

## The Genetic Diversity of Seven Pig Breeds in China Evaluated by Means of Microsatellites

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**ABSTRACT:** The polymorphism of six microsatellites was investigated in four indigenous pig breeds (Erhualian, Tongcheng, Qingping and Wannanhua) and three introduced breeds (Large White, Landrace and Duroc) in China. 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 approximately consistent with their geographical distribution. Estimated time of breed divergence ranged from 653 to 1856 years.

Key Words: Chinese Pig, Microsatellites, Genetic Diversity

### INTRODUCTION

Microsatellites are very useful markers in assessing population relationships because of their abundant, even distribution in the genome and high polymorphism. Many studies using microsatellites were reported on genetic variations within and among breeds in different livestock. However, there are few reports about microsatellite diversity of pig breeds in China. In the present paper, the genetic variations within and among seven pig breeds in China were conducted by using six microsatellite loci.

### MATERIALS AND METHODS

#### Materials

Blood samples were collected from 96 unrelated pigs belonging to 7 breeds: Erhualian (n=24), Tongcheng (n=16), Qingping (n=11), Wannanhua (n=10), Duroc (n=10), Landrace (n=12), Large White (n=13). The blood samples were lysed and then taken to laboratory.

#### Methods

DNA was extracted as described by X. Li *et al.* (1997). PCR primer pairs of the six microsatellites (SW24, SW2019, SW1876, SW605, SW1653 and SW1355) were synthesized. The PCR reaction conditions were as described by Rohrer *et al.* (1994). The PCR products were analyzed on an 8% polyacrylamide denaturing sequencing gel, which was then dyed in AgNO<sub>3</sub> solution. PCR product size was 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 and TREEVIEW (Page, 1996).

In addition, polymorphism information content (PIC) (Botstein *et al.*, 1980) and effective number of alleles (Kimura & 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 t is the estimated microsatellite mutation rate, assuming the average generation interval of pig is 1.5 years.

### RESULTS AND DISCUSSION

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 in Duroc to 6.500 in Erhualian (see Table 1).

Some alleles were only found in a single breed, such as the allele 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 the shared alleles appeared in all of the seven breeds 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). Comparing these means, 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 of introduced breed.

Nei's standard distance and UPGMA clustering result are shown in Table 2 and Figure 1. Result of clustering is consistent with their geographical distribution approximately, the indigenous pig breeds are grouped 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 which belongs to Lower-Changjiang River Basin Type. Therefore, our result is consistent with

such classification. When a mutation rate of  $4.5 \times 10^{-4}$  per microsatellite locus was assumed, the estimate 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 the 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 calculated from phenotypes can not 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.

**Table 1.** Mean number 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      |
|-------------------|-------------------------------|--------------|--------------|--------------|--------------|
| <b>Indigenous</b> |                               |              |              |              |              |
| Erhuanlian        | 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) |
| <b>Introduced</b> |                               |              |              |              |              |
| 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) |

**Table 2.** Nei's (1972) standard distance (below the diagonal) and the estimate time (years) of divergence (above the diagonal) among seven pig populations

| Breeds    | Erhualian | Wannanhua | Qingping | Tongcheng | Landrace | Yorkshire | Duroc |
|-----------|-----------|-----------|----------|-----------|----------|-----------|-------|
| Erhualian | ***       | 973       | 736      | 896       | 925      | 1138      | 971   |
| Wannanhua | 0.584     | ***       | 653      | 983       | 1270     | 1856      | 718   |
| Qingping  | 0.442     | 0.392     | ***      | 773       | 800      | 1205      | 721   |
| Tongcheng | 0.538     | 0.590     | 0.464    | ***       | 948      | 1411      | 1225  |
| Landrace  | 0.555     | 0.762     | 0.480    | 0.569     | ***      | 815       | 318   |
| Yorkshire | 0.683     | 1.114     | 0.723    | 0.847     | 0.489    | ***       | 1260  |
| Duroc     | 0.583     | 0.431     | 0.433    | 0.735     | 0.191    | 0.756     | ***   |

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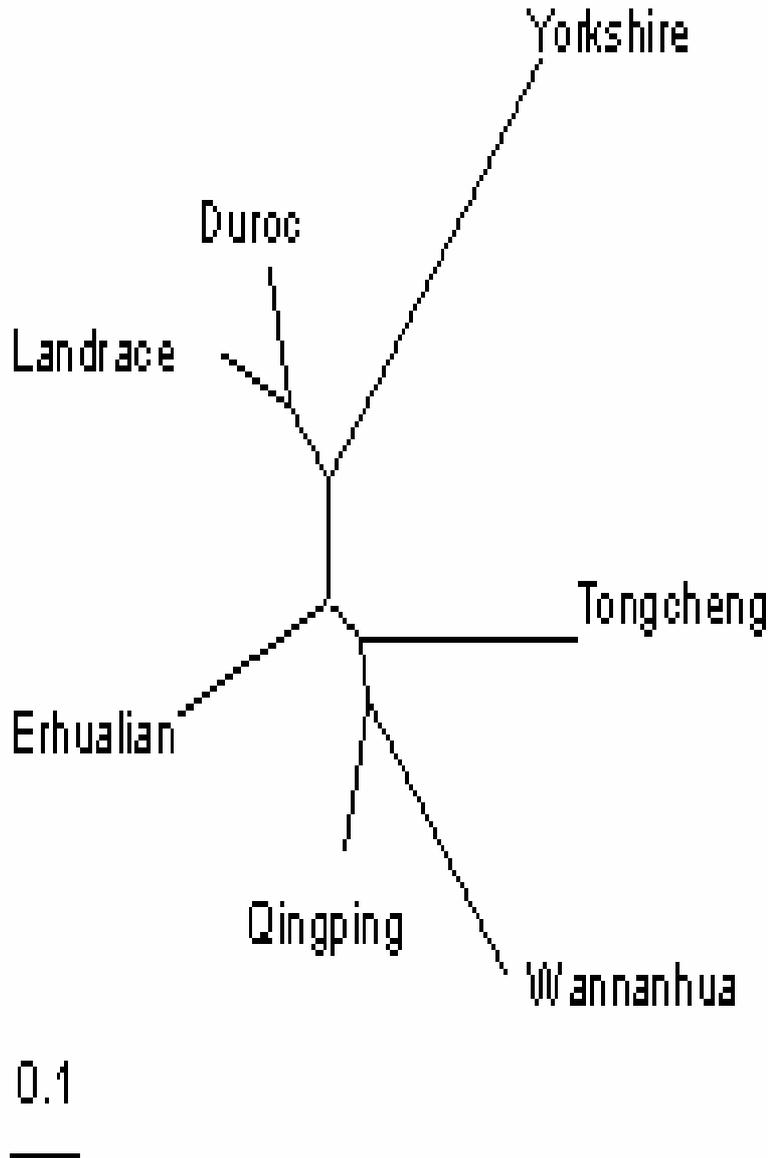
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**Figure.1** Dendrogram of seven pig breeds by UPGMA from Nei's standard genetic distance.