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Genetic diversity in the mitochondrial DNA D-loop region of global swine (*Sus scrofa*) populations

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ABSTRACT

Increased global use of highly productive commercial breeds has reduced genetic diversity in indigenous breeds. It is necessary to protect local porcine breeds. We therefore assessed the level of genetic diversity in global swine populations. In this study, the mitochondrial DNA D-loop region was examined in 1010 sequences from indigenous pigs and commercial swine as well as 3424 publicly available sequences. We identified 334 haplotypes and 136 polymorphic sites. Genetic diversity was analyzed based on basic parameters, including haplotype diversity, nucleotide diversity and the average number of nucleotide differences, and also assessed by principal component analysis. A comparison of nucleotide diversity and the average number of nucleotide differences between indigenous breeds and commercial breeds showed that indigenous pigs had a lower level of diversity than commercial breeds. The principle component analysis result also showed the genetic diversity of the indigenous breeds was lower than that of commercial breeds. Collectively, our results reveal the Southeast Asian porcine population exhibited the higher nucleotide diversity, whereas Chinese population appeared consistently lower level in Asia. European, American and Oceanian pigs had a relatively higher degree of genetic diversity compared with that of Asian pigs. In conclusion, our findings indicated that the introgression of commercial into indigenous breeds decreased indigenous breeds' genetic diversity.

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1. Introduction

Animal production has significantly increased during the last couple of decades. However, the downside is that increased global use of highly productive breeds has been coupled to loss of genetic diversity in most species of farm animals [1]. Indigenous breeds are often replaced by globally used highly productive breeds [2]. Indigenous pig populations are an important genetic resource worldwide. The development and distribution of the populations is shaped by environmental factors, resulting from extremes of climate and geography, leaving many populations with special and unique characteristics. Unfortunately, these populations are under increasing pressure from integration or replacement with commercial breeds with fast growth rates and a very high lean content. In fact, many of the indigenous pig breeds are at risk for extinction [3]. The genetic diversity of the commercial breeds may be quite

small, because a small number of sires are selected to have a multitude of progeny [1]. The decline in the genetic diversity of pig population is a serious problem. It is, therefore, important to study the genetic diversity of both indigenous and commercial breeds.

Porcine mtDNA is a 16 kb circular molecule. It contains a non-coding region, the displacement (D)-loop, which contains regulatory sequences controlling both replication and transcription of mtDNA [4,5]. Analysis of mtDNA sequence diversity has provided important insights into the origin and diversification of modern pig populations. Previous studies of porcine mtDNA have revealed that domestic pigs (*Sus scrofa domestica*) originated from wild boars (*Sus scrofa scrofa*) and the independent domestication of pigs in Asia and Europe [4,6–8]. Other studies have examined mtDNA diversity in European and Asian pigs [9–11]. In the present study, we examined sequence variation in mtDNA from global pig populations, specifically focusing on nucleotide polymorphisms in the D-loop region. We also assessed the relative impact of commercial pigs on indigenous pig populations. This study will acquire the information of the porcine genetic diversity worldwide. The findings will be helpful for conservation and sustainable use of these

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resources.

2. Materials and methods

2.1. Sampling and DNA extraction

A total of 11 breeds, including 1010 individuals distributed in Sichuan province, Tibetan highlands, Gansu province, Yunnan province, Shandong province and Qinghai Province of China, were collected (Information on collected samples provided in [Table S1](#)). Blood samples were collected from the jugular vein into ACD-containing vacuum tubes and preserved at -20°C . And 10 mL blood were collected from each pig. Ear tissues were collected into 1.5 mL microcentrifuge tubes containing 75% ethanol, and preserved at -80°C until DNA extraction. Animals were released immediately following treatment of the wounds with antiseptic.

DNA was extracted and purified using the phenol-chloroform extraction method [12].

2.2. PCR and sequencing

A fragment of the D-loop region was amplified using the primers: Forward strand 5'-CCAAAAACAAAGCAGAGTGTAC-3' and Reverse strand 5'-CGTTATGAGCTACCGTTATA-3'. PCR reactions were performed in a final volume of 60 μL with the following: 30 μL 2 \times *Eco Taq* PCR Supermix containing 1 U *Taq* polymerase, 500 mM dNTPs, and 10 \times *Taq* buffer (Beijing TransGen Biotech Co., Ltd, China), 0.2 μg template DNA, 0.5 μL 10 pmol/mL of each primer and 28 μL ddH₂O. PCR conditions included an initial denaturation at 94 $^{\circ}\text{C}$ for 5 min, followed by 34 cycles of 94 $^{\circ}\text{C}$ for 20 s, 58 $^{\circ}\text{C}$ for 60 s and 72 $^{\circ}\text{C}$ for 60 s, and a final extension for 10 min at 72 $^{\circ}\text{C}$. Amplified DNA fragments were purified following agarose gel electrophoresis and sequenced using the ABI 3130 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

2.3. Data analysis

A total of 4434 mtDNA D-Loop sequences were analyzed in this study. Sampling provided 1010 sequences, including 905 sequences from indigenous pigs and 105 sequences from commercial pigs. Considering the quantitative limitation of testing sequences, an additional 3424 sequences were downloaded from GenBank to provide a more comprehensive analysis of the genetic diversity of global pig populations. The downloaded sequences included 3070 sequences from indigenous pigs and 354 sequences from commercial pigs. Distribution, sampling size on all downloaded sequences is provided in [Table S2](#), [Fig. S1](#) and [Fig. S2](#). GenBank accession numbers and other detailed information of all sequences in this study are listed in [Table S3](#).

