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# Genetic structure of pig breeds from Korea and China using microsatellite loci analysis<sup>1</sup>

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**ABSTRACT:** To understand molecular genetic characteristics of Korean pigs, the genetic relationships of nine pig breeds including two Korean pigs (Korean native pig and Korean wild pig), three Chinese pigs (Min pig, Xiang pig, and Wuzhishan pig), and four European breeds (Berkshire, Duroc, Landrace, and Yorkshire) were characterized from a 16-microsatellite loci analysis. The mean heterozygosity within breeds ranged from 0.494 to 0.703. Across multiple loci, significant deviation from Hardy-Weinberg equilibrium was observed in most pig breeds, except for two Chinese pigs (Min pig and Wuzhishan pig). This deviation was in the direction of heterozygote deficit. Across population loci, 36 of 144 significantly deviated ( $P < 0.05$ ) from Hardy-Weinberg equilibrium. The mean  $F_{ST}$ , a measure of genetic divergence among subpopulations, of all loci indicated that 26.1% of total variation could be attributed

to the breed difference. Relationship trees based on the Nei's  $D_A$  genetic distance and scatter diagram from principal component analysis consistently displayed pronounced genetic differentiation among the Korean wild pig, Xiang pig, and Wuzhishan pig. Individual assignment test using a Bayesian method showed 100% success in assigning Korean and Chinese individual pigs into their correct breeds of origin and 100% exclusion success from all alternative reference populations at  $P < 0.001$ . These findings indicate that the Korean native pig has been experiencing progressive interbreeding with Western pig breeds after originating from a North China pig breed with a black coat color. Considering the close genetic relationship of Korean pigs to the Western breeds such as Berkshire and Landrace, our findings can be used as valuable genetic information for the preservation and further genetic improvement of the Korean native pig.

Key Words: Genetic Relationship, Genetic Structure, Korean Native Pig, Microsatellite

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## Introduction

The original Korean native pigs had long black coarse hair, long straight noses, and small BW. It was presumed that Korean native pigs came to Korea via north China approximately 2,000 yr ago (Kim and Choi, 2002). Since 1910, the Korean native pigs have been crossed with European pig breeds, such as Berkshire,

to improve their productivity. In addition, many commercial pig breeds have been introduced into Korea. As a consequence, the number of Korean native pigs decreased dramatically until the 1980s and faced the brink of extinction. The National Livestock Research Institute in Korea began restoring the genetic characteristics of Korean native pigs in 1988. To date, the restoration of Korean native pigs is being performed based on morphological characteristics; however, recently, DNA-based research to study genetic differentiation of pig breeds is rapidly replacing or complementing the morphology-based approach.

The genetic relationship between the Asian and the European pig breeds has been evaluated using both mitochondrial DNA and nuclear DNA analyses (Kim et al., 2002a,b; Kim and Choi, 2002). Phylogenetic analysis of mitochondrial D-loop DNA (Kim et al., 2002a) revealed that Asian native pigs are closely related but differ from European pigs, except for the Berkshire and Large White breeds. Amplified fragment-length poly-

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morphism (Kim et al., 2002b) and microsatellite markers (Kim and Choi, 2002) together showed that the Korean native pig has a low level of genetic diversity and is distinct from the Western pig breeds. Nonetheless, population genetics information, especially in a population management context, was limited because of small sample size as well as a limited number of microsatellite markers.

In this study, we used 16 microsatellite markers to evaluate the genetic structure of Korean and Chinese breeds, to explain the genetic relationships between Asian pig and European pig breeds, and to determine the assignment accuracy of individuals into their correct breeds of origin.

## Materials and Methods

### *Sample Collection and DNA Extraction*

A total of 242 animals representing the nine pig breeds examined was distributed as follows: Korean native pig ( $n = 32$ ); Korean wild pig ( $n = 22$ ); Min pig ( $n = 12$ ); Wuzhishan pig ( $n = 22$ ); Xiang pig ( $n = 28$ ); Berkshire ( $n = 30$ ); Duroc ( $n = 32$ ); Landrace ( $n = 32$ ); and Yorkshire ( $n = 32$ ). Samples of DNA from Chinese pig breeds were obtained from China Agricultural University. Blood samples for Korean native pigs unrelated at the grandparental level were collected from the National Livestock Research Institute and three different private pig farms in Korea. Korean wild pig samples were collected from four private wild pig farms located at different locations in Korea. The other blood samples, including Berkshire, Duroc, Landrace, and Yorkshire pigs that were unrelated based on pedigree information, were collected from the National Livestock Research Institute and five different private pig farms in Korea. Number of individuals sampled in some pig breeds, especially in the Min pig, was quite low; thus, caution should be used when interpreting analyses. Genomic DNA was extracted from blood samples with Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

