

## Evaluation of the Genetic Relationship among Ten Chinese Indigenous Pig Breeds with Twenty-six Microsatellite Markers

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**ABSTRACT** : The genetic diversities and relationships of 10 Chinese indigenous pig breeds and three exotic pig breeds have been evaluated using 26 microsatellites recommended by the Food and Agriculture Organization & the International Society of Animal Genetics (FAO-ISAG). The allele frequencies, genetic heterozygosity (H) and polymorphism information content (PIC) have been calculated. The results showed that genetic diversity of Chinese indigenous pig breeds is higher than that of the introduced pig breeds. The clustering of 10 breeds is generally consistent with their geographical distribution. (*Asian-Aust. J. Anim. Sci.* 2004. Vol 17, No. 4 : 441-444)

**Key Words** : Microsatellites, Chinese Indigenous Pig Breeds, Genetic Variation

### INTRODUCTION

Chinese indigenous pig breeds possess the most abundant genetic resource of pig breeds in the world. 118 Chinese indigenous pig breeds were documented in the domestic animal diversity information system (DAD-IS), which take up one-third of the world total. Chinese indigenous pig breeds, given their own special characters, such as prematurity, prolific and delicious pork, should be a source of great interest, appeal and curiosity to pig breeders of the world. However, in the past twenty years, most indigenous pig breeds have been facing the challenge of modern commercial pig breeds. The population size of many breeds has become very small or even being effectively lost. Therefore, evaluation and conservation have been recognized as an essential task for breeders and geneticists.

In recent years, the genetic variations of some Chinese indigenous pig breeds have been evaluated by cytogenetic and biochemical methods, randomly amplified polymorphic DNA (RAPD) and microsatellite markers (Li et al., 2000).

Microsatellite markers, with the characters of high polymorphism, evenly distributed in the whole genome, easy for genotyping, had been recommended by the Food and Agriculture Organization and the International Society for Animal Genetics (FAO-ISAG) to study the diversity of

global pig breeds, and the method was used extensively to construct structure of the closely related populations and breed allocating of cattle, pig and sheep (MacHugh et al., 1998; Diez-Tascón et al., 2000; Li et al., 2000; Martínez et al., 2000; Fan et al., 2002).

### MATERIALS AND METHODS

Blood samples were collected from 572 unrelated Chinese indigenous pigs and 184 unrelated introduced pigs. Chinese pigs belong to 10 breeds: Yimeng Black (YM), Laiwu (LA), Huai (HA), Putian (PT), Lantang (LT), Dahuabai (DH), Luchuan (LC), Longlin (LL), Donglan (DL), Dongshan (DS); and introduced pig breeds include 3 breeds: Duroc (DR), Large White (LW) and Landrace (LD). More information about these breeds can be seen in Table 1. Genomic DNA was prepared from whole blood using the procedure described by Zhao (1999).

#### Methods

Primers of 26 microsatellites recommended by ISAG-FAO were synthesized by Beijing Sangon Biotechnology Co.. DNA amplifications were performed with PE480 and Amp9600 Thermal Cycler (PE Company, USA). PCR conditions were optimized according to the referenced protocols; the denatured PCR products were analyzed on 8% polyacrylamide denaturing sequencing gel and then dye the gel in AgNO<sub>3</sub> solution and scanned the gel by Gel Analyzer of Fotodyne (Incorporated Hartland Company, USA). To ensure the accuracy of allele calling, in our studies, microsatellites products of two samples were cloned and sequenced, which were used as the control animals (DR and LD pigs). The control DNAs, F9110010 and F9110012, kindly provided by Dr Denis Milan of INRA,

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Received January 25, 2003; Accepted November 27, 2003

**Table 1.** The detail information of 10 Chinese indigenous pig breeds and 3 introduced pigs included in the study

Breeds	Samples locations (Provinces)	Total numbers
Yimeng Black (YM)	Shandong	60
Laiwu (LA)	Shandong	59
Huai (HA)	Fujian	60
Putian (PT)	Fujian	61
Lantang (LT)	Guangdong	60
Dahuabai (DH)	Guangdong	56
Luchuan (LC)	Guangxi	58
Longlin (LL)	Guangxi	56
Donglan (DL)	Guangxi	46
Dongshan (DS)	Guangxi	56
Duroc (DR)	Beijing	62
Large White (LW)	Beijing	66
Landrace (LD)	Beijing	56
Total		756

France, were also used as the control animals (DR and LD pigs) to adjust our microsatellite data.