Original sequence data obtained using the 3130 ABI PRISM DNA sequencer. software. DNASTAR was used to align sequences, MEGA 5.0 was used to collect sequences [13]. ClustalX 1.81 was utilized to align D-Loop sequences [14]. All sequences were expected to be approximately 435 bp in length. Thus, sequences that were significantly shorter than 435 bp were excluded from further study. In addition, DnaSP 5.0 software was used to analyze the haplotypes of all sequences and genetic diversity [15]. Spss16.0 was used to conduct correlation analysis and principal component analysis (PCA).

Based on the distribution, source and characteristics of the global populations, 4434 individuals from 128 local breeds were divided into 80 population for statistical analysis to further illustrate the distribution of pig breeds worldwide and to examine genetic diversity.

3. Results

3.1. Single nucleotide polymorphism (SNP) analysis

We examined 1010 D-loop fragment sequences isolated from pigs, in addition to 3424 mtDNA sequences previously deposited in GenBank. A total of 435 bp were analyzed for single nucleotide polymorphisms (SNPs). No insertion/deletions (indels) were detected in our 1010 novel sequences, whereas the downloaded 3424 sequences had several indels compared with our novel sequences, and these indels were excluded for the alignment step and subsequent analyses. We identified a total of 136 polymorphic sites, representing 31.7% of the total sites analyzed, including 55 single variable sites and 81 parsimony informative sites. The four types of nucleotide mutations identified were transitions, transversions, insertions and deletions. Except for eight of the 136 variable sites representing insertions/deletions of single base pairs. In the remaining 128 variable sites, 89.59% were single nucleotide transitions and 10.41% were transversions. The transition:transversion ratio R (Ts/Tv) was 8.61:1, indicating a strong transitional bias that is common in mammalian mitochondrial evolution [16]. We detected that there were no nucleotide mutations from site 1 to 50, and represented that this was a relatively conservative region, and the variable region was found between sites 51 and 400. The highly variable region ranged from sites 30 to 330.

3.2. Haplotype analysis

In total, 334 haplotypes were identified from the 4434 samples according to the characters of variable sites distribution ([Table S4](#)). Distribution frequencies of haplotypes indicated no equilibrium. The highest frequency haplotype was Hap226 which was shared by 658 sequences. The lowest frequency haplotypes were 168 which harbored a sequence respectively. And other haplotypes 166 haplotypes were shared by two or more sequences. Hap8, Hap3, Hap78, Hap181, Hap24, Hap251, Hap268 and Hap226 were advantageous haplotypes which were present in more than 100 sequences. 47 haplotypes were identified in commercial pigs, and 287 haplotypes were found in indigenous pigs. The indigenous porcine population with the most haplotypes was in Indonesia, which shared sixty haplotypes. The population with a haplotype showed in Croatia, Denmark, Iceland, Iran, Macedonia, Mexico, Morocco, the Netherlands, Pakistan, Turkey and Shanxi Province in China.

3.3. Genetic diversity analysis

The basic parameters that were used to assess genetic diversity in the global pig population included haplotype diversity (Hd), nucleotide diversity (Pi) and average number of nucleotide differences (K; [Table 1](#)). Hd is a measure of the uniqueness of a particular haplotype in a given population (Masatoshi Nei), which reflects haplotype abundance in a population. Pi measures the degree of polymorphism within a population [17]. Pi and K represent the degree of haplotype mutation intrapopulation. Hd of the global pig populations was between 0.491 and 1.000; Pi was between 0.00127 and 0.02590; K ranged from 0.551 to 11.267. Hd of the commercial pigs were higher than that of indigenous pigs. Hd distributed in 0.674–0.856. Yorkshire had the highest Hd (0.856), while Hampshire had the lowest Hd. Hd of indigenous pigs had a highly variable range (0.491–1.000). Norway, Vanuatu, Mongolia, Japan and Chinese Hainan had the highest level of Hd (1.000), while the Chinese region Rkaze had the lowest Hd (0.420). Pi ranged from 0.00127 to 0.02590, and K from 11.267 to 0.551 in the global pig populations. Pi and K of the commercial pigs were higher than those of indigenous pigs. In commercial pigs, Large Black had the highest Pi(0.01579)

Table 1
Parameters for determination of genetic diversity of global pig populations.