### *Microsatellite Genotyping*

Sixteen microsatellites were selected based on their genomic location, allele number, and ease of scoring (Table 1). Microsatellite markers that labeled with fluorescence were amplified by PCR using 10 ng of pig genomic DNA as a template. The polymerase chain reaction was performed in a 10- $\mu$ L final volume with 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M of each nucleotide (dNTP), 3 pmol of each primer, and 0.5 units of Taq DNA polymerase (TaKaRa Shuzo Co., Shiga, Japan). Thermal cycling conditions in GeneAmp PCR System 9600 (Applied Biosystems, Foster City, CA) included an initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature (Table 1), 1 min at 72°C, and a final extension step of 72°C for 10 min.

For genotyping of samples, PCR products of up to nine markers were combined and electrophoresed using an ABI 310 DNA sequencer (Applied Biosystems). Fragment length of the PCR products was determined with Genescan software (Version 2.1, Applied Biosystems), and marker genotypes were assigned to the animals using Genotyper software (Version 2.5, Applied Biosystems).

### *Data Analyses*

Allele frequencies (available from the authors upon request), the mean number of alleles per locus ( $M_N$ ), observed heterozygosity ( $H_O$ ), and heterozygosity expected from Hardy-Weinberg ( $H_W$ ) assumptions ( $H_E$ ) for each locus were computed using the GENETIX software package (Belkhir et al., 1996). The two measures of heterozygosity are highly correlated, but in this study, we focused on  $H_E$  because it is considered a better estimator of the genetic variability present in a population (Nei and Kumar, 2000). To compare the number of alleles between different sample sizes, allelic richness ( $A_R$ ), which measures the number of alleles independent of sample size, was calculated using the program FSTAT v. 2.9.3 software package (Goudet, 2001).

The program FSTAT was used to calculate two different measures of the genetic differentiation over subpopulations,  $F_{ST}$  (Weir and Cockerham, 1984) and  $R_{ST}$  (Rousset, 1996), where measures of  $F_{ST}$  and  $R_{ST}$  are based on the infinite allele model (Kimura and Crow, 1964) and the stepwise mutation model (Kimura and Ohta, 1978), respectively. Pairwise  $F_{ST}$  and inbreeding coefficients ( $F_{IS}$  and  $F_{IT}$ ) were calculated using the program FSTAT (Goudet, 2001). The sequential Bonferroni correction was applied to derive significance levels for the analysis involving multiple comparisons (Rice, 1989).

The probability test approach described by Guo and Tomson (1992) and implemented in the GENEPOP software (Raymond and Rousset, 1995) was employed to test for HW equilibrium. The HW test for each locus in each population and global tests for all populations were performed to investigate whether there was a heterozygote excess or deficit.

The genetic divergence between the populations based on allele frequencies was calculated according to  $D_A$  genetic distance (Nei et al., 1983) using the DISPAN computer program (Ota, 1993). Phylogenetic trees were constructed by using the neighbor-joining (NJ) clustering (Saitou and Nei, 1987) and the unweighted pair group method with the arithmetic mean (UPGMA; Sneath and Sokal, 1973) from  $D_A$  distance. Bootstrap re-sampling ( $n = 1,000$ ) was performed to test the robustness of the dendrogram topologies.

To represent geometric relationships among the pig breeds, a principal component analysis (PCA) was applied using gene frequencies of all variable loci. The frequencies of all alleles at a single locus were considered to be independent variables, even though they

**Table 1.** List of microsatellite markers used in this study

Locus	Chromosomal location	Size range, bp	Annealing temperature, °C	Reference
S0036	2	114 to 132	58	Brown et al. (1994)
SW1695	2	160 to 210	58	Alexander et al. (1996)
SW902	3	188 to 210	55	Rohrer et al. (1994)
S0301	4	246 to 258	55	Hoyheim et al. (1994)
SW2409	4	77 to 93	55	Alexander et al. (1996)
SW445	4	184 to 208	58	Robic et al. (1996)
SW2	5	83 to 119	55	Rohrer et al. (1994)
SW71	6	81 to 121	60	Rohrer et al. (1994)
SW205	8	144 to 160	58	Rohrer et al. (1994)
SW61	8	227 to 259	55	Rohrer et al. (1994)
S0070	10	260 to 294	55	Ellegren et al. (1994)
SW874	12	189 to 217	55	Rohrer et al. (1994)
SW2612	14	150 to 172	60	Alexander et al. (1996)
SW510	14	148 to 158	60	Rohrer et al. (1994)
SW1119	15	144 to 184	60	Rohrer et al. (1994)
SW936	15	85 to 133	58	Rohrer et al. (1994)