### Statistical analyses

Allele frequencies and observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) at each locus for each population, polymorphic information content (PIC) and effective numbers of alleles, fixation indices ( $F_{IT}$ ,  $F_{IS}$ ,  $F_{ST}$ ), total population heterozygosity ( $H_t$ ) and Nei's standard genetic distance were obtained using GENEPOP ver.3.3 package (Raymond et al., 1995). The Neighbor-Joining tree was constructed using DISPAN (Ota, 1993) and TreeView (Page 1996).

## RESULTS

The electrophoresis results, allele frequencies (the part of gene frequency is shown in Table 2) and the number of alleles are available from the corresponding author upon request. All loci were polymorphic and the number of alleles per locus ranged from 11 (SW951) to 42 (CGA) in

whole population, and the mean number of the alleles observed in 26 microsatellite loci was 20.2. The Fixation indices ( $F_{IT}$ ,  $F_{ST}$ ,  $F_{IS}$ ) in all populations, total population heterozygosity ( $H_T$ ) and sub-population heterozygosity ( $H_S$ ) were shown in Table 3. The total heterozygosity per locus ranged from 0.4892 (SW951) to 0.9308 (CGA) and fixation indices of  $F_{ST}$  per locus varied from 0.0327 (SW951) to 0.3725 (CGA). The average  $F_{ST}$  of all loci is 0.1626, which means most of the genetic variation generated within breeds and only a few existed among populations (Table 3). Table 3 also gives other Fixation indices ( $F_{IT}$ ,  $F_{IS}$ ) and sub-population heterozygosity ( $H_S$ ). The mean of unbiased expected heterozygosity ( $H$ ) and polymorphism information content (PIC) of all breeds were showed in Table 4. The mean heterozygosity ( $H$ ) of all breeds varied from 0.8114 (Dongshan, DS) to 0.8803 (Donglan, DL). The mean PIC of Chinese indigenous pig breeds was prominent higher than exotic pig breeds. The change trends of the mean  $H$  of all breeds were identical with that of the mean PIC of all pig breeds.

The Neighbor-Joining (NJ) tree was constructed based on Nei's standard genetic distances (Table 5). The clustering result is shown in Figure 1. Three exotic breeds used as out-groups, are grouped into a branch and the Chinese indigenous pig breeds into another branch. Among all the Chinese indigenous pig breeds, the two pig breeds (LT and DH) in Guangdong province are firstly clustered with the two pig breeds (HA and PT) in Fujian province, they have formed the first branch. The second branch is the cluster of the four pig breeds (LL, DS, LC and DL) distributed in Guangxi province. And then, the first branch and the second one form a large branch. However, the only exception is that the two pig breeds (LA and YM) in Shandong province are not firstly clustered together.

## DISCUSSIONS

### Genetic variation within breed

The genetic variation within breed was estimated by the

**Table 2.** The gene frequency of 10 Chinese indigenous pig breeds and 4 exotic pig breeds

	CGA	IGF <sub>1</sub>	S0026	S0068	S0090	S0128	S0355	S0386	SW951	Total	Mean
LA	0.92562	0.86609	0.88592	0.91687	0.85494	0.88747	0.86675	0.84411	0.77752	22.13549	0.8514
LT	0.91084	0.83094	0.86382	0.89154	0.89343	0.88743	0.79464	0.86884	0.68635	21.65629	0.8329
LC	0.91698	0.84408	0.8513	0.92385	0.87829	0.83602	0.71412	0.90778	0.70112	21.07012	0.8104
HA	0.94805	0.82546	0.83133	0.93402	0.88258	0.8207	0.89903	0.86938	0.80196	21.68187	0.8339
PT	0.93767	0.69358	0.72433	0.92079	0.86889	0.89464	0.88621	0.83716	1.57E-02	20.83098	0.8012
DH	0.86979	0.75289	0.78004	0.92431	0.87979	0.87294	0.9223	0.88164	0.43408	21.20617	0.8156
LL	0.94989	0.87817	0.84477	0.91002	0.8553	0.85545	0.90912	0.8785	0.56727	21.37457	0.8221
DL	0.90546	0.85766	0.73771	0.92027	0.86531	0.92088	0.87346	0.86226	0.65779	21.79122	0.8381
DS	0.93569	0.8617	0.85703	0.91141	0.84956	0.84886	0.87	0.89774	0.62197	22.24033	0.8554
LW	0.91635	0.8943	0.819	0.7838	0.88919	0.89903	0.90024	0.86916	5.12E-02	20.51014	0.7889
LD	0.89267	0.35149	0.75428	4.46E-02	0.15433	0	0.13353	5.74E-02	0.16297	11.48947	0.4419
DR	0.91886	0.75817	0.53895	0.28949	0	3.52E-02	3.47E-02	6.74E-02	0.29693	12.52577	0.4818
Total	0.53538	0.5855	0.575	0.87861	0.62248	0.46226	0.10684	5.62E-01	0.4844	15.57528	0.599
Mean	0.8893	0.7285	0.836	0.7613	0.7807	0.6537	0.8096	6.39E-01	0.7981	0	0

