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Evaluation of Genetic Diversity and Genetic Distance Between Twelve Chinese Indigenous Chicken Breeds Based on Microsatellite Markers

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Abstract: A total of 720 individuals of 12 indigenous chicken populations, geographically localized in Southern China were genotyped for 30 microsatellite markers in polymerase chain reaction (PCR) to evaluate the genetic variation and genetic distance between populations. All microsatellites were found to be polymorphic. Heterozygosity was calculated to determine the genetic variation. Of the 30 microsatellite loci, number of alleles per locus (Na) and effective number of alleles per locus (Ne) ranged from 4 to 11 and 2.157 to 8.019, respectively. The average expected heterozygosity (H_E) was 0.669, while the average observed heterozygosity (H_O) was 0.764. The polymorphism information content (PIC) has values between 0.560 and 0.641. Using Nei`s standard distance, genetic distance (D_A) calculated ranged between 0.088 (Guanxi Sanhuang *vs.* Nandan Yao) and 0.495 (Huiyang Beard *vs.* Zhangzhou Game). The topology of phylogenetic trees constructed showed general patterns of relationship and genetic differentiation among the indigenous populations studied, however, both trees from Neighbor-Joining method and Unweighted Pair Group method showed a similar topology. The results provided evidence of the applicability of microsatellite to determining the genetic relatedness among different Chinese indigenous chicken populations and evaluating of genetic variations.

Key words: Microsatellite, chicken breeds, genetic distance, genetic diversity

Introduction

During thousands years of domestication, the chicken has been considerably differentiated by natural and artificial selections (Romanov and Weigend, 2001). With its long history of animal husbandry and diversified geographical conditions, China has a wide variety of indigenous poultry resources, most of them are local and fancy breed populations (Ji *et al*., 2005). Such indigenous breeds may contain genes and alleles pertinent to the adaptation to particular environments and local breeding goals, and needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and a source of research materials (Romanov *et al*., 1996).

Mitochondrial DNA (Dong *et al*., 2002), random amplified polymorphic DNA (RAPD) (Smith *et al*., 1996 and Singh and Sharma, 2002), DNA fingerprinting (Siegel *et al*., 1992 and Ye *et al*., 1998) and microsatellites (Kaiser *et al*., 2000 and Takahashi *et al*., 1998) were widely used to study genetic variability among populations. Currently, microsatellites have been widely applied in the genetic appraisal, since they are abundant, randomly distributed in the genome, highly polymorphic, and show co-dominant inheritance (Groenen *et al*., 2000 and Crooijmans *et al*., 1993). Recent information in literature have revealed that microsatellite markers are more accurate and efficient method for estimating genetic variation than other methods that have been used previously (Chen *et al*., 2004b and Takezaki and Nei, 1996). However, only a limited number of investigations have used microsatellites across Chinese indigenous chicken populations in Southern China (Zhang *et al*., 2002).

The objective of this study was to evaluate the genetic variability and genetic divergence of twelve indigenous chicken breeds in Southern China, and construct phylogenetic tree to visualize the results.

Materials and Methods

Chicken populations: In total, 720 unrelated birds of 12 indigenous chicken populations were examined. Sixty birds per population (12 males and 48 females) were collected from private farms located at different areas in Southern China; sampling was in consistent to Barker (1994) for samples requirements for genetic diversity evaluation. Table 1 showed the description of the chicken populations.

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Table 1: Description of the 12 chicken populations

Population name	Abbreviation	Origin (Province) *	Specific features**
Hetian	HT	A	1
Zhangzhou Game	ZZG	A	2
Jinhu Silky	JHS	A	3
Fujian Silky	FJS	a	3
Huiyang Beard	HYB	B	1
Qingyuan Partridge	QYP	B	1
Xinghua	XH	B	1
Yangshan	YS	B	1
Huaixiang	HX	C	1
Xiayan	XY	C	1
Nandan Yao	NDY	C	1
Guangxi Sanhuang	GXS	c	1

*Origin: A = Fujian, B = Guangdong and C = Guangxi.
**Specific features: 1= broiler, 2 = game-purpose breed and 3 = dual-purpose breed

DNA Isolation: Blood samples of 1.5 ml were collected by brachial venipuncture aseptically into haemotocrit tubes using EDTA and heparin as anticoagulant. Blood samples were stored at -20°C. DNA was extracted from the whole blood using exactly the phenol/chloroform method previously described by Miller *et al.* (1988). DNA was quantified spectrophotometrically and the concentration was adjusted to 50ng/µl.

Thirty pair highly polymorphic microsatellite markers were chosen based on their genomic location (Table 2). The PCR products were obtained in a total volume of 25 µl reaction. The reaction contained 100 ng of genomic DNA, 5 pmol of each forward and reverse primers (Sangon Biotechnology Co, China), 2 mM $MgCl_2$, 5 mM dNTP, 1 unit Taq polymerase (Sangon Biotechnology Co, China). The amplification condition was 5 min initial denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 40 s, primer annealing at 52 to 64°C for 45 s (depending on locus), and extension at 72°C for 1 min., follow by final extension at 72°C (5 min.). Fluorescent end-labeled (fluorescent dye: FAM, TET, HEX) an external molecular size standard (Augct Biotechnology Co. Ltd, China) PCR primers were used, and size characterization of PCR product was performed by an ABI 310 DNA Genetic Analyzer (Applied Biosystem, Foster City, CA).