were not independent from each other, as their sum is unity. Accordingly, correlation matrices were computed from the gene frequencies of all loci. In addition, the eigenvalues of all principal components, the proportions of individual eigenvalues to the total variance (contribution rates of components), and the factor scores of every pig for each of the principal components were computed. A scattergram of the score data was examined to visualize the geometric relationship among pig breeds. The PCA was performed using the XLSTAT program (Agresti, 1990).

The statistical certainty of assignment or exclusion for individuals into their reference populations was evaluated using the program GeneClass v.2.0 (Piry et al., 2004). The exclusion method was carried out using the Bayesian approach developed by Rannala and Mountain (1997) because the Bayesian method showed better accuracy than the frequency- and distance-based methods (Cornuet et al., 1999; Koskinen, 2003).

Assignment of each individual was tested using the "leave one out" procedure (Efron, 1983), which means each individual was excluded from the data set when performing its assignment.

The principle of the exclusion method has been well described elsewhere (Cornuet et al., 1999; Koskinen, 2003). Frequency probabilities of multilocus genotypes in each reference population were performed using Monte Carlo simulations of 10,000 independent individuals for the population. The assignment criterion estimate of an individual in question was then compared with the frequency distribution of simulated genotypes of each reference population, allowing exclusion of individuals from populations with a specified degree of confidence (e.g., 0.001).

## Results

### Genetic Variability

The allele frequencies of 16 microsatellite loci were analyzed in 242 unrelated pigs from nine European,

Chinese, and Korean pig breeds. A total of 186 alleles was observed at the 16 loci distributed on 10 chromosomes. The average number of alleles per locus was 11.6, ranging from 6 (S0301, SW510) to 17 (SW1695; Tables 2 and 3).

As shown in Table 2, the Korean wild pig, Landrace, and Min pig exhibited a high degree of genetic diversity with mean expected heterozygosities of 0.698, 0.702, and 0.703, respectively. In contrast, the lowest genetic diversity was observed in the Korean native pig in all measures of genetic diversity (MNA, allelic richness, and observed and expected heterozygosities).

Across multiple loci, the Korean wild pig, Berkshire, and Landrace showed a significant value of inbreeding coefficient ( $F_{IS}$ ) after correction for multiple tests. Most pig populations, except for two Chinese native pigs (Min pig and Wuzhishan pig), showed a deviation ( $P < 0.05$ ) from HW equilibrium. Across populations and loci, 6 of 144 deviated from the HW equilibrium ( $P < 0.05$ ) after correction for multiple tests (Table 2). All of these deviated cases are related to the positive  $F_{IS}$ , indicating HW equilibrium deviation in the direction of heterozygote deficit.

The overall  $F_{IS}$  values per locus ranged from  $-0.0435$  (SW2409) to  $0.1393$  (SW2), showing an overall  $F_{IS}$  of  $0.067$  (Table 3). The  $F_{ST}$  and  $R_{ST}$  estimates of genetic differentiation were similar for all 16 microsatellites loci (Table 3). The  $F_{ST}$  values ranged from  $0.2189$  (SW2612) to  $0.3308$  (SW510). The mean  $F_{ST}$  value of  $0.261$  from all loci indicated that 73.9% of the genetic variation was caused by the differences among individuals and 26.1% was due to the differentiation among breeds.

### Genetic Distances

Nei's  $D_A$  genetic distance and mean  $F_{ST}$  estimates between each pair of nine porcine populations are shown in Table 4. The genetic distance ranged from  $0.139$  (between Landrace and Yorkshire) to  $0.684$  (be-

**Table 2.** Number of samples, mean number of alleles per locus (MNA), mean allelic richness ( $A_R$ ), expected heterozygosity ( $H_E$ ) at Hardy-Weinberg equilibrium, observed heterozygosity ( $H_O$ ), inbreeding coefficient ( $F_{IS}$ ), and number of loci (in parentheses) showing a significant departure from Hardy-Weinberg equilibrium for 16 microsatellite loci