Code	Breed/Population	Number	S	H	Hd	Pi	K
NOR	Norway native pig	2	3	2	1.000 ± 0.500	0.00688	3.000
VAN	Vanuatu native pig	2	2	1	1.000 ± 0.500	0.00688	3.000
MGL	Mongolia native pig	2	2	1	1.000 ± 0.500	0.00688	3.000
RUS	Russia native pig	3	2	2	0.667 ± 0.314	0.00307	1.333
HAI	Haiti native pig	4	12	2	0.667 ± 0.204	0.01839	8.000
FIN	Finland native pig	5	6	4	0.900 ± 0.161	0.00688	3.000
PNG	Papua New Guinea native pig	4	2	2	0.833 ± 0.222	0.00230	1.000
ARM	Armenia native pig	6	12	4	0.800 ± 0.172	0.01070	4.667
CAM	Cambodia native pig	6	8	4	0.800 ± 0.172	0.00858	3.733
CHI	Chile native pig	6	12	5	0.933 ± 0.122	0.01716	7.467
MAS	Malaysia native pig	6	22	5	0.933 ± 0.122	0.02590	11.267
USA	USA native pig	5	18	4	0.900 ± 0.161	0.01885	8.200
ROM	Romania native pig	7	5	4	0.857 ± 0.102	0.00568	2.476
FRA	France native pig	7	15	6	0.952 ± 0.096	0.01379	6.000
JAP	Japan native pig	6	7	6	1.000 ± 0.096	0.00628	2.733
IND	India native pig	8	10	6	0.929 ± 0.084	0.00952	4.143
VIE	Vietnam native pig	5	4	4	0.900 ± 0.161	0.00460	2.000
AUS	Australia native pig	41	24	16	0.854 ± 0.04	0.0146	6.278
BHU	Bhutan native pig	147	27	18	0.850 ± 0.014	0.01083	4.709
CUB	Cuba native pig	30	19	10	0.834 ± 0.045	0.00613	2.667
GER	Germany native pig	21	15	9	0.757 ± 0.088	0.01036	4.505
HUN	Hungary native pig	213	18	8	0.800 ± 0.012	0.00467	2.033
IPL	Iberian Peninsula native pig	288	26	20	0.792 ± 0.013	0.00385	1.666
INA	Indonesia native pig	149	60	59	0.953 ± 0.009	0.02164	9.412
ITA	Italy native pig	73	19	16	0.881 ± 0.025	0.00887	3.868
KOR	Korea native pig	43	22	12	0.760 ± 0.048	0.01542	6.707
LAO	Laos native pig	55	10	12	0.821 ± 0.037	0.00563	2.448
MYA	Myanmar native pig	16	15	14	0.983 ± 0.028	0.00826	3.592
NEP	Nepal native pig	40	24	12	0.819 ± 0.053	0.01187	5.164
NZL	New Zealand native pig	17	15	5	0.750 ± 0.069	0.01535	6.676
SRI	Sri Lanka native pig	24	25	10	0.841 ± 0.062	0.00941	4.094
THA	Thailand native pig	68	19	14	0.862 ± 0.025	0.00925	4.026
GBR	United Kingdom native pig	22	16	9	0.853 ± 0.052	0.0169	7.351
T1	Aba Tibetan pig	70	6	8	0.839 ± 0.018	0.00359	1.563
T2	Ganzi Tibetan pig	133	15	14	0.596 ± 0.044	0.00333	1.449
T3	Diqing Tibetan pig	178	12	16	0.717 ± 0.032	0.00479	1.458
T4	Linzhi Tibetan pig	241	12	14	0.538 ± 0.036	0.00198	0.86
T5	Shannan Tibetan pig	91	13	14	0.816 ± 0.029	0.00352	1.525
T6	Changdu Tibetan pig	90	16	17	0.883 ± 0.014	0.00431	1.876
T7	Rkaze pig	24	3	2	0.420 ± 0.110	0.00127	0.551
T8	Hezuo Tibetan pig	386	23	31	0.873 ± 0.00809	0.00468	2.036
T9	Qinghai Tibetan pig	106	11	10	0.725 ± 0.0013	0.00317	1.38
CHN-GX	Guangxi native pig	25	6	6	0.763 ± 0.054	0.00414	1.8
CHN-QH	Qinghai native pig	115	10	10	0.491 ± 0.055	0.00264	1.15
CHN-YWN	Yunnan westnorth native pig	64	12	14	0.818 ± 0.04	0.00391	1.701
CHN-YS	Yunnan south native pig	68	8	10	0.778 ± 0.032	0.00524	2.28
CHN-YE	Yunnan east native pig	128	13	18	0.733 ± 0.039	0.00295	1.285
CHN-SC	Sichuan native native pig	189	8	13	0.647 ± 0.031	0.00215	0.935
CHN-AH	Anhui native pig	33	6	7	0.727 ± 0.067	0.00316	1.375
CHN-ZJ	Zhejiang native pig	100	8	11	0.833 ± 0.018	0.00391	1.699
CHN-JS	Jiangsu native pig	71	20	15	0.871 ± 0.021	0.00684	2.975
CHN-SD	Shandong native pig	260	8	12	0.718 ± 0.024	0.00321	1.397
CHN-GZ	Guizhou native pig	90	14	16	0.861 ± 0.017	0.00421	1.833
CHN-GD	Guangdong native pig	45	5	6	0.687 ± 0.038	0.0036	1.568
CHN-JX	Jiangxi native pig	57	10	11	0.758 ± 0.050	0.0037	1.609
CHN-NE	Northeast native pig	37	15	8	0.697 ± 0.061	0.0048	2.09
CHN-FJ	Fujian native pig	15	7	7	0.867 ± 0.057	0.00473	2.057
CHN-HB	Hebei native pig	31	6	7	0.845 ± 0.028	0.00471	2.047
CHN-HUN	Hunan native pig	9	3	5	0.806 ± 0.12	0.00294	1.278
CHN-HN	Hainan native pig	2	1	2	1.000 ± 0.5000	0.0023	1
CHN-TW	Taiwan native pig	20	13	8	0.816 ± 0.071	0.00489	2.126
BER	Berkshire commercial pig	13	15	6	0.821 ± 0.082	0.01314	5.718
DUR	Duroc commercial pig	101	18	12	0.732 ± 0.033	0.00947	4.12
HAM	Hampshire commercial pig	20	15	5	0.574 ± 0.121	0.00724	3.147
LAN	Landrace commercial pig	108	18	18	0.853 ± 0.02	0.01567	6.815
LAB	Large Black commercial pig	6	13	3	0.733 ± 0.155	0.01579	6.867
PIE	Pietrain commercial pig	41	23	12	0.854 ± 0.037	0.00959	4.171
YOR	Yorkshire commercial pig	165	27	24	0.856 ± 0.018	0.01559	6.768
MIW	Middle White commercial pig	5	11	2	0.6 ± 0.175	0.01517	6.6