Statistical analysis: The microsatellites obtained were used to analyze the genetic variation between and within populations. Observed heterozygosity (H_o) and heterozygosity expected from Hardy - Weinberg Equilibrium (HWE) for each population were calculated (Levene, 1949). PopGene 1.31 and Cervus 2.0 computer packages were employed to analyze other genetic parameters including number of alleles (Na), effective allele number (Ne) and polymorphism information content (PIC). Wright's fixation index (F_{IS}), the measurement of heterozygote deficiency was calculated by Wright (1978).

An unweighted pair group method (UPGMA; Sneath and Sokal, 1973) and a neighbor-joining method (Saitou and Nei, 1987) with arithmetic mean based on genetic distance (D_A) were used to construct the phylogenetic trees using DISPAN program (Ota, 1993). Bootstrap re-sampling 1,000 was performed to test the percentage of a group's occurrence.

Results

Genetic variation: Mean observed heterozygosities (H_O), mean expected heterozygosities (H_E), mean polymorphic information content (PIC) and number of alleles in 12 populations were shown in Table 3. The Hardy-Weinberg Equilibrium (HWE) test showed that all loci were in HWE when analyzed across population.

The expected mean heterozygosity was lower than the observed mean heterozygosity for all populations. Average mean expected heterozygosities (H_E) within the 12 chicken populations across all 30 loci was 0.669, ranged between 0.703 and 0.651 for Huiyang Beard and Xiayan, respectively, while the observed average mean heterozygosities (H_O) was 0.767, Fujian Silky scored the highest (0.780) and Jinhu Silky was the lowest (0.747). Observed heterozygosities (H_T) and expected heterozygosities (H_S) for each locus were depicted in Table 4. H_T values were higher than H_S values for all loci except MCW330 ranged from 0.620 (MLW330) to 0.903 (ADL136) and 0.537(MCW174) to 0.877 (ADL136) for H_T and H_S, respectively. For the 30 microsatellite loci examined, the number of alleles was 238 across all populations, with an average of 7.933. The observed number of alleles per locus ranged from 4 (ADL201 and MCW150) to 11 (ADL136, ADL 166 and LEI0094).

Genetic distance: Nei's (1972) genetic identity and genetic distance of 12 indigenous chicken populations were listed in Table 5. The genetic distance ranged from 0.088 (between Nandan Yao and Guangxi Sanhuang) to 0.495 (between Zhangzhou Game and Huiyang Beared). Phylogenetic trees of the 12 chicken populations were constructed based on Nei's genetic distance D_A (Fig. 1). Both trees from Neighbor-Joining method and Unweighted Pair-Group methods showed a similar topology. Zhangzhou Game formed its own branch in both dendrograms.

Discussion

Genetic diversity: It is considered that, loci are highly polymorphic when PIC > 0.5 (Vanhala *et al.*, 1998), all loci studied were highly polymorphic, and with the highest value 0.877 (ADL136). The levels of genetic diversity estimated in this study were higher than other values reported for different chicken populations using microsatellite markers, Jossi *et al.* (2003) for some European chicken populations, and Zhang *et al.* (2002), Shen (2004) and Wu (2004) for Chinese chicken

Table 2: Characterization of the 30 microsatellite analyzed in 12 chicken populations

Locus	Chromosomal location	Primer sequence (5'-3')	TA (°C)	Reference
ADL136	9	F: TGTCAAGCCCATCGTATCAC R: CCACCTCCTTCTCCTGTTCA	59	e, d
ADL166	5	F: TGCCAGCCCGTAATCATAGG R: AAGCACCACGACCCAATCTA	55	e, d
ADL185	2	F: CATGGCAGCTGACTCCAGAT R: AGCGTTACCTGTTCGTTTGC	60	e, h
ADL195	1	F: AGATGGAAGACAGGACAAAT R: TAGCACAGACAATGTTATGC	52	d
ADL210	11	F: ACAGGAGGATAGTCACACAT R: GCCAAAAAGATGAATGAGTA	55	b, d
ADL212	2	F: TTTCAAAAGTGCCCTCACAC R: TTCCTCCCTAAACTATGCTG	52	d
ADL225	13	F: CCAAAAAGCTGTATCACCTT R:GCCTGTTGTAAACCACCTGA	59	b, d
MCW104	13	F: TAGCACAACTCAAGCTGTGAG R: AGACTTGCACAGCTGTGACC	64	c, f
MCW145	1	F: ACTTTATTCTCCAAATTTGGCT R: AAACACAATGGCAACGGAAAC	61	e, g
MCW150	3	F: TCCTGACTGAAATGGTACAGC R: CATGAAAACCTTTGCCCTCAG	61	d
MCW32	5	F: AAGTTCCTTGTACAATTGTTA R: TCATTACTAGTACAATCAAGATGG	56	g, d
MCW4	