 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 dog breeds. Further investigation are 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 in Korean native dogs, more extensive investigation might be required. Thus, a comparative research on the genetic diversity of Korean native dogs, such as Sapsaree, Jeju dog, and some other foreign dogs will be necessary. As this data show, microsatellites are a useful tool for studying the genetic relationship among closely related dog breeds. Since the microsatellites in this study are highly polymorphic, they can also be applied for parentage testing in those dog breeds.

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