S: Number of polymorphic (segregating) sites.

Pi: Nucleotide diversity, Nei 1987, equations 10.5 or 10.6 (Masatoshi Nei).

K: Average number of nucleotide differences; Tajima 1983, equation A3 (Tajima).

Hd ± SD: Haplotype (gene) diversity and sampling variance, Nei 1987, equations 8.4 and 8.12 but replacing 2n by n. The standard deviation (or standard error) is the square root of the variance (Masatoshi Nei) [17].

Table 2
Correlation matrix between indexes.

Item	Hd	Pi	K
Hd	1	0.278	0.281
Pi	0.278	1	0.999
K	0.281	0.999	1

and K (6.867), while Hampshire had the lowest Pi(0.00724) and K (3.147). In indigeous pigs, Malaysia had the highest Pi (0.02590) and K (11.267). Sequences from pigs in Rkaze of China had the lowest Pi (0.00127) and K (0.551). The number of domestic pigs from Croatia,

Table 3
Scores and general PCA scores of different populations.

Code	Breed/Population	F ₁	F ₂	F _z
NOR	Norway native pig	0.259	1.604	0.646
VAN	Vanuatu native pig	0.259	1.604	0.646
MGL	Mongolia native pig	0.259	1.604	0.646
RUS	Russia native pig	-1.573	-0.669	-1.313
HAI	Haiti native pig	2.153	-1.967	0.969
FIN	Finland native pig	-0.012	0.825	0.228
PNG	Papua New Guinea native pig	-1.310	0.691	-0.735
ARM	Armenia native pig	0.647	-0.279	0.381
CAM	Cambodia native pig	0.128	-0.099	0.063
CHI	Chile native pig	2.575	0.212	1.896
MAS	Malaysia native pig	4.700	-0.528	3.197
USA	USA native pig	2.896	-0.188	2.010
ROM	Romania native pig	-0.421	0.591	-0.130
FRA	France native pig	1.807	0.646	1.473
JAP	Japan native pig	0.111	1.656	0.555
IND	India Native pig	0.707	0.828	0.742
VIE	Vietnam native pig	-0.569	1.018	-0.113
AUS	Australia native pig	1.718	-0.180	1.172
BHU	Bhutan native pig	0.810	0.101	0.606
CUB	Cuba native pig	-0.375	0.374	-0.160
GER	Germany native pig	0.444	-0.585	0.148
HUN	Hungary native pig	-0.822	0.232	-0.519
IPL	Iberian Peninsula	-1.046	0.240	-0.676
INA	Indonesia native pig	3.718	-0.011	2.646
ITA	Italy native pig	0.421	0.508	0.446
KOR	Korea native pig	1.682	-0.990	0.914
LAO	Laos native pig	-0.533	0.315	-0.289
MYA	Myanmar native pig	0.546	1.356	0.779
NEP	Nepal native pig	0.980	-0.229	0.632
NZL	New Zealand native pig	1.638	-1.062	0.862
SRI	Sri Lanka native pig	0.441	0.151	0.358
THA	Thailand native pig	0.460	0.328	0.422
GBR	United Kingdom native pig	2.294	-0.390	1.523
T1	Aba Tibetan pig	-0.979	0.628	-0.517
T2	Ganzi Tibetan pig	-1.701	-1.245	-1.570
T3	Diqing Tibetan pig	-1.193	-0.365	-0.955
T4	Linzhi Tibetan pig	-2.187	-1.583	-2.014
T5	Shannan Tibetan pig	-1.061	0.455	-0.625
T6	Changdu Tibetan pig	-0.685	0.910	-0.226
T7	Rkaze pig	-2.680	-2.443	-2.612
T8	Hezuo Tibetan pig	-0.622	0.801	-0.213
T9	Qinghai Tibetan pig	-1.390	-0.226	-1.056
CHN-GX	Guangxi native pig	-1.052	-0.011	-0.753
CHN-QH	Qinghai native pig	-2.154	-2.006	-2.111
CHN-YWN	Yunnan westnorth pig	-0.959	0.437	-0.557
CHN-YS	Yunnan South pig	-0.744	0.013	-0.526
CHN-YE	Yunnan east pig	-1.422	-0.145	-1.055
CHN-SC	Sichuan native pig	-1.850	-0.747	-1.533
CHN-AH	Anhui native pig	-1.388	-0.209	-1.049
CHN-ZJ	Zhejiang native pig	-0.919	0.554	-0.495
CHN-JS	Jiangsu native pig	-0.103	0.602	0.100
CHN-SD	Shandong native pig	-1.400	-0.284	-1.079
CHN-GZ	Guizhou native pig	-0.769	0.747	-0.333
CHN-GD	Guangdong native pig	-1.389	-0.558	-1.150
CHN-JX	Jiangxi native pig	-1.173	-0.013	-0.839
CHN-NE	Northeast native pig	-1.070	-0.582	-0.930
CHN-FJ	Fujian native pig	-0.627	0.750	-0.231
CHN-HB	Hebei native pig	-0.691	0.580	-0.326

