

Genetic Diversity of Four Pig Breeds in Thailand Based on Microsatellite Analysis

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ABSTRACT

Fourteen microsatellite loci were analyzed in 79 random individuals to characterize the genetic variability of Thai native pig breed (TN), Large White (LW), Spotted Large White (SLW), and Pietrain (PT). Mean numbers of allele per locus, mean numbers of shared allele per locus and average gene diversity per locus were 8.86, 1.86 and 0.7875, respectively. Polymorphism information content (PIC), observed and expected heterozygosity of TN (0.7555, 0.5335 and 0.7925) were higher than the values in LW (0.5468, 0.5571 and 0.6707), SLW (0.5300, 0.5800 and 0.6492) and PT (0.4874, 0.4545 and 0.5986). Base on Nei' standard genetic distance and phylogenetic tree constructed using Nei' standard genetic distance matrix, there were a great genetic differentiation between TN and the other three breeds.

Key words: genetic diversity, pig, microsatellite

INTRODUCTION

Knowledge of genetic variation within and among different breeds is very important for understanding and developing endogenous economic genetic traits of breeds. Microsatellites or simple tandem repeatedly genetic markers have been used to define genetic structures and genetic relationships among different breeds. Microsatellites are highly polymorphic, they provide extremely useful markers for comparative studies of genetic variation, parentage assessment and studies of gene flow and hybridization. However, most of the studies using microsatellites have concentrated on cattle, sheep, and pig (Buchanan *et al.*, 1994; MacHugh *et al.*, 1998; Martinez *et al.*, 2000; Hanslik *et al.*, 2000), while not much information is available about the genetic diversity of native pigs. Some studies reported variation between Chinese indigenous Meishan and western breeds (Paszek *et*

al., 1998), and four indigenous pig breeds and three introduced breeds in China (Li *et al.*, 2000), but no information is available on the population structure and genetic diversity of Thai native pigs. In this study, the genetic variability and population structures of four pig breeds in Thailand were conducted using 14 microstellite loci. Gene differentiation and the genetic relationship within and between these four pig breeds are estimated.

MATERIALS AND METHODS

Sample collection and DNA extraction

Blood samples stabilized with EDTA were collected from a total of 79 individuals belonging to four breeds: Thai native pig (n=49), Spotted Large White (n=10), Large White (n=10) and Pietrain (n=10). Genomic DNA was extracted by an organic solvent method. The white cells were washed in phosphate-buffered saline (PBS) centrifugation and

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resuspension, then lysed with SDS detergent in the present of 10 mM Tris buffer (pH 8.0), 25 mM EDTA and 100 mg/ml proteinase K. The samples were incubated at 55°C for 60 min, and overnight at 37°C. Then, the mixture was filled with 500 µl phenol:chloroform:isoamyl alcohol (25:24:1), centrifuged and resuspended after that it was extracted with 500 µl chloroform:isoamyl alcohol (24:1), centrifuged and resuspended two times. The aqueous phase of this extraction was combined with 1 µl 95% ethanol to precipitate the DNA, which was washed in 70% ethanol and, finally, resuspended in 10 mM Tris/25 mM EDTA buffer.

Microsatellite analysis

Fourteen microsatellite markers (ATP2, IGF1, PgHAS, S0010, S0086, S0107, S0151, S0227, S0352, SS13N17R, SS13R44R, SW957, SW1066 and TNFB) were used for the analysis of the pig breeds (Table 1). Samples were adjusted to a concentration of 5 ng/µl before PCR amplification. The PCR amplification was performed on individual animal DNA. The PCR reaction was accomplished

in a total volume of 5 µl using 5 ng of genomic DNA, 1 mM dNTP, 5 µM of each primer, 1 unit of *Tag* DNA polymerase and 10X PCR buffer. The 10X PCR buffer consisted of 100 mM Tris-HCl, 15 mM MgCl₂, 500 mM KCl and pH 8.3. The PCR reaction cycle was accomplished by an initial denaturation at 94°C for 3 min, second denaturation at 94°C for 30 sec, primer annealing for 30 sec at the desired temperature, extension at 72°C for 1 min and final extension at 72°C for 5 min. The PCR were analyzed on 4.5% polyacrylamide denaturing sequencing gel, which was then dyed in AgNO₃ solution.

Data analysis

Allele frequency, the mean number of alleles per locus, average gene diversity (Nei, 1973), polymorphism information content (Botstein *et al.*, 1980), observed and expected heterozygosity and Nei' standard genetic distance (Nei, 1978) were computed. UPGMA dendrogram was constructed using PHYLIP software package (Felsenstein, 1993).

Table 1 Microsatellite marker names, primer oligonucleotide sequences and PCR conditions.

