Kasetsart J. (Nat. Sci.) 36 : 138 - 143 (2002)

Genetic Diversity of Spotted Large White, Large White and Pietrain Using Microsatellite Analysis

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ABSTRACT

Thirty-six microsatellite loci were analyzed in 30 random individuals to characterize the genetic variability of Spotted Large White (SLW), Large White (LW) and Pietrain(PT). Mean numbers of allele per locus, mean numbers of shared allele per locus and average gene diversity per locus were 5.39, 2.14 and 0.6646, respectively. Polymorphism information content (PIC), observed and expected heterozygosity of SLW (0.5138, 0.5667 and 0.6344, respectively) were lower than the values in LW (0.5685, 0.5706 and 0.6943, respectively) and PT (0.5236, 0.4806 and 0.6439). Base on Nei' standard genetic distance, there was a great genetic differentiation between SLW and the other two breeds. Similarly, individual clustering based on the proportion of shared alleles showed that SLW individuals formed a single cluster separated from the other two pig breeds.

Key words: genetic diversity, microsatellite, pig

INTRODUCTION

Many studies of genetic structure of livestock breeds have used allele frequency data protein coding (primarily allozyme) loci. Since the late 1970s, molecular methods have provided new markers for the study of genetic variation even to the level of analysis at the DNA sequence itself (Avise, 1994). 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, we estimated the genetic variations and population structures of Spotted Large White, Large White and Pietrain using 36 microstellite loci. We also estimated genetic relationship within and between these three pig breeds.

MATERIALS AND METHODS

Sample collection and DNA extraction

Blood samples were collected from a total of 30 individuals belonging to three breeds: Spotted Large White (n=10), Large White (n=10) and Pietrain (n=10). 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

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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 mg/ml proteinase K. The samples were incubated at 55°C for 60 min, following which incubation was continued overnight at 37°C. Then, the mixture was filled 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

Thirty six microstellite markers (ATP2, DAGK, IGF1, L30149, OPN, PgHAS, PIGCGT21, PICMICWA08, PIGREPD, PIGS0088X, PIGS0094X, S0010, S0086, S0097, S0107, S0151, S0213, S0219, S0227, S0229, S0352, S0358, SS121, SS13N05R, SS13N17R, SS13N18R, SS13N32R, SS13R44R, SSMDNAX7, SSC133243, SSS0313, SSU24283, SSU24286, SW957, SW1066 and TNFB) were used for the analysis of the pig breeds. Samples were adjusted to a concentration of 5 µg/ml 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 $MgCl_2$, 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 dyed 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 in Spotted Large White (SLW), Large White (LW) and Pietrain (PT) breed, and the numbers shared among the three breeds, are given in Table 1. A total of 194 alleles were detected across the 36 loci analyzed. The number of alleles per locus varied form two (S0227, SS13N17R, SS13N18R, SS13R44R and ATP2) to 11 (SW1066) with a mean value of 5.39 (the mean of SLW, LW and PT were 3.47, 4.00 and 3.60, respectively). But for locus SS13N17R, SS13N18R and ATP2 were all detected alleles (2 alleles) found in the three breeds. The mean number of alleles per locus is similar in the three breeds and mean number of shared alleles was 2.14. Average gene diversity over all loci was 0.6646 (Table 1) while, for individual loci, average gene diversities ranged from 0.0647 (SS13R44R) to 0.9176 (S0097). Across loci, average gene diversity increases with increasing number of alleles (regression coefficient = 0.0593 ± 0.009, $P < 0.001$).

Thirty-two loci were polymorphic in all breeds, while four were monomorphic in one to two breeds (namely S0227 in SLW, S0219 in PT, and 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) are given in Table 2. PIC of LW(0.5685) was higher than the value of SLW (0.5138) and PT (0.5236). Observed heterozygosity (of SLW, LW and PT were 0.0.5667, 0.5706 and 0.4806, respectively) was less than

Table 1 Number of microstellite alleles at each locus in Spotted Large White (SLW), Large White (LW) and Pietrain (PT), number shared between the three breeds and average gene diversity within populations.

