Genetic Diversity of 10 Indigenous Pig Breeds in China by Using Microsatellite Markers

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ABSTRACT : The genetic diversities of 10 Chinese pig populations were analyzed by using microsatellite DNA polymorphisms. The results showed that the mean heterozygosities of the 10 populations were between 0.4561 and 0.6446, the mean polymorphism information contents were 0.4241-0.6184 and the mean effective number of alleles were 2.4295-3.7573. These indicated that the genetic diversity of local Chinese pigs was high. The clustering of the 10 populations was nearly inaccordance with their geographical distributions. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1219-1222)***

Key Words : Microsatellite DNA, Genetic Diversity, Chinese Indigenous Pig

INTRODUCTION

China has many indigenous pig breeds. In recent years, more and more introduced breeds were used to cross with local Chinese pigs, so how to conserve them has become of increasing international concern. The genetic diversity of local Chinese pigs had been evaluated by cytogenetic and biochemical genetic means (Nie et al., 1995; Huang et al., 1998; Li et al., 2001). With the development of molecular biology, many technologies based on DNA amplification by PCR have been introduced. Microsatellite DNA has high polymorphism, and it is widely used in assessing population relationships, analyzing inbreeding in populations and for examining the genetic diversity of populations because of its abundant distribution in the genome and high levels of polymorphisms. Fan et al. (1999), Li et al. (2000a) and Li et al. (2000b) had reported on microsatellite variations in some indigenous Chinese pig breeds. However, other breeds in China have not yet been studied by using microsatellite markers. The purpose of this study is to find the relationships among 10 Chinese pig breeds and to assess the intra- and inter-population variations by using 10 microsatellite loci. The results would be useful for the protection and conservation of pig genetic resources in China.

MATERIALS AND METHODS

Materials

536 individual blood samples were collected from 10 pig breeds (see Table 1).

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Methods

DNA was extracted as described by Sambrock et al. (1992). The 10 microsatellite primer pairs used were synthesized by the Beijing SBS biotechnology Co.. The primer sequences and PCR annealing temperatures were as described by Genebank and Roher et al. (1996), and shown in Table 2. The PCR amplification reaction system consisted of genomic DNA 50 ng, dNTP 200 μM, primers 10 pmol, 250 μM $MgCl_2$, 1 U Taq DNA polymerase. The PCR products were analyzed on an 8% polyacrylamide denaturing sequencing gel which was stained in 0.1% $AgNO_3$ solution. The PCR product size was calculated according to the PBR322 DNA/mspI marker on the computer (see Figures 1 and 2).

Statistical analysis

The mean heterozygosity, polymorphism information content, effective number of alleles and the number of alleles at each locus in each population were calculated. The genetic distances were abtained by using the PHYLIP software, and the phylogenetic tree was constructed by using NJ from Nei's standard genetic distance (Felsenstein, 1995).

RESULTS AND DISCUSSION

It could be seen from Table 3 that for the 10 Chinese pig breeds we studied, the mean effective number of alleles observed per locus ranged from 2.4295 to 3.5930, the mean polymorphism information contents from 0.4241 to 0.6184, and the mean heterozygosities from 0.4561 to 0.6446. The mean number of alleles of each breed were between 4.1 and 7.2. These results showed that the diversity of indigenous Chinese pig breeds was high. The results were as the same as those reported by Li et al. (2000a) and Li et al. (2000b). Wuzhishan had the highest intra-breed variation, while

Table 1. The name, sources and sample numbers of the 10 breeds

Breed	Source	Sample number
Wuzhishan miniature pig	Haikou Wuzhishan pig breeding farm of Hainan province	60
Diannan xiaoer pig	Xishuang banna state pig breeding farm	59
Guizhou miniature pig	Guizhou animal and poultry excellent breeds farm	60
Guizhou miniature pig (inbreeding)	The third army medical university	46
Bama Xiang pig (inbreeding)	The third army medical university	46
Rongchang pig	Sichuan pig institution and Chongqing breeding farm	58
Yimeng black pig	Linyi pig breeding farm of Shandong province	60
Hanzhong black pig	Heihe pig breeding farm of Shannxi province	60
Erhualian pig	Changshu animal and poultry breeds farm of Jiangsu	49
Jinhua pig	Jinhua pig breeding farm of Zhejiang province	38