Population	No.	MNA	$A_R$	$H_E$	$H_O$	$F_{IS}^a$
Korean breeds						
Korean native pig	32	3.44	2.96	0.494	0.497	-0.007 (1) <sup>b</sup>
Korean wild pig	22	5.81	4.98	0.698	0.607	0.133* (0)
Chinese breeds						
Min pig	12	4.69	4.62	0.703	0.735	-0.048 (1)
Wuzhishan pig	22	3.44	3.08	0.502	0.515	-0.026 (0)
Xiang pig	28	4.69	4.01	0.619	0.599	0.033 (1)
European breeds						
Berkshire	30	4.88	3.82	0.566	0.501	0.117* (0)
Duroc	32	4.31	3.78	0.599	0.557	0.071 (0)
Landrace	32	5.69	4.86	0.702	0.624	0.113* (2)
Yorkshire	32	5.13	4.27	0.637	0.577	0.095 (1)

<sup>a</sup>Probability from multi-locus test that there is no heterozygote deficiency (\* $P < 0.05$  after correction for multiple tests).

<sup>b</sup>Number in parentheses indicates the number of loci showing a significant departure ( $P < 0.05$ ) from Hardy-Weinberg equilibrium after correction for multiple tests.

tween Duroc and Wuzhishan pig). Pairwise  $F_{ST}$  estimates ranged from 0.092 (between Landrace and Yorkshire) to 0.438 (between Korean native pig and Wuzhishan pig). Genetic divergence of Asian native pig breeds from European breeds was pronounced, whereas genetic divergence among European breeds was relatively small (0.092 to 0.279). Phylogenetic trees of the nine porcine breeds were reconstructed based on Nei's  $D_A$  genetic distances (Figure 1). Trees from both NJ and UPGMA methods showed a similar topology, but the bootstrap values were slightly different. The Korean

native pig and Min pig were grouped into the same branches with commercial western pig breeds with high bootstrap support values (93% in NJ and 98% in UPGMA). Conversely, the Xiang pig, Wuzhishan pig, and Korean wild pig were clustered into different branches.

Figure 2 shows the relative position of the nine porcine breeds defined by principal component factor scores based on correlation matrix from allele frequency of the 16 microsatellites. The x- and y-axes represented 25 and 17% of the total variation, respectively. These axes separated the porcine breeds into three blocks: Block I

**Table 3.** Characterization of the 16 microsatellites analyzed in nine pig populations<sup>a</sup>

Locus	No. of alleles	$F_{IS}$	$F_{IT}$	$F_{ST}$	$R_{ST}$
S0036	9	-0.0281	0.2010***	0.2228***	0.343
SW1695	17	0.1043**	0.3070***	0.2264***	0.251
SW902	12	0.1026**	0.3463***	0.2716***	0.084
S0301	6	0.0120	0.2361***	0.2268***	0.115
SW2409	9	-0.0435	0.2036***	0.2368***	0.209
SW445	11	0.1251**	0.3953***	0.3088***	0.253
SW2	13	0.1393***	0.3509***	0.2458***	0.256
SW71	13	0.0721	0.3535***	0.3033***	0.398
SW205	9	0.0865*	0.3680***	0.3081***	0.414
SW61	16	0.0780*	0.2710***	0.2093***	0.266
S0070	16	0.0279	0.3612***	0.3429***	0.260
SW874	13	0.0692	0.2519***	0.1963***	0.373
SW2612	9	0.1095*	0.3044***	0.2189***	0.312
SW510	6	0.0676	0.3760***	0.3308***	0.405
SW1119	14	0.0764*	0.3448***	0.2907***	0.316
SW936	13	0.0902*	0.3129***	0.2448***	0.282
All loci	186	0.067***	0.311***	0.261***	0.280

<sup>a</sup> $F_{IS}$  and  $F_{IT}$  are measures of the deviation from Hardy-Weinberg proportions within subpopulations and in the total population, respectively.  $F_{ST}$  and  $R_{ST}$  are measures of the genetic differentiation over subpopulations.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**Table 4.** Nei's  $D_A$  genetic distance (below the diagonal) and mean  $F_{ST}$  estimates (above the diagonal) between each pair of nine pig populations<sup>a</sup>