Locus	Number of alleles					Average gene
	Total	SLW	LW	PT	Shared	diversity
ATP2	2	2	2	2	2	0.3229
DAGK	4	2	4	3	2	0.5226
IGF1	4	2	3	4	2	0.6360
L30149	3	2	2	2	-	0.6729
OPN	6	6	5	5	5	0.8340
PgHAS	5	2	5	2	1	0.6968
PIGCGT21	5	2	4	2	1	0.7038
PICMICWA08	6	5	4	3	2	0.7680
PIGREPD	10	6	6	5	4	0.8662
PIGS0088X	3	2	3	3	2	0.6439
PIGS0094X	9	5	8	6	4	0.8232
S0010	6	5	5	3	2	0.7959
S0086	4	1	4	1	1	0.3416
S0097	10	8	7	7	3	0.9176
S0107	9	7	7	6	4	0.9018
S0151	9	6	4	5	2	0.8280
S0213	6	5	4	5	3	0.8118
S0219	4	2	3	1	1	0.3374
S0227	2	1	2	2	1	0.4223
S0229	5	3	3	4	2	0.6905
S0352	5	3	5	3	2	0.6299
S0358	6	2	6	6	2	0.7913
SS121	5	3	3	3	2	0.4640
SS13N05R	5	4	4	4	3	0.7231
SS13N17R	2	2	2	2	2	0.5082
SS13N18R	2	2	2	2	2	0.5159
SS13N32R	6	5	4	5	3	0.8218
SS13R44R	2	1	2	1	1	0.0647
SSMDNAX7	7	4	4	4	2	0.8471
SSC133243	5	4	4	4	3	0.7507
SSS0313	5	3	3	5	3	0.7375
SSU24283	3	3	2	2	1	0.5528
SSU24286	5	2	3	3	1	0.6700
SW957	4	3	3	2	1	0.5483
SW1066	11	5	6	8	1	0.9026
TNFB	8	5	6	5	4	0.8603
Mean/locus	5.39	3.47	4.00	3.60	2.14	0.6646
(SE)	(0.41)	(0.30)	(0.27)	(0.29)	(0.19)	

Table 2 Sample size, polymorphism information content (PIC) and heterozygosity (standard errors in parenthesis) averaged over 36 microstellite loci.

Breed	Sample size	PIC	Mean heterozygosity	
			Observed	Expected
SLW	10	0.5138 (0.06)	0.5667 (0.09)	0.6344 (0.06)
LW	10	0.5685 (0.05)	0.5706 (0.09)	0.6943 (0.05)
PT	10	0.5236 (0.06)	0.4806 (0.09)	0.6439 (0.06)
Average		0.5353	0.5393	0.6576

expected (0.6344, 0.6943 and 0.6439, respectively) in all breeds. The lowest heterozygosity was found in SLW, while the highest heterozygosity was found in LW. The genetic diversity of LW (H_O= 0.5706) in this study is higher than that of Yorkshire in China (H_O=0.526) 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. In conclusion, the results indicated that genetic diversity of SLW was lower than that of LW and PT, and had been genetically subdivided from other pig breeds. It may be that SLW had been bred without crossbreeding with any pig breeds.

Nei' standard genetic distance based on allele frequencies between SLW and LW, SLW and PT, and LW and PT were 0.1683, 0.3414 and 0.2776, respectively (Table 3). The value showed a high degree of genetic divergence between SLW and PT, and LW and PT. An individual tree based on the proportion of shared alleles is showed in Figure 1. Each breed was formed a clear cluster.

CONCLUSION

Thirty-six microsatellite loci were analyzed in 30 random individuals to characterize the genetic variability of SLW, LW and PT. Mean numbers of allele per locus, mean numbers of shared allele per locus and average gene diversity per locus were 5.39, 2.14 and 0.6646, respectively. The lowest genetic diversity was found in SLW (PIC, H_O, and of SLW were 0.5138, 0.5667 and 0.6344, respectively), while the highest value was found in LW (PIC, H_O, and of LW 0.5685, 0.5706 and 0.6943, respectively). Base on Nei' standard genetic distance, there was a great genetic differentiation between SLW and the other two breeds. Similarly, individual clustering based on the proportion of shared alleles showed that SLW individuals formed a single cluster separated from the other two pig breeds.

