The Genetic Diversity of Seven Pig Breeds in China, Estimated by Means of Microsatellites

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ABSTRACT : The polymorphisms of six microsatellites were investigated in four indigenous pig breeds (Erhualian, Tongcheng, Qingping and Wannanhua) and three introduced breeds (Large White, Landrace and Duroc) in China, and the genetic variations within and among populations were analyzed. The results showed that genetic diversity of Chinese indigenous pig breeds is higher than that of the introduced pig breeds. The clustering of seven breeds is consistent with their geographical distribution approximately. Estimated time of breed divergence ranged from 653 to 1856 years. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 9 : 1193-1195*)

Key Words : Chinese Pig, Microsatellites, Genetic Diversity

INTRODUCTION

There are many indigenous pig breeds in China. Conservation of them has been recognized as an increasing international concern. In recent years, the genetic diversities of those breeds have been evaluated using cytogenetic and biochemical genetic methods (Hu et al., 1998). There are also some reports on using mitochondrial DNA and randomly amplified polymorphic DNA (RAPD) (Zhou et al., 1998). Microsatellites are very useful markers in assessing population relationships because of their abundant, even distribution in the genome and high polymorphism. Paszek et al. (1998a, b) reported variation between Chinese indigenous Meishan and western breeds. However, few reports are found about microsatellite diversity of other Chinese local pig breeds. In the present paper, the genetic variations within and among seven pig breeds in China by using six microsatellite loci are reported.

MATERIALS AND METHODS

Materials

Blood samples were collected from 96 unrelated pigs belonging to 7 breeds: Erhualian (n=24) from Changshu city breeding farm, Xishan city pig breeding farm, Yixing city pig breeding farm and Jiangpu breeding farm of Nanjing Agricultural University in Jiangsu province; Tongcheng (n=16) from Tongcheng county pig breeding farm in Hubei province; Qingping (n=11) from Dangyang city pig breeding farm in Hubei; Wannanhua (n=10) from Huangshan city pig breeding farm, Xiuning county pig breeding farm and Xi county pig breeding farm in Anhui province; Duroc (n=10) from Huangpi foreign trade pig breeding farm in Hubei province and Tanghe foreign trade pig breeding farm in Henan provine; Landrace (n=12) from Runan foreign trade pig breeding farm in Henan province and pig breeding farm of Henan academy of agricultural science; Large White (n=13) from two pig breeding farms of Hubei academy of agricultural science and Runan foreign trade pig breeding farm in Henan province. The blood samples were lysed and then taken to laboratory.

Methods

DNA was extracted as described by Li et al. (1997). PCR primer pairs of the six microsatellites (SW24, SW2019, SW1876, SW605, SW1653 and SW1355) were synthesized by Shanghai Sangon Co. and Yuanpinghao Biotech. Co.. The PCR primer sequences and reaction conditions were as described by Alexander et al. (1996) and Rohrer et al. (1994). The primer sequences, chromosome localization and PCR conditions of the microsatellites used in this study are shown in table 1. The PCR products were analyzed on an 8% polyacrylamide denaturing sequencing gel, and then dye the gel in $AgNO_3$ solution. PCR product size is calculated according to Rickwood and Hames (1989).

Statistical analyses

Allele frequencies and observed heterozygosity (Ho), expected heterozygosity (Hs) at each locus for each population, and genetic distances among populations (Nei, 1972) were obtained using the GENES IN POPULATION (May et al., 1995). The phylogenetic tree was constructed using UPGMA from Nei's (1972) standard genetic distance by PHYLIP

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Table 1. The primer sequences, chromosome localization and PCR conditions of the microsatellites used in this study

Microsatellite loci	Chromosome location	Primer sequences	Mg^{2+}	Anneal temp.
SW24	17	CTTTGGGTGGAGTGTGTGC ATCCAAATGCTGCAAGCG	1.5 mM	58℃
SW2019	7	ATGATGCGAACCTGGAACTC TATGTGTAACTTGGTCCCATGC	1.5 mM	60℃
SW1876	13	TGTCACTGCTGTGGCTTAGG CTGGCTACTCCAAGACATTGG	1.5 mM	62℃
SW605	12	AGCCTTCTGTGAAGAAAAGC CCCCAGGTTCTCTGCTCTC	4.5 mM	58℃
SW1653	1	TTAAGCTCTCATTTGCTATGAGG CCCACATTAAGAGACGTACGC	1.5 mM	52℃
SW1355	6	ACTGGCTGACATGGGTTCTC GTCTGCGGCTAAGAGGGTC	1.5 mM	62℃

(Felsenstein, 1995) and TREEVIEW.

In addition, polymorphism information content (PIC) (Botstein et al., 1980) and effective number of alleles (Kimura and Crow, 1964) at each locus in each population were calculated. Time of breed divergence was estimated based on the equation D=2at (Nei, 1972), where D is Nei's (1972) standard genetic distance and is the estimated microsatellite mutation rate, assuming the average generation interval of pig is 1.5 years.