Breed	K	K <sup>W</sup>	M	W	X	B	D	L	Y
Korean native pig (K)	—	0.304**	0.249**	0.438**	0.359**	0.324**	0.512**	0.234**	0.270**
Korean wild pig (K <sup>W</sup> )	0.563	—	0.538**	0.632**	0.524**	0.565**	0.650**	0.511**	0.553**
Min pig (M)	0.382	0.177	—	0.307**	0.225**	0.292**	0.412**	0.278**	0.134**
Wuzhishan pig (W)	0.672	0.311	0.535	—	0.350**	0.514**	0.684**	0.600**	0.344**
Xiang pig (X)	0.594	0.217	0.497	0.587	—	0.555**	0.596**	0.526**	0.270**
Berkshire (B)	0.266	0.276	0.174	0.324	0.322	—	0.279**	0.159**	0.162**
Duroc (D)	0.344	0.278	0.208	0.398	0.312	0.337	—	0.187**	0.196**
Landrace (L)	0.378	0.178	0.116	0.316	0.237	0.237	0.343	—	0.092**
Yorkshire (Y)	0.414	0.231	0.276	0.595	0.522	0.219	0.320	0.139	—

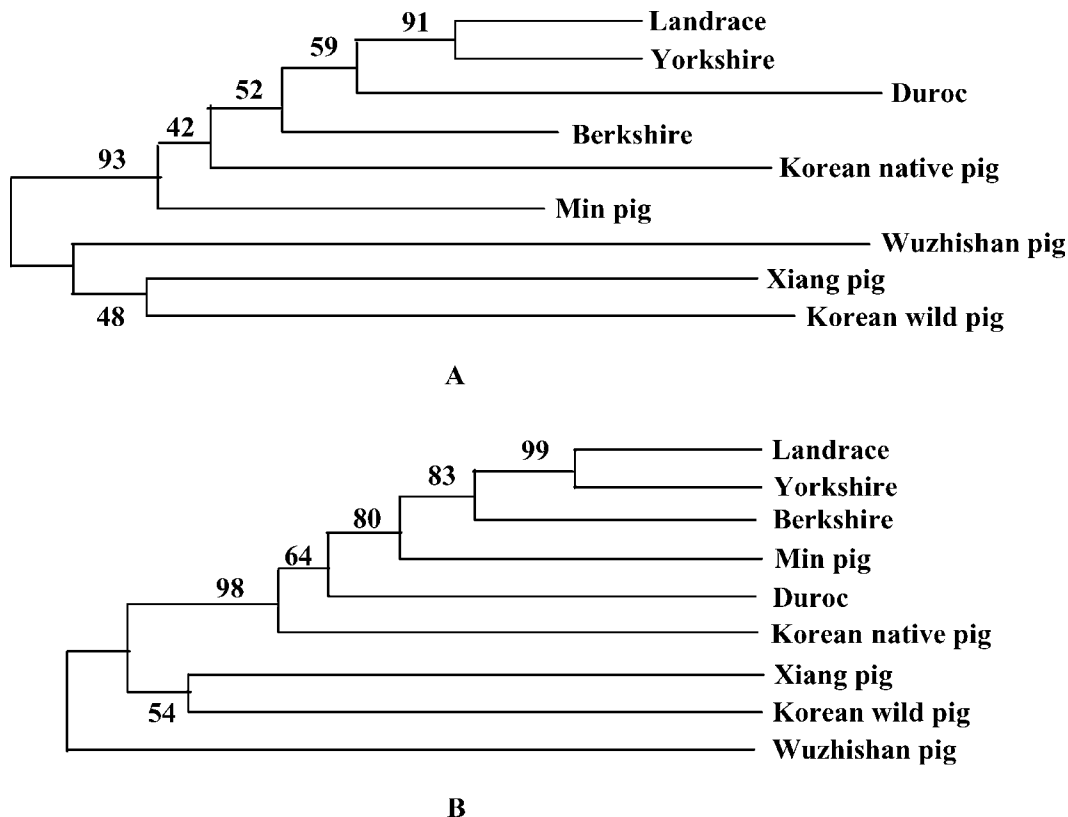
<sup>a</sup>Nei's  $D_A$  (Nei et al., 1983) and pairwise  $F_{ST}$  (Weir and Cockerham, 1984) are measures of genetic distance and genetic differentiation between populations, respectively.

\*\* $P < 0.01$  from a multi-locus test that there is no genetic differentiation in population pair after corrections for multiple comparisons.