Table 3 Matrix of Nei' standard genetic distance among Spotted Large White, Large White and Pietrain.

Breed	Spotted Large White	Large White	Pietrain
Spotted Large White	-		
Large White	0.1683	-	
Pietrain	0.3414	0.2776	-

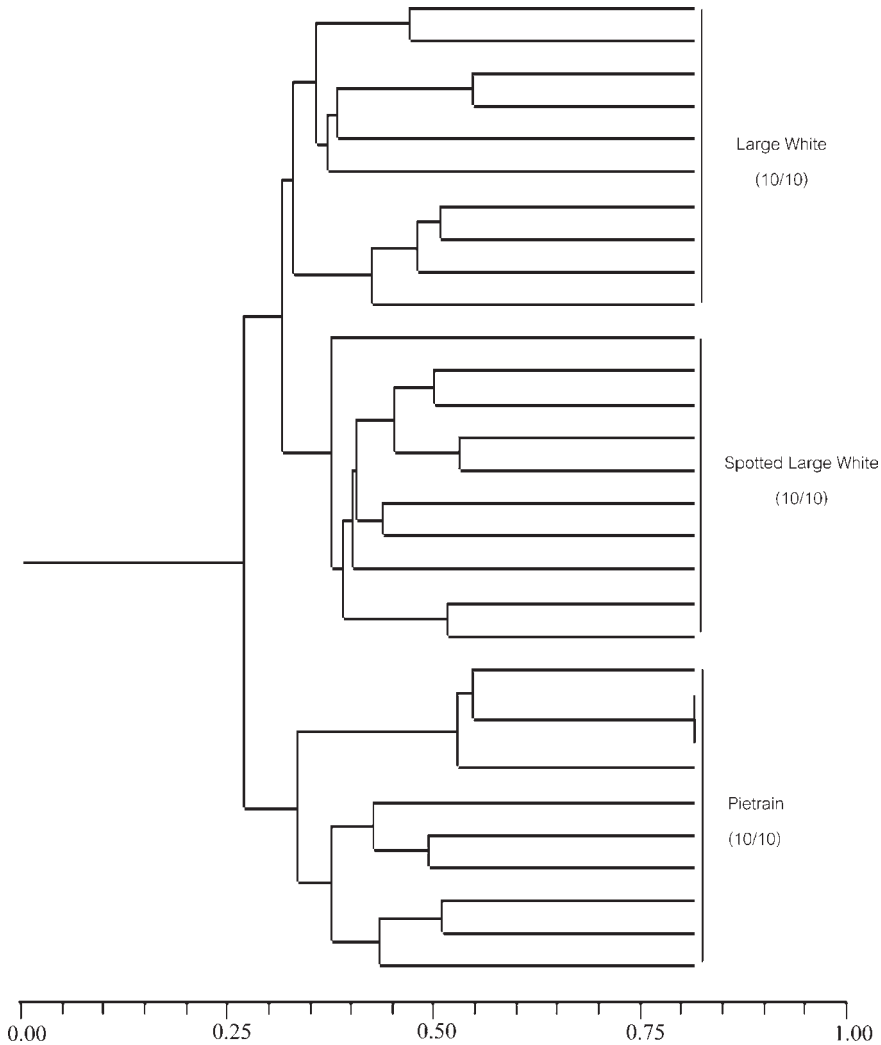

Figure 1 Neighbour-joining dendrogram constructed from allele-sharing distances inferred from microstellite data among 30 individuals from three pig breeds. Numbers to the right indicate the fraction of individuals from the breed found in a cluster.

ACKNOWLEDGEMENTS

This work was supported by Kasetsart University Research and Development Institute.

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Received date : 1/05/02
Accepted date : 27/06/02