Table 3 (continued)

Code	Breed/Population	F ₁	F ₂	F _z
CHN-HUN	Hunan native pig	-1.227	0.426	-0.752
CHN-HN	Hainan native pig	-0.857	1.993	-0.038
CHN-TW	Taiwan native pig	-0.726	0.339	-0.420
BER	Berkshire	1.294	-0.321	0.830
DUR	Duroc	0.160	-0.704	-0.088
DUR				
HAM	Hampshire	-0.811	-1.748	-1.080
LAN	Landrace	1.995	-0.286	1.340
LAB	Large Black	1.699	-1.232	0.857
PIE	Pietrain	0.520	0.237	0.438
YOR	Yorkshire	1.980	-0.254	1.338
MIW	Middle White	1.188	-2.217	0.210

Denmark, Iceland, Iran, Macedonia, Mexico, Morocco, Netherland, Pakistan, Turkey and the Chinese province of Shanxi sampled in this study was small, therefore, the genetic diversity in these regions was not examined.

Principal component analysis (PCA) is a statistical procedure to reduce the dimensionality of a data set by transforming to a new set of variables (the principal components) to summarize the features of the data [18]. In order to assess porcine diversity worldwide, we analyzed Hd, Pi and K using correlation analysis and PCA. Table 2 presents the correlation analysis results from indigenou pigs. All three parameters were positively correlated, and the correlation degree of Pi and K was 0.999. This reflects the degree of mtDNA diversity in the hypervariable segment 1 (HVS1) region.

Genetic diversity was analyzed by the PCA and the results were showed at Table 3. It shows the score of the porcine breeds/population defined by principal component factor scores based on a components matrix (Table 4) from Hd, Pi and K. We extracted two principal components (F1 and F2). F1 reflects the variation in Pi and K, and F2 shows variation in Hd. Based on the PCA pattern, we obtained an synthesized assessment score (Fz). The Fz score indicated higher genetic diversity in Malaysian native pig relative to other breeds worldwide and lower genetic diversity for Rkaze Tibetan pigs of China and most Chinese populations/breeds (Fig. 1).

3.4. Shared haplotypes between commercial and indigenou breeds

Haplotypes in 3975 individuals from 72 native breeds/populations and 459 individuals from eight commercial breeds were identified. Three hundred and fourteen haplotypes were identified in indigenou pigs and 47 haplotypes were identified in commercial pigs. Twenty-seven shared haplotypes were identified between indigenou and commercial pigs distributed among 2132 indigenou and 425 commercial pigs. Shared haplotype between indigenou and commercial pigs were be counted in our study. The ratio of the number of indigenou pigs with shared haplotypes and total of indigenou pigs (Sc/S) showed the degree of indigenou pigs affected by commercial pigs. The average percentage of Sc/S was 53.64% and ranged from 0 to 97.73% (Table 5). Our data showed that

Table 4
Component matrix.^a

	Component	
	1	2
Hd	0.480	0.877
Pi	0.976	-0.217
K	0.977	-0.215

^a 2 components extracted.