Marker name	Forward primer	Reverse primer	PCR temp.
ATP2	GCTGCATAGGGAGCTGTAGG	TAATGATGGTGGTGGTTAGTGC	65
IGF1	GCTTGGATGGACCATGTTG	CACTTGAGGGGGCAATGATT	60
PgHAS	GTCACAGTGGATGGCATTG	ACATCCCTAAGGTCGTGGC	60
S0010	TTAACATGGCTGTCTGGACC	GTCCCTGTCCAACCATAAGA	60
S0086	GCACAGTCTATTGATACTGGCGTC	CTGAGAACTTCCATATGCTCCTGG	65
S0107	CAAGGATGCCTGTAAGTGGTGAG	TCCTTAAGGCCTCGTAGGATCTG	65
S0151	CACCACACAACCACAGACTCCA	ACTTGAAGCCATAGCCACACCT	65
S0227	GATCCATTATATAATTTAGCACAAAGT	GCATGGTGTGATGCTATGTCAAGC	60
S0352	CGTTAAGCCACTTTCTGTGG	AAGGGGGAGGGCGAGGGA	60
SS13N17R	ATGTGCCGCAGTGCCTGCTAG	GCCAGTGTCCCCACAAGGG	60
SS13R44R	CCTCCTGGTGACCTGACAGG	GTGTGATATGGAAGGAGGAC	60
SW957	AGGAAGTGAGCTCAGAAAGTGC	ATGGACAAGCTTGTTTTCC	65
SW1066	GCAGGATGAACCAACCTG	CTCTTGAGGCAACCTGCTG	65
TNFB	CTGGTCAGCCACCAAGATTT	GGAAATGAGAAGTGTGGAGACC	65

RESULTS AND DISCUSSION

The number of alleles observed at each locus in Thai native pig (TN), Large White (LW), Spotted Large White (SLW) and Pietrain (PT) and the numbers shared among the four breeds, were given in Table 2. A total of 124 alleles were detected across the 14 loci analyzed. The number of alleles per locus varied from three (SS13N17R) to 15 (PgHAS) with a mean value of 8.86 (the mean of TN, LW, SLW and PT were 8.64, 4.00, 3.29 and 3.36). The mean number of alleles per locus was similar in all breeds. But there were marked differences for some loci, e.g. S0086 with 10 alleles in TN and only four, one, and one in LW, SLW and PT, respectively; PgHAS with 15 alleles in TN and five, two and two in LW, SLW and PT, respectively. The mean number of shared alleles was 1.86. Average gene diversity over all loci was 0.7875

(Table 2) while, for individual loci, average gene diversities ranged from 0.5635 (SS13N17R) to 0.9068 (S0107). Across loci, average gene diversity increased with increasing number of alleles (regression coefficient = 0.0282 ± 0.004 , $P < 0.001$).

Eleven loci were polymorphic in all breeds, while three were monomorphic in one to two breeds (namely S0227 in SLW, S0086 and SS13R44R in SLW and PT). Measures of genetic variation for each population (polymorphism information content, PIC; observed, H_O ; and expected, H_E heterozygosity) were given in Table 3. The highest PIC was found in TN (0.7555), while the lowest PIC was found in PT (0.4874). Observed heterozygosity of TN, LW, SLW and PT (0.5335, 0.5571, 0.5800 and 0.4545) was less than expected heterozygosity (0.7925, 0.6707, 0.6492 and 0.5986) in all breeds. The lowest heterozygosity was showed in PT, while the highest heterozygosity was showed

Table 2 Number of microstellite alleles at each locus in Thai native pig (TN), Large White (LW), Spotted Large White (SLW) and Pietrain (PT), number shared among the four breeds and average gene diversity within populations.

Locus	Numbers of alleles						Average gene diversity
	Total	TN	LW	SLW	PT	Shared	
ATP2	5	5	2	2	2	2	0.7010
IGF1	10	10	3	3	4	2	0.8592
PgHAS	15	15	5	2	2	1	0.8815
S0010	12	12	5	2	3	2	0.8399
S0086	10	10	4	1	1	1	0.7978
S0107	12	12	7	7	6	4	0.9068
S0151	9	7	4	6	5	2	0.8514
S0227	5	5	2	1	2	1	0.7006
S0352	7	7	5	3	3	2	0.7406
SS13N17R	3	3	2	2	2	2	0.5635
SS13R44R	6	6	2	1	1	1	0.6484
SW957	8	8	3	3	2	1	0.7661
SW1066	11	11	6	5	8	1	0.8800
TNFB	11	10	6	5	6	4	0.8876
Mean/locus	8.86	8.64	4.00	3.29	3.36	1.86	0.7875
(SE)	0.30	0.30	0.23	0.29	0.31	0.20	

Table 3 Sample size, polymorphism information content (PIC) and heterozygosity (standard errors in parenthesis) averaged over 14 microsatellite loci.