**Table 3.** The Fixation indices (Fit, Fst, Fis), heterozygosity of total population ( $H_T$ ) and subpopulation ( $H_S$ )

Loci	Fis	Fst	Fit	$H_T$	$H_S$
CGA	0.4229	0.0327	0.4417	0.9308	0.5372
IGF1	0.4465	0.1711	0.5412	0.7438	0.4117
S0002	0.0978	0.0802	0.1702	0.8846	0.7981
S0005	0.3315	0.0890	0.3910	0.8551	0.5716
S0026	0.2521	0.0829	0.3141	0.8052	0.6022
S0068	0.2885	0.1817	0.4177	0.7827	0.5569
S0090	0.6944	0.2173	0.7608	0.6900	0.2109
S0155	0.2536	0.1076	0.3339	0.8092	0.6040
S0178	0.2297	0.1418	0.3389	0.7674	0.5911
S0215	-0.0113	0.2577	0.2493	0.6751	0.6827
S0218	-0.0004	0.2437	0.2434	0.6913	0.6916
S0225	-0.0274	0.1732	0.1506	0.7397	0.7600
S0226	0.2916	0.1689	0.4112	0.7669	0.5433
S0227	0.4546	0.2223	0.5759	0.7117	0.3881
S0228	0.0992	0.1157	0.2034	0.8298	0.7475
S0355	0.1866	0.2614	0.3992	0.6806	0.5536
S0386	0.3568	0.2535	0.5198	0.6856	0.4410
SW122	0.4111	0.2025	0.5303	0.7037	0.4144
SW24	0.2000	0.1497	0.3197	0.7877	0.6302
SW240	0.2984	0.1519	0.4050	0.7565	0.5308
SW632	0.0880	0.0701	0.1520	0.8638	0.7878
SW72	0.4051	0.0974	0.4631	0.8161	0.4855
SW857	0.3297	0.1264	0.4144	0.7929	0.5315
SW911	0.3426	0.2038	0.4766	0.6601	0.4339
SW936	0.0750	0.0937	0.1617	0.8155	0.7544
SW951	0.3639	0.3725	0.6008	0.4892	0.3112
Mean	0.2612	0.1626	0.3813	0.7590	0.5604

number of alleles, their frequencies, H, PIC and fixation indices (Fit, Fst, Fis). The results illustrated that all loci were deviated from Hardy-Weinberg equilibrium in at least 1 population ( $p < 0.05$ ). Seven loci (IGF1, S0090, S0227, S0386, SW122, SW911, SW951) were disequilibria in all

populations. The number of the loci deviated from equilibrium ranged from 4 to 11 in each population. This might be caused by population subdivision owing to genetic drift, the effect of a bottleneck through the reproductive isolation of rare populations or sample collection (Li et al., 2002).

The average  $F_{ST}$  of all loci was 0.1626, it means 83.7% of the genetic variation is within breeds, only 16.3% of the genetic variation exists between populations and this might be resulted from the natural and artificial selection, inbreeding cross, and mutation.