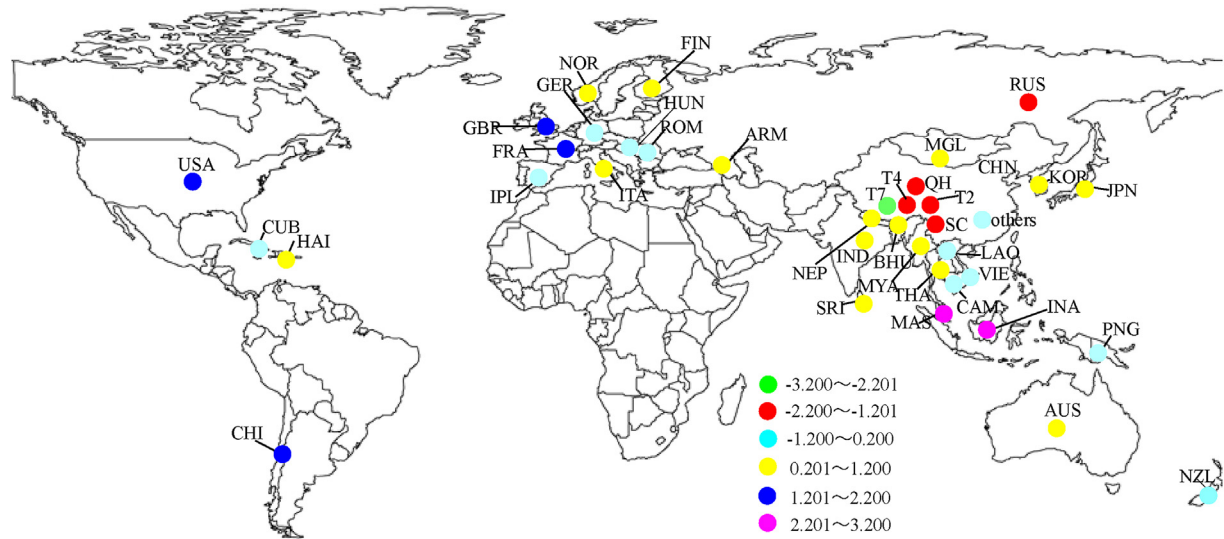


Fig. 1. Global distribution of genetic diversity as assessed by PCA(TIFF). Fz represented the degree of genetic diversity worldwide Based on the principle component analysis, which ranged from -3.200 – 3.200 . The figure showed the distribution and degree of genetic diversity by Fz value.

indigenous pigs were impacted by commercial pigs. If the number of sequences from a breed/population was less than five, Sc/S was not calculated due to the small sample size.

4. Discussion

4.1. Genetic diversity of the global pig population

The genetic diversity of global livestock populations is declining [2]. Our study examined this trend in global pig populations. We analyzed the mtDNA D-Loop for haplotypes in 4434 samples, and found 334 haplotypes, including 166 shared haplotypes found in all sequences. We also analyzed genetic diversity by basic parameters (Hd, Pi and K), and principal component analysis. Previous studies established that the level of genetic variation among Asian pigs was lower than that among European domestic pigs [7,11], and the results of this study are consistent with those findings. The global pig population is approximately one billion, two thirds of this population is found in Asia, with the majority found in China [2]. Genetic diversity is low in these pigs, due to commercial hybridization. Chinese indigenous pigs show some unfavorable traits such as slow growth, small body weight, low dressing percentage and high back fat thickness. To improve production, these populations are crossed with commercial pigs (Duro, Landrace and Yorkshire) [19]. Diversity of indigenous porcine populations is, therefore, impacted by commercial breeds. There are a considerable number of pigs in Vietnam and India [2], and most of them were indigenous pigs. Genetic diversity in these countries was high. Europe and the Caucasus have approximately one fifth of the global pig population, while America has an additional 15% [2]. Genetic diversity is relatively high in these populations. No analysis was done for African pig populations due to a lack of data. Based on principle component analysis, Fz ranged from -3.200 – 3.200 , indicating that European, American and Oceanian pigs had a similar level of diversity which was slightly higher than the Asian pig population. This is consistent with the analysis of the parameters Pi and K. The majority of populations/breeds focused in -1.200 – 1.200 , indicating a low level of genetic diversity in pig populations globally. In conclusion, the mtDNA diversity observed underscores the significance of the indigenous breeds/populations as an important genetic resource.

A summary comparison of Pi and K between indigenous breeds

Table 5

Analysis of native pigs haplotypes shared with commercial pigs.