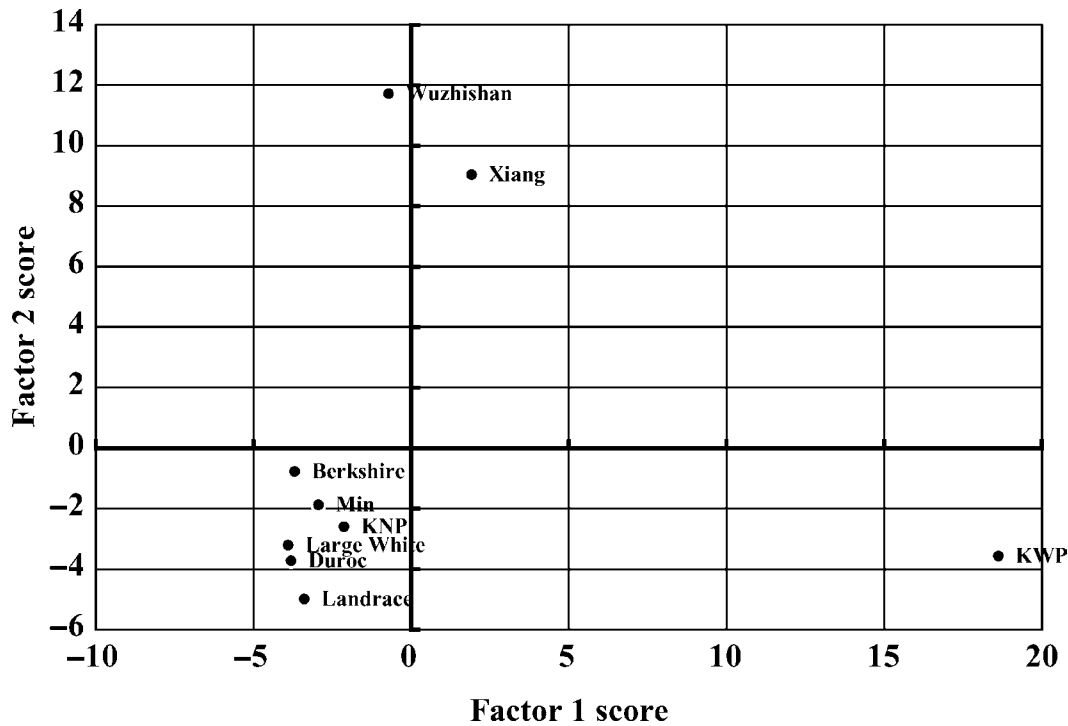
(Wuzhishan pig and Xiang pig), Block II (Berkshire, Min pig, Korean native pig, Yorkshire, Duroc, and Landrace), and Block III (Korean wild pig). Korean native pig and Min pig also were grouped with commercial breeds, supporting the result obtained from the phylogenetic tree. The Korean wild pig was separated from the other pig breeds and accounted for 18.5% of the total variation with respect to the other pig breeds.

Table 5 shows the percentage of individuals correctly assigned to their population of origin and excluded from reference populations based on the Bayesian method.

All individuals from the Chinese and Korean breeds were correctly assigned to their breed of origin by using a direct approach, whereas the accuracy of assignment of individuals from Landrace, Yorkshire, and Berkshire was 81, 94, and 97%, respectively. Similarly, by using a simulation approach, all Chinese and Korean pigs showed a 100% exclusion success from all alternative reference pig breeds with a high level of confidence ( $P < 0.001$ ); however, individuals from Landrace and Yorkshire could not be excluded successfully from their reference breeds.



**Figure 1.** Dendrograms showing the genetic relationships among nine pig breeds based on  $D_A$  genetic distance (Nei et al., 1983). The numbers at the nodes are the percentage bootstrap values from 1,000 replications of re-sampled loci. A: Neighbor-joining dendrogram; B: unweighted pair group method with the arithmetic mean dendrogram.



**Figure 2.** Scatter diagram showing relative position of nine pig breeds defined by principal component factor scores based on correlation matrix from allele frequency of the 16 microsatellites. Factors 1 and 2 accounted for 25 and 17% of the total variance in the correlation matrix, respectively. KNP = Korean native pig; KWP = Korean wild pig.