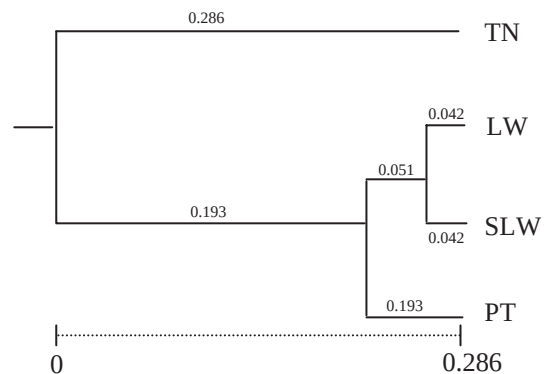
Breed	Sample size	PIC	Mean heterozygosity	
			Observed	Expected
TN	49	0.7555 (0.02)	0.5335 (0.03)	0.7925 (0.01)
LW	10	0.5468 (0.06)	0.5571 (0.10)	0.6707 (0.07)
SLW	10	0.5300 (0.06)	0.5800 (0.09)	0.6492 (0.07)
PT	10	0.4874 (0.07)	0.4545 (0.09)	0.5986 (0.08)
Average		0.5799	0.5313	0.6778

in TN. The genetic diversity of LW ($H_O=0.5571$) in this study was higher than that of Yorkshire in China ($H_O=0.526$), while genetic variations of TN ($H_O=0.5335$) was lower than that of indigenous pig breeds in China ($H_O=0.561-0.719$) reported by Li *et al.* (2000). The difference of genetic diversity value obtained could be due to the choice of microsatellite loci as well as the choice of population. All of the results indicated that diversity of TN was higher than that of exotic breeds. It may be long-time selection that resulted in the low variation in the exotic breeds.

Nei' standard genetic distance based on allele frequencies between TN and LW; TN and SLW; TN and PT; LW and SLW; LW and PT; and SLW and PT; were 0.6141, 0.5299, 0.5697, 0.0849, 0.2426 and 0.1297, respectively (Table 4). The value showed a high degree of genetic divergence between TN and the other three pig breeds and the closest was found to be LW and SLW.

Nei' standard genetic distance matrix was used in order to build phylogenetic tree with the

UPGMA method (Figure 1). In this tree, the TN population were clearly differentiated from the remaining breeds.

**Figure 1** Dendrogram showing the genetic relationship among four pig breeds in Thailand. Phylogenetic tree was constructed from Nei' standard genetic distance by the UPGMA method, which was based on 14 microsatellite loci. Numbers were the branch lengths.**Table 4** Matrix of Nei' standard genetic distance among Thai native pig (TN), Large White (LW), Spotted Large White (SLW), and Pietrain (PT).

Breed	TN	LW	SLW	PT
Thai native pig (TN)	-			
Large White (LW)	0.6141	-		
Spotted Large White (SLW)	0.5299	0.0849	-	
Pietrain (PT)	0.5697	0.2426	0.1297	-

CONCLUSION

Fourteen microsatellite loci were analyzed in 79 random individuals to characterize the genetic variability of TN, LW, SLW and PT. Mean numbers of allele per locus, mean numbers of shared allele per locus and average gene diversity per locus were 8.86, 1.86 and 0.7875. The highest genetic diversity was found in TN (PIC, H_O and H_E of TN), while the lowest value was found in PT (PIC, H_O and H_E of PT). However, the genetic variations of TN were lower than that of native pigs in China. Base on Nei' standard genetic distance, there was a great genetic differentiation between TN and the other three breeds. Similarly, phylogenetic tree was constructed using Nei' standard genetic distance matrix showed that TN was clearly distinct from the other three breeds.

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LITERATURE CITED

- Boststein, D., R. White, and M. Skolnick. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32 : 314-331.
- Buchanan, F.C., L.J. Adams, R.P. Littlejohn, J.F. Maddox, and A.M. Crawford. 1994. Determination of evolutionary relationships among sheep breeds using microsatellites. *Genomics* 22 : 397-403.
- Felsenstein, J. 1993. PHYLIP-phylogenetic inference package, version 3.5c. University of Washington, Seattle, Oregon.
- Hanslik, S., B. Harr, G. Brem, and C. Schlotterer. 2000. Microsatellite analysis reveals substantial genetic differentiation between contemporary New World and Old World Holstein Friesian populations. *Anim. Genet.* 31 : 31-38.
- Li X., K. Li, B. Fan, Y. Gong, S. Zhao, Z. Peng, and B. Liu. 2000. The genetic diversity of seven pig breeds in China, estimated by means of microsatellite. *Asian-Aus. J. Anim. Sci.* 13 : 1193-1195.
- MacHugh, D.E., R.T. Loftus, P. Cunningham, and D.G. Bradley. 1998. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. *Anim. Genet.* 29 : 333-340.
- Martinez, A.M., J.V. Delgado, A. Rodero, and J.L. Vega-Pla. 2000. Genetic structure of the Iberian pig breed using microsatellites. *Anim. Genet.* 31 : 295-301.
- Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 26 : 355-9.
- Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science of the USA* 70 : 3321-3.
- Paszek, A.A., G.H. Flickinger, L. Fontanesi, G.A. Rohrer, L. Alexander, C.W. Beattie, and L.B. Schook. 1998. Livestock variation of linked microsatellite markers in diverse swine breeds. *Anim. Biotech.* 9 : 55-66.

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