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Genetic Diversity of Native Pig in Thailand Using Microsatellite Analysis

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ABSTRACT

Fifteen microsatellite loci were analyzed in 49 random individuals to characterize the genetic variability of native pig breed (native pig from the north, 22 and native pig from the northeast, 27) in Thailand. Mean numbers of allele per locus, mean numbers of shared allele per locus and average gene diversity per locus were 8.60, 4.27 and 0.7742, respectively. Polymorphism information content (PIC), observed and expected heterozygosity of native pig from the north (0.5630, 0.4496 and 0.6003, respectively) were less than the values of native pig from the northeast (0.7439, 0.5570 and 0.7743, respectively). Nei' standard genetic distance between native pig from the north and native pig from the northeast was 0.5510. These results indicated that genetic diversity of native pig from the northeast was higher than that from the north.

Key words: native pig, microsatellite, genetic diversity

INTRODUCTION

Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives (Notter, 1999). Molecular methods have provided new markers for the study of genetic variation. Among these molecular markers, microsatellite loci have been found to be common in all eukaryotic genomes so far examined, with frequencies as high as one every 6 kb (Beckmann and Weber, 1992). As microsatellites are also highly polymorphic, they provide extremely useful markers for comparative studies of genetic variation, and could well be the markers of choice for analyses genetic structures and genetic relationships among different breeds (Bruford and Wayne, 1993). In this study, the genetic variability and population structures of native pig breed in Thailand were conducted using 15 microstellite loci. We also estimated the genetic relationship within these pigs.

MATERIALS AND METHODS

Sample collection and DNA extraction

Blood samples were collected from a total of 49 individuals belonging to two group of native pigs in Thailand: native pig from the north (n=22) and native pig from the northeast (n=27). Genomic DNA was extracted from the EDTA stabilized blood samples of all the pigs by an organic solvent method. The white cells were washed in phosphate-buffered saline (PBS) by alternate centrifugation and resuspension, then lysed with SDS detergent in the present of 10 mM Tris buffer (pH 8.0), 25 mM EDTA and 100 μg/ml proteinase K. The samples

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were incubated at 55°C for 60 min, follow by incubation at 37°C overnight. Then, the mixture was added with 500 µl phenol:choloform:isoamyl alcohol (25:24:1), centrifuged and resuspended after that it was extracted with 500 µl choloform:isoamyl alcohol (24:1), centrifuged and resuspended 2 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

Fifteen microstellite markers (S001, S0010, S0086, S0107, S0151, S0227, S0352, SW957, SW1066, ATP2, IGF1, TNFB, PgHAS, SS13N17R and SS13R44R) were used for the analysis of native pig breeds. Samples were adjusted to a concentration of 5 ng/µl before PCR amplification. The PCR amplification was performed on individual 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 $MgCl_2$, 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 stained in $AgNO_3$ solution.

Data analysis

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

RESULTS AND DISCUSSION

The number of alleles observed at each locus of native pig from the north and northeast, and the numbers shared between the two groups, are given in Table 1. A total of 129 alleles were detected across the 15 loci analyzed. All loci were polymorphic. The number of alleles per locus varied form three (SS13N17R) to 15 (PgHAS) with a mean value of 8.60 (the mean of native pig from the north and northeast were 5.33 and 7.53, respectively). The mean number of alleles per locus is similar in the two groups, but there are marked differences for locus PgHAS with 15 alleles in native pig from the northeast and only six alleles in native pig from the north. The mean number of shared alleles was 4.27. Average gene diversity over all loci was 0.7742 (Table 1) while, for individual loci, average gene diversities ranged from 0.5899 (SS13N17R) to 0.8952 (PgHAS). Across loci, average gene diversity increases with increasing number of alleles (regression coefficient = 0.0266 ± 0.004, $P < 0.001$).

Measures of genetic variation for each population (polymorphism information content, PIC; and observed and expected heterozygosity) are given in Table 2. PIC of native pig from the northeast (0.7439) was higher than the value of native pig from the north (0.5630). Observed heterozygosity (of native pig from the north and northeast were 0.4496 and 0.5770, respectively) was less than expected (0.6003 and 0.7743, respectively) in both groups, and heterozygosity of Thai native pig was lower in the north group than in the northeast group. Comparing these means (Table 1 and Table 2), more alleles were detected in native pig from the northeast for most loci (IGF1 was exceptional) and average of 49.6% of the alleles detected were shared between the two groups. These results indicated that genetic diversity of native pig from the north was lower than from the northeast. It may be that native pig from the north has been bred with non-random mating.

Table 1 Number of microstellite alleles at each locus in Thai native pig from the north and northeast, number shared between the two groups and average gene diversity within populations.