RESULTS AND DISCUSSIONS

A table of allele frequencies at six loci for each population is available from the corresponding author. It is clear that the distribution of allele number and frequencies varied in different breeds. Mean numbers of allele observed per locus ranged from 3.667 of Duroc to 6.500 of Erhualian (table 2).

Some alleles were only found in a single breed, such as the allele of 155 of Sw1653 in Tongcheng and 129 of Sw1355 in Landrace. Of 54 alleles only eight were detected in all of the seven breeds at six microsatellite loci (such as 123 of SW605, 134 of SW2019, 113 of SW1355), and the distribution of these shared alleles is also different (x^2=149.15, df=72, p<0.01). Table 1 also gives mean observed heterozygosity (Ho), mean expected heterozygosity (Hs), mean polymorphism information content (PIC) and mean number of alleles (Ne). In comparison of these indices, diversity of Chinese indigenous pig breeds is higher than that of the introduced breeds. Erhualian has the largest within-breed variation, while Duroc is the smallest, and the order of other populations is Tongcheng, Wannanhua, Qingping, Yorkshire and Landrace. It may be long-time selection that resulted in the low variation in the introduced breeds.

Nei's standard distance and UPGMA clustering result are shown in figure 1. Result of clustering is consistent with their geographical distribution approximately, the indigenous pig breeds are grouped

Table 2. Mean no. of alleles per locus, mean observed heterozygosity (Ho), mean expected heterozygosity (Hs), mean polymorphism information content (PIC), mean effective number of alleles (Ne) at six microsatellites for seven populations

Breed	Mean no. of alleles per locus	Mean Ho	Mean Hs	Mean PIC	Mean Ne
Indigenous					
Erhualian	6.500±0.563	0.576±0.087	0.741±0.029	0.705±0.032	4.117±0.450
Wannanhua	4.667±0.211	0.600±0.113	0.660±0.045	0.616±0.045	3.185±0.386
Qingping	4.667±0.211	0.561±0.043	0.628±0.048	0.575±0.048	2.906±0.341
Tongcheng	6.000±0.730	0.719±0.042	0.727±0.028	0.687±0.032	3.908±0.486
Introduced					
Landrace	4.000±0.577	0.500±0.116	0.538±0.111	0.501±0.105	2.755±0.517
Yorkshire	4.000±0.447	0.526±0.061	0.593±0.048	0.539±0.051	2.617±0.277
Duroc	3.667±0.422	0.550±0.112	0.538±0.101	0.493±0.095	2.685±0.529

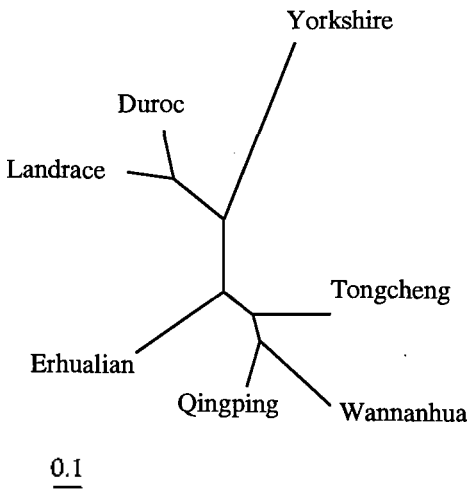

Figure 1. Dendrogram of seven pig breeds by UPGMA from Nei's standard genetic distance

into a branch and the introduced breeds into another branch. Chinese indigenous breeds have been provisionally grouped into North-China Type, South-China Type, Central-China Type, Lower-Changjiang River Basin Type, Southwest Type and Plateau Type (Zhang et al., 1986). In this study, Central-China Type breeds, Wannanhua, Qingping and Tongcheng were grouped firstly, then grouped with Erhualian belonged to Lower-Changjiang River Basin Type. Therefore, our result is consistent with such classification. When a mutation of 4.5×10^{-4} per microsatellite locus was assumed, the estimation of the time of these seven breed divergence ranged from 653 years for separation of Wannanhua and Qingping to 1856 years for separation of Wannanhua and Yorkshire, which were in accordance with their known history.

Microsatellites may reveal the variation and divergence among the pig breeds more objectively because mammalian genome has about 10% structure and 90% non-structure genes. Natural and artificial selection are conducted according to adaptation and phenotype of livestock, that is, according to structure gene. A livestock species is composed of many different breeds. When two breeds diverge from a common ancestor, artificial selection is extremely affected by the variation of structure gene. Sometimes, people select the same phenotype in two populations so that the genetic distance from phenotype cannot reveal the divergent time accurately. While microsatellites mainly belong to non-structure gene, its variation is rarely affected by selection, and variation will be accumulated in populations during their evolution. So diversity between two populations is determined by divergent time, and the microsatellite variation is not equal to the structure gene variation. Consequently, the genetic distance using microsatellites can reflect the divergent time more accurately.

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