**Table 5.** Percentage of individual pigs assigned to and excluded from reference populations based on the Bayesian approach<sup>a</sup>

Breed	B	D	K	L	M	W	X	Y	K <sup>W</sup>
Berkshire (B)									
Assignment	97	3	—	—	—	—	—	—	—
Exclusion	10	100	100	100	100	100	100	97	100
Duroc (D)									
Assignment	—	100	—	—	—	—	—	—	—
Exclusion	100	0	100	100	100	100	100	100	100
Korean native pig (K)									
Assignment	—	—	100	—	—	—	—	—	—
Exclusion	100	100	9.7	100	100	100	100	100	100
Landrace (L)									
Assignment	—	—	—	81	—	—	—	19	—
Exclusion	100	100	100	0	100	100	100	81	100
Min pig (M)									
Assignment	—	—	—	—	100	—	—	—	—
Exclusion	100	100	100	100	0	100	100	100	100
Wuzhishan pig (W)									
Assignment	—	—	—	—	—	100	—	—	—
Exclusion	100	100	100	100	100	0	100	100	100
Xiang pig (X)									
Assignment	—	—	—	—	—	—	100	—	—
Exclusion	100	100	100	100	100	100	0	100	100
Yorkshire (Y)									
Assignment	—	—	—	6	—	—	—	94	—
Exclusion	100	100	100	47	100	100	100	6.3	100
Korean wild pig (K <sup>W</sup> )									
Assignment	—	—	—	—	—	—	—	—	100
Exclusion	100	100	100	100	100	100	100	100	0

<sup>a</sup>The assignment test was carried out using the direct approach, with the assignment threshold of 0.05 (Cornuet et al., 1999); the exclusion test was done using a simulation approach, with the confidence level of 0.001 (see Materials and Methods).

## Discussion

This study defined the genetic structure of the Korean native pig by analyzing 16 microsatellite markers in 242 animals from nine pig breeds. The mean allele number and heterozygosity were used as estimators to evaluate genetic characteristics and diversity. Korean native pigs exhibited consistently low levels of allelic diversity and heterozygosity, whereas Chinese pig breeds, except for Wuzhishan pig, seemed to have a relatively high degree of genetic diversity compared with that of commercial and Korean native pig breeds. Low genetic diversity of Korean native pigs is consistent with the previous findings reported by Kim and Choi (2002), and genetic diversity level observed in other pig breeds is within the range of diversity of pig breeds reported in previous studies (Fredholm et al., 1993; Laval et al., 2000; Li et al., 2000; Martinez et al., 2000; Lemus-Flores et al., 2001; Fan et al., 2002; Fang et al., 2005).

The low genetic diversity in the Korean native pig can be attributed to its breeding history for improved traits. Because the Korean native pig's original characteristics were not economically favorable (black coat, small BW, slow growth, and small litter size), since 1910, animal breeders tried to improve its productivity by crossing it with commercial breeds such as Berkshire.

Recently, the Korean native pig has been recognized as an important genetic resource because of its indigenous adaptation and specific traits. When the Korean native pig faced the threat of extinction during the 1980s, because of crossbreeding with commercial breeds and the introduction of many commercial breeds, several animal breeders and geneticists tried to restore the genetic characteristics of the Korean native pig. A small number of pigs were used as founder animals for the genetic restoration of the Korean native pig; however, the founder effects and closed breeding resulted in the loss of genetic variation. These facts further explain why the Korean native pig had the lowest heterozygosity and was clustered with the commercial pig breeds owing to its close genetic distance with Berkshire.

Both phylogenetic trees and PCA revealed that Korean native pig and Min pig (North Chinese pig breed) were grouped together with commercial pig breeds, whereas the Xiang pig and Wuzhishan pig were clustered distantly from other pig breeds.

The current findings confirm previous results, indicating the close genetic distance between the Korean native pig and the North Chinese breed (Kim et al., 2002a; Kim and Choi, 2002). Korean native pigs also were placed on the same branch of the phylogenetic tree as the Berkshire and Yorkshire based on the analysis of mitochondrial DNA, even though the Korean native pig was distinct from the European pig breeds (Swedish, Landrace, Duroc, Welsh, and Yucatan; Kim et al., 2002a). However, our findings largely differ from the

previous reports in terms of genetic relationships. Fang et al. (2005) reported that all Chinese pig breeds, including Min pig, are distinct from Western pig breeds based on a comprehensive microsatellite loci survey for 32 Chinese local pig breeds as well as three Western pig breeds. Moreover, Kim and Choi (2002) reported that the Korean native pig was placed in a different branch from commercial breeds such as Duroc, Landrace, and Yorkshire (no analysis was made for Berkshire). The discrepancy in the relationship studies could be more likely due to the use of different pig samples, although the use of different kinds and numbers of microsatellites cannot be ruled out. Indeed, the sample size of Min pig in this study was relatively small, so we acknowledge that our sample might not represent the real population of Min pig.