**Genetic variation among breeds**

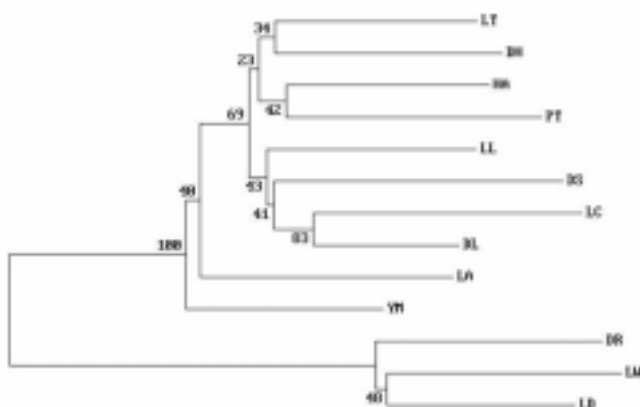
NJ tree derived from Nei's (1978) standard genetic distance was used for describing Genetic relationship among the populations. The cluster result was consistent with their geographical distribution, but was not completely consistent with their classification in the book of *Pig Breeds in China*. DH of the Central China Type in Guangdong province and LT of the South China Type in Guangdong province did not belong to the same type as classified by *The Pig Breeds in China* were firstly clustered together and then they clustered with HA and PT of the Central China Type in Fujian province. The DL, DS, LC and LL of South China Type in Guangxi province were clustered together. This research also showed that the genetic distances among Chinese indigenous breeds were quite close, which may indicate that Chinese indigenous breeds had a common ancestor. But according to the classification in *The Pig Breeds in China*, Chinese indigenous pig breeds had several originators. The two different conclusions implied that there would be further related research to do for understanding the real genetic relationship of Chinese indigenous pig breeds.

**Table 4.** The mean heterozygosity (H) and polymorphism information content (PIC) of 13 breeds in 26 microsatellite loci

	YM	LA	LT	LC	HA	PT	DH	LL	DL	DS	LW	LD	DR
H	0.8717	0.8601	0.8297	0.8499	0.8222	0.8401	0.8511	0.8606	0.8803	0.8114	0.4605	0.5187	0.5012
PIC	0.8602	0.8294	0.8205	0.8309	0.8101	0.8210	0.8221	0.8381	0.8554	0.7889	0.4419	0.4909	0.4605

**Table 5.** Nei's genetic distance (da, 1978) of 13 pig breeds

	YM	LA	LT	LC	HA	PT	DH	LL	DL	DS	LW	LD
LA	0.2648											
LT	0.2793	0.2688										
LC	0.3374	0.3406	0.2871									
HA	0.2612	0.3010	0.2560	0.3350								
PT	0.2784	0.3400	0.3000	0.4039	0.2573							
DH	0.2987	0.3353	0.2409	0.3294	0.2701	0.2675						
LL	0.2704	0.3096	0.2652	0.3008	0.2598	0.2814	0.2641					
DL	0.2761	0.2892	0.2474	0.2309	0.2487	0.2909	0.2584	0.2118				
DS	0.3464	0.3346	0.3082	0.3375	0.3022	0.3324	0.3127	0.2856	0.2619			
LW	0.5568	0.5833	0.6101	0.6463	0.6428	0.6309	0.6376	0.5828	0.6015	0.6510		
LD	0.5554	0.5572	0.5620	0.6135	0.5871	0.6203	0.6247	0.5575	0.5759	0.6196	0.2358	
DR	0.5257	0.5797	0.5951	0.6388	0.6065	0.6229	0.6427	0.5845	0.6135	0.6246	0.2568	0.2446



**Figure 1.** The Neighbor-Joining dendrogram of 13 breeds based on Nei's genetic distances.

### ACKNOWLEDGEMENT

This project was supported by the Center of Preservation and Utilization of Germplasm Resource of Animal Husbandry and Forage Grass, National Animal Husbandry and Veterinary Service (NAVS), Ministry of Agriculture, P. R. China and the National Outstanding Youth Science Foundation of China (39925027). This Project also belongs to the EU-China collaboration project (QLRT-2001-01059).

We thank Dr Denis Milan (INRA, France) for providing the control animal DNA, Prof. S. J. Guo, G. F. Xu, J. H. Zhang (NAVS, China), H. H. Cao (Chinese Academy of Agricultural Sciences, China) and Y. Zhang (China Agricultural University, China) for their help and useful suggestions.

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