Table 2. The name, sequence structure, annealing temperature and loading in chromosomes of the 10 primers

Primer name	Forward 5'-3'	Reverse 5'-3'	PCR temp.	Chrom
IGF-1	GCTTGGATGGACCATGTTG	CATATTTTTCTGCATAACTTGAACCT	62	5
S0003	GAAGTGTTAAGGAAAGCCTT	AGCCTCAGTTTCTCTACCTA	60	6
S0005	TCTTCCCTCCTGGTAACTA	GCACTTCCTGATTCTGGGTA	60	5
S0008	GAGGCAGTGTGTTCTATTCA	GCCATGTGTAAAGTGTTGCT	58	1
S0010	TTAACATGGCTGTCTGGACC	GTCCCTGTCCAACCATAAGA	60	2
SW769	GGTATGACCAAAAGTCCTGGG	TCTGCTATGTGGGAAGAATGC	60	13
SW781	CAACTACGTCCTTCTTTTTGCC	GATCCTTGGTCTGGAAACTTG	62	1
SW790	CTGTGGGAGTGTAGCATCTTTG	CATACACCCCAGATGTGG	62	8
SW995	TTAAGCACTTCATGGAGCTTTG	CATAATGGAAATACCGGGTCC	58	5
SW1032	ATTGGGTGGACTGATATGGT	GATCTATAAAGTGTAAATTGTGTGTG	58	14

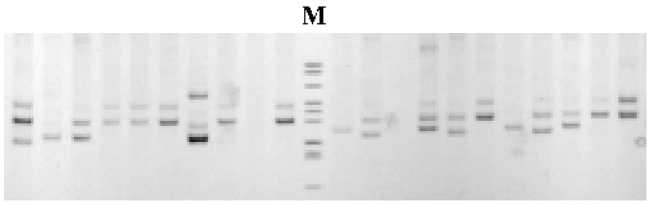

Figure 1. Phenotypes of the animals for the Sw1032 locus. M: molecular weight marker of PBR322DNA/mspI.

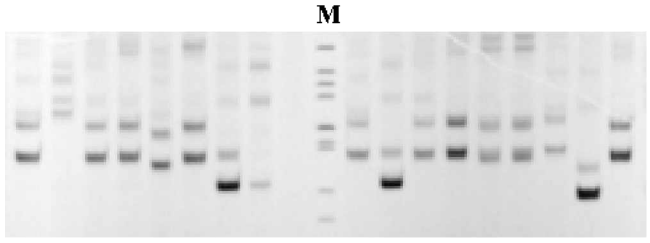

Figure 2. Phenotypes of the animals for the Sw790 locus. M: molecular weight marker of PBR322DNA/mspI.

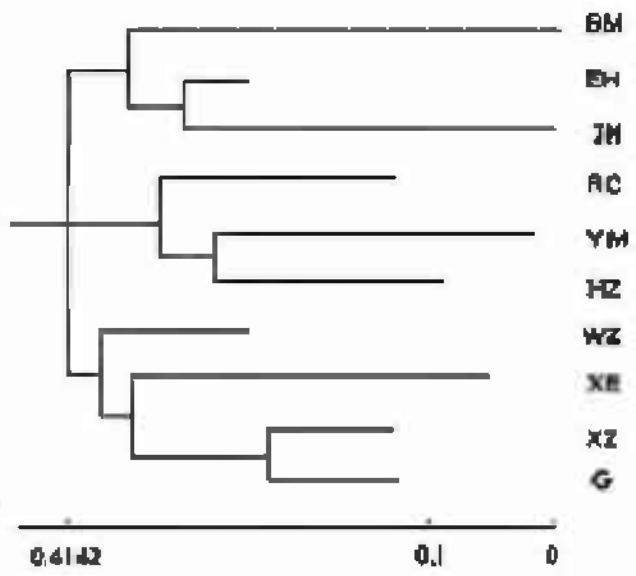

Figure 3. Dendrogram showing the genetic relationships among 10 indigenous pig breeds.