Despite these discrepancies, the close genetic relationship between the Korean native pig, and Berkshire, followed by Min pig, provided evidence supporting the historical hypothesis that the Korean native pig originated from North China, spread to South Korea via North Korea, and then experienced introgression of European alleles through progressive interbreeding with Western pig breeds. Chinese breeds were classified into six types according to their geographic origin, distribution, body conformation, and coat color (Li et al., 2004). According to their classification, the Min pig belongs to Type I (North China), and the Xiang pig and Wuzhishan pig are classified into Type IV (South China). Therefore, the results of the present study support the historical hypothesis with respect to the North China origin of the Korean native pig. To address this issue fully, however, more rigorous investigation with a greater number of pig samples will be needed.

According to some researchers, domestication of the Asian and European pigs was done independently from wild boar subspecies in Asia and Europe (Giuffra et al., 2000; Okumura et al., 2001). Conversely, more recent research revealed that domestication of pigs was undertaken at multiple centers across Eurasia (Larson et al., 2005). Jones (1998) insisted that Chinese pig breeds were used to develop European pig breeds such as Berkshire, Small White, and Middle White. This might explain why the Min pig was clustered with commercial breeds. Although the Min pig did not contribute directly to the development of commercial breeds, it is possible that a Chinese pig breed that is close to the Min pig was used for the development of commercial breeds. Once again, however, detailed scrutiny from a larger number of pig samples will be required to answer this issue.

The close relationship between the Korean pig breed and Western pig breeds could be explained by two possible hypotheses: 1) the Korean breed has been introgressed with European breeds or 2) a significant number of European pigs used in our study have Asian haplotypes; thus, they cluster with Min and Korean. Introgression of European alleles into Korean native pigs, as well as the relationship between Min pig and

Korean native pig, could benefit from mitochondrial DNA sequence comparison between pig breeds sampled in this study.

All pig breeds, with the exception of two Chinese pigs, deviated significantly from HW equilibrium ( $P < 0.05$ ). Random mating without artificial selection could cause the HW equilibrium in the two Chinese native pigs, or genetic drift might not have a detectable effect on the genotypic frequencies of these populations. Deviation from HW equilibrium in other pig breeds could be the result of the sampling of a single population with different allele frequencies in the subpopulations (Wahlund effect), nonrandom sampling, and/or inbreeding. In the present study, at least three populations, the Korean wild pig, Berkshire, and Landrace, showed evidence of significant inbreeding (Table 2). Deficiency of heterozygotes caused by the Wahlund effect has been proposed in other domestic animals, particularly in the Mexican hairless pig population (Lemus-Flores et al., 2001) and Iberian cattle breeds (Martin-Burriel et al., 1999).

Our estimation (overall  $F_{ST} = 0.261$ ) of genetic differentiation indicates significant population subdivision over pig breeds. This value belongs in the high category of genetic differentiation between pig breeds examined thus far. The greatest differentiation is observed in European pig breeds ( $F_{ST} = 0.27$ ; Laval et al., 2000) and the least in Chinese breeds ( $F_{ST} = 0.077$ ; Yang et al., 2003). Nonetheless, these differences are most likely to be explained by the different types of markers analyzed as well as the current genetic status of source population sampled.

The high level of genetic differentiation among pig breeds in this study increases success rate for individual assignment. This study was able to assign 91.3% of 242 animals into their correct reference breeds. This result was very similar to a previous finding of 92.14% of 420 animals (Yang et al., 2003). All individuals from Korean and Chinese native pigs showed 100% exclusion success from all alternative reference pig breeds with a high level ( $P < 0.001$ ) of confidence; however, some portion (9.7%) of Korean native pigs also was excluded from their source population, indicating the need for more efforts in genetic refinement of Korean native pigs. In contrast, Western pig breeds, except for Duroc, could not be excluded successfully from their alternative reference breeds. In particular, Landrace and Yorkshire could not successfully exclude each other by either direct or simulation approaches, suggesting the possibility of an admixture of gene pools between these two samples studied.

In conclusion, the Korean native pig had low heterozygosity and clustered with the Min pig and Western pig breeds; however, the Korean native pig was distinct from the Korean wild pig and South China pig breeds (Xiang pig and Wuzhishan pig). The findings suggested that the Korean native pig originated from a North China pig breed with a black coat color similar to the Min pig, but which possessed the traits of Western breeds such as Berkshire and Landrace because of

crossbreeding. The results of this study provide evidence supporting the fact that commercial breeds were used mainly as sires for improving Korean native pigs. These findings can be used as genetic information for the preservation and further genetic improvement of the Korean native pig.

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