Code	Breed/population	Sc	S	Sc/S (%)
FIN	Finland native pig	3	5	60.00
USA	USA native pig	3	5	60.00
ROM	Romania native pig	5	7	71.43
FRA	France native pig	4	7	57.14
JAP	Japan native pig	2	6	33.33
VIE	Vietnam native pig	2	5	40.00
AUS	Australia native pig	18	41	43.90
BHU	Bhutan native pig	58	147	39.46
CUB	Cuba native pig	18	30	60.00
GER	Germany native pig	18	21	85.71
HUN	Hungary native pig	89	213	41.78
IPL	Iberian Peninsula native pig	229	288	79.51
INA	Indonesia native pig	47	149	31.54
ITA	Italy native pig	40	73	54.79
KOR	Korea native pig	31	43	72.09
LAO	Laos native pig	35	56	62.50
MYA	Myanmar native pig	4	15	26.67
NEP	Nepal native pig	23	40	57.50
NZL	New Zealand native pig	16	17	94.12
SRI	Sri Lanka native pig	12	24	50.00
THA	Thailand native pig	34	68	50.00
GBR	United Kingdom native pig	19	22	86.36
T1	Aba Tibetan pig (T1)	46	70	65.71
T2	Ganzi Tibetan pig (T2)	23	133	17.29
T3	Diqing Tibetan pig (T3)	113	178	63.48
T4	Linzhi Tibetan pig (T4)	58	241	24.07
T5	Shannan Tibetan pig (T5)	43	91	47.25
T6	Changdu Tibetan pig (T6)	52	90	57.78
T7	Rkaze Tibetan pig (T7)	6	24	25.00
T8	Hezuo Tibetan pig (T8)	237	386	61.40
T9	Qinghai Tibetan pig (T9)	48	106	45.28
CHN-GX	Guangxi native pig	24	25	96.00
CHN-QH	Qinghai native pig	96	115	83.48
CHN-YWN	Yunnan westnorth pig	14	68	20.59
CHN-YS	Yunnan South pig	19	64	29.69
CHN-YE	Yunnan east pig	31	128	24.22
CHN-SC	Sichuan native pig	139	189	73.54
CHN-AH	Anhui native pig	26	33	78.79
CHN-ZJ	Zhejiang native pig	67	101	66.34
CHN-JS	Jiangsu native pig	28	68	41.18
CHN-SD	Shandong native pig	172	260	66.15
CHN-GZ	Guizhou native pig	13	45	28.89
CHN-GD	Guangdong native pig	43	44	97.73
CHN-JX	Jiangxi native pig	47	58	81.03
CHN-NE	Northeast native pig	6	37	16.22

Table 5 (continued)

Code	Breed/population	Sc	S	Sc/S (%)
CHN-FJ	Fujian native pig	13	15	86.67
CHN-HB	Hebei native pig	20	31	64.52
CHN-HUN	Hunan native pig	9	11	81.82
CHN-HN	Hainan native pig	2	2	—
CHN-TW	Taiwan native pig	10	20	50.00

Sc: Number of indigenous pigs sharing haplotypes with commercial pigs.
 S: Number of indigenous pigs.

[20,21]. Domestic pigs have a lower level of genetic diversity than wild boars as a general consequence of domestication [21] and indigenous pigs similarly have a lower level of genetic diversity [20,22,23]. Genetic diversity is essential for continued breeding. This is especially true in the situation where future breeding goals differ from current goals [1]. Porcine genetic diversity could be useful for sourcing future breeds for livestock production, and supplements biodiversity databases being accumulated on populations and breeds throughout the world [23].

and commercial breeds showed that indigenous pigs have a lower level of diversity than commercial breeds. Principle component analysis results also showed the indigenous breeds had lower diversity. Previous studies revealed a loss of porcine genetic diversity

4.2. Introgression between commercial breeds and indigenous breeds

Shared haplotypes were identified in 53.64% of indigenous pigs.

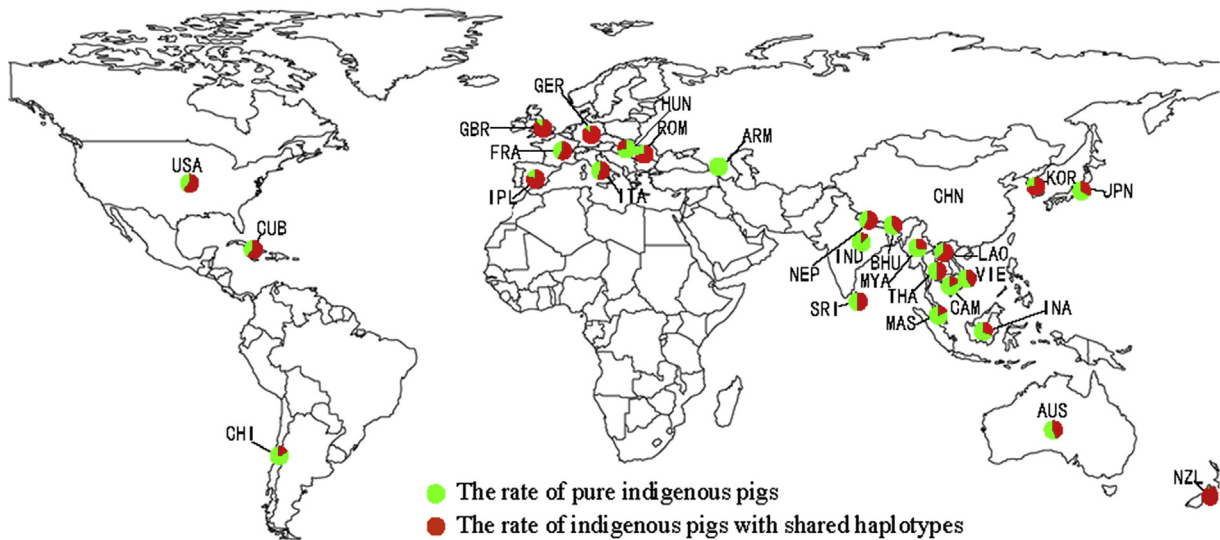


Fig. 2. Comparison of shared haplotype frequency between indigenous pig populations and commercial pigs (TIFF). Each pie chart showed the ratio of indigenous pigs shared haplotypes with commercial pigs and the ratio of pure indigenous pig. Figure showed that introgression of commercial pigs into indigenous in different regions.