Jinhua had the lowest. Studies using other genetic markers had also showed that the variation in Jinhua was low (Zhang et al., 1998; Sun et al., 2001). The levels of variation we found in Chinese pigs were comparable to these reported by of Arranz et al. (1996) and Barker et al. (1997) in cattle and buffalo respectively.

Nei's standard genetic distance and Cavalli-Sforza and Edwards distance are shown in Table 4. Above the diagonal is Nei's distance, and below the diagonal is that of Cavalli-Sforza and Edwards'. NJ clustering result is shown in Figure 3. The clustering result is consistent with the populations' geographical distributions except for the Bama Xiang pig. The 10 indigenous pig breeds are grouped into 3 clusters. The Wuzhishan miniature pig, Diannan xiaoer pig, Guizhou miniature pig and its inbreeding population are in a group. The Yimeng black pig, Hanzhong black pig and Rongchang pig are in another group, while the Erhualian, Jinhua and the inbreeding population of Bama xiang pig are in the third group. This result is accordance with the results

Table 3. The genetic variation of the 10 local pig breeds for 10 microsatellite loci

Breed	Mean heterozygosity	Mean number of alleles of each breed	Mean effective number of alleles	Mean polymorphism information content
WZ	0.6446	7.2	3.5930	0.6184
XE	0.5950	5.6	2.9139	0.5462
XZ	0.5941	4.0	2.9734	0.5395
G	0.6349	5.5	3.4164	0.5950
BM	0.5897	4.5	3.3552	0.5088
RC	0.6309	7.3	3.7573	0.6004
YM	0.5573	5.6	3.3589	0.5289
HZ	0.5470	5.8	2.8716	0.5161
EH	0.5798	5.8	3.6570	0.5670
JH	0.4561	4.1	2.4295	0.4241

WZ stands for Wuzhishan miniature pig; XE: Diannan xiaoer pig; XZ: Guizhou miniature pig; G: the inbreeding population of Guizhou miniature pig; BM: Bama Xiang pig; RC: Rongchang pig; YM: Yimeng black pig; HZ: Hanzhong black pig; EH: Erhualian pig, JH: Jinhua pig. The following is the same to that.

Table 4. The genetic distance of 10 indigenous pig breeds based on 10 microsatellite loci

Breed	WZ	XE	XZ	G	BM	RC	YM	HZ	EH	JH
WZ	0.0000	0.4172	0.3612	0.4148	0.5795	0.4893	0.3207	0.4612	0.3080	0.5224
XE	0.1026	0.0000	0.5293	0.4855	0.6749	0.6913	0.8223	0.6836	0.4817	0.7554
XZ	0.0979	0.1243	0.0000	0.1974	0.8085	0.4531	0.5299	0.7009	0.4042	0.6846
G	0.1180	0.1284	0.0710	0.0000	0.6569	0.4619	0.6949	0.6857	0.3039	0.6330
BM	0.1315	0.1573	0.1584	0.1462	0.0000	0.6449	0.9221	0.7164	0.4705	0.7028
RC	0.1072	0.1432	0.0988	0.1040	0.1372	0.0000	0.5623	0.3812	0.3860	0.7411
YM	0.0937	0.1359	0.1039	0.1353	0.1594	0.1207	0.0000	0.4546	0.5189	0.7300
HZ	0.1195	0.1398	0.1326	0.1421	0.1472	0.1125	0.1068	0.0000	0.4342	0.6005
EH	0.0954	0.1279	0.0983	0.0964	0.1286	0.0957	0.1034	0.1017	0.0000	0.3441
JH	0.1312	0.1637	0.1265	0.1410	0.1424	0.1338	0.1419	0.1374	0.1031	0.0000

obtained by using blood protein markers and mtDNA diversity (Huang et al., 1998; Geng, 2001; Mo et al., 2003). The clustering of the Bama Xiang pig is not consistent with its geographical distribution, perhaps because of its inbred mode of reproduction.

CONCLUSIONS

The mean heterozygosities of the 10 indigenous pig breeds were between 0.4561 and 0.6446; the mean polymorphism contents were 0.4241-0.6184; and the mean effective number of alleles 2.4295-3.7573. These results indicated that the genetic diversity in local Chinese pigs was high. The clustering of the 10 populations was nearly in accordance with their geographical distributions.

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