Locus	Numbers of alleles				Average gene diversity
	Total	North	Northeast	Shared	
S0001	8	4	6	2	0.6268
S0010	12	6	8	2	0.7877
S0086	10	5	9	4	0.8475
S0107	12	11	11	10	0.8823
S0151	7	7	7	7	0.8216
S0227	5	3	5	3	0.5978
S0352	7	5	6	4	0.7414
SW957	8	4	8	4	0.8136
SW1066	11	9	7	5	0.8533
ATP2	5	2	5	2	0.6505
IGF1	10	6	9	5	0.8574
PgHAS	15	6	15	6	0.8952
TNFB	10	8	8	6	0.8690
SS13N17R	3	2	3	2	0.5899
SS13R44R	6	2	6	2	0.7784
Mean/locus	8.6	5.33	7.53	4.27	0.7742
(SE)	(0.83)	(0.69)	(0.74)	(0.59)	

Table 2 Sample size, polymorphism information content (PIC) and heterozygosity (standard errors in parenthesis) averaged over 15 microstellite loci.

Thai native pig group	Sample size	PIC	Mean heterozygosity	
			Observed	Expected
North	22	0.5630 (0.05)	0.4496 (0.06)	0.6003 (0.05)
Northeast	27	0.7439 (0.02)	0.5770 (0.03)	0.7743 (0.02)
Average		0.6535	0.5133	0.6873

Nei' standard genetic distance based on allele frequencies between both of Thai native pig groups was 0.5510. The value showed a high degree of genetic divergence between native pig from the north and northeast. An individual tree based on the proportion of shared alleles supported the relatively high heterozygosity of native pig from the northeast, which formed two distinct clusters (Figure 1). The native pig from the north formed a clear level of clustering within the native pig in northeast.

CONCLUSION

Fifteen microstellite markers were used to characterize the genetic variability of 49 individuals of Thai native pig. Allele frequency, the mean

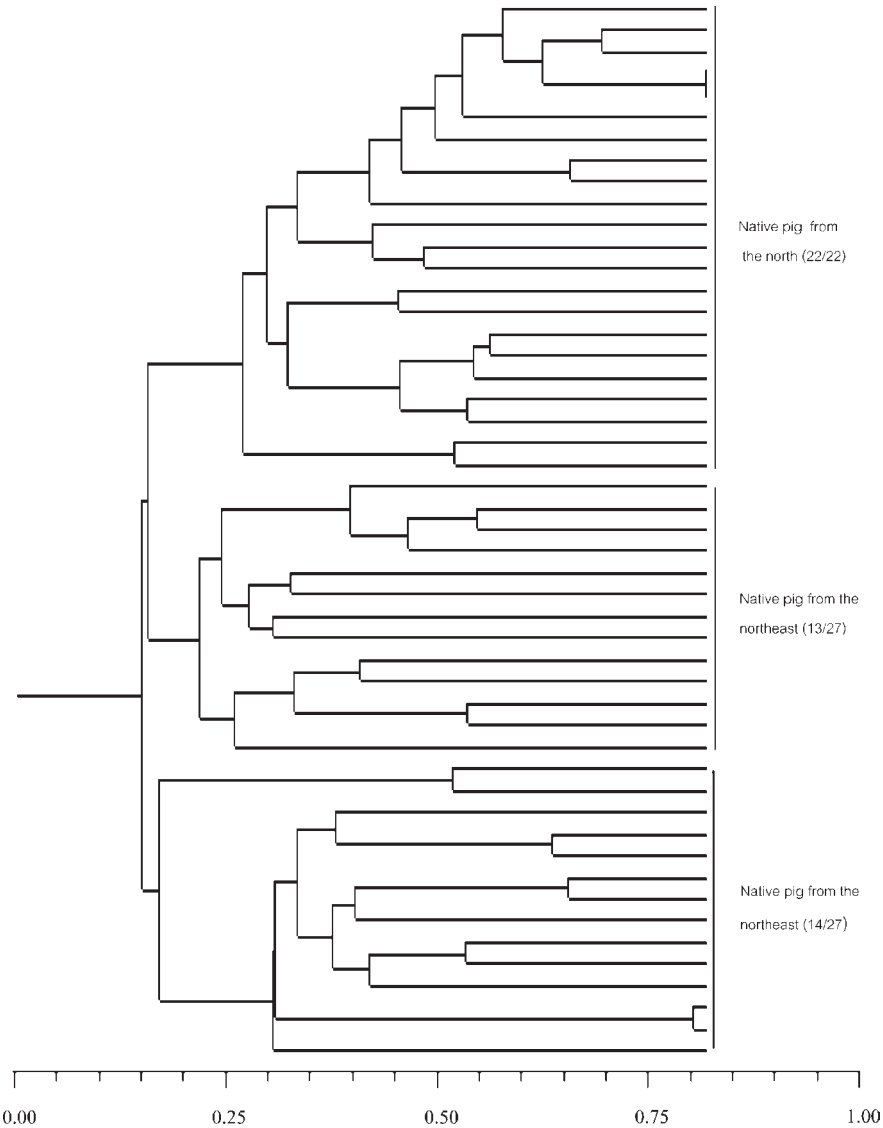

Figure 1 Neighbour-joining dendrogram constructed from allele-sharing distances inferred from microstellite data among 49 individuals from native pig breed. Numbers to the right indicate the fraction of individuals from the breed found in a cluster.

number of alleles per locus, average gene diversity, polymorphism information content (PIC), observed and expected heterozygosity and Nei' standard genetic distance were calculated. Neighbour-joining dendrogram was constructed. The results showed that the native pig from the north has a lower level of genetic diversity than native pig from the northeast.

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