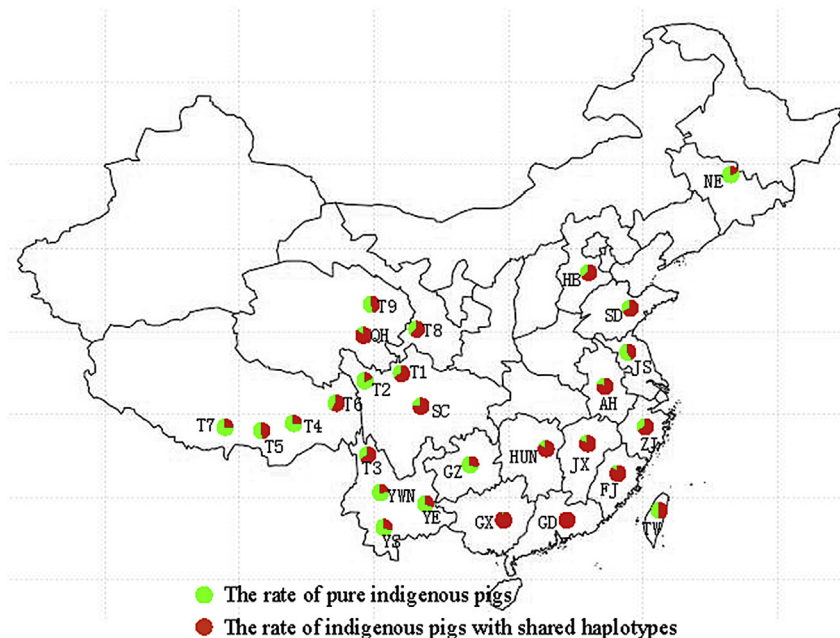


Fig. 3. Comparison of shared haplotype frequency between indigenous pig populations and commercial pigs in China (TIFF). The figure showed that introgression of commercial pigs into indigenous in different regions of China.

Twenty-seven haplotypes that were shared with commercial pigs were identified in 2132 indigenous pigs. The frequency of shared haplotypes between indigenous and commercial pigs showed an unequal global distribution (Fig. 2). Frequency of shared haplotypes was low in Southeast Asia, where the native pig population was seldom affected by commercial pigs. In contrast, there was a high frequency of shared haplotypes in China, especially in Southeast China (Fig. 3). European, American and Oceanian pig populations also had a relatively high shared haplotype frequency. The reason for a higher shared haplotypes frequency in these indigenous populations was the introgression of Asian pigs into Europe, primarily during the eighteenth and nineteenth centuries [6,9] and crossbreeding with commercial lines. Some reports, however, show that the frequency of Asian haplotypes is low or absent in Duroc and Hampshire lines. Landrace lines were less affected by Asian introgression than Large White lines and Pietrains [9]. In addition, current commercial lines were introduced and crossed with indigenous breeds, increasing the lineage of commercial lines.

The indigenous pig population has been recognized as an important genetic resource, despite their potentially economically unfavorable characteristics, such as slow growth, small body weight, and black coat, amongst other unfavorable traits. Previously, breeders improved indigenous porcine production by crossbreeding or breeding commercial lines directly [20,24]. In many regions, including Europe, the Caucasus, Africa and North America, relatively few indigenous pig populations exist. Conversely, there are many indigenous pig populations in Eastern Asia [2], although indigenous populations face the threat of extinction due to the introgression of commercial pigs. Increasing use of commercial lines threatens indigenous breeds and decreases genetic diversity. Recently, many countries have recognized the indigenous pig population as an important genetic resource due to specific traits (indigenous adaptation, strong adversity resistance, high meat quality and so on). The small number of the indigenous pigs could be used as founders to maintain the genetic characteristics of the indigenous population. The founder effect also results in a reduction in genetic variation, which may explain the low genetic diversity between indigenous pigs worldwide.

5. Author contributions

Conceived and designed the experiments: JXZ SGZ. Performed the experiments: JXZ SGZ TJ. Analyzed the data: JXZ SGZ. Contributed reagents/materials/analysis tools: JXZ SGZ TJ. Wrote the paper: JXZ SGZ.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at [http://](http://dx.doi.org/10.1016/j.bbrc.2016.03.125)

dx.doi.org/10.1016/j.bbrc.2016.03.125.

Transparency document

Transparency document related to this article can be found online at 10.1016/j.bbrc.2016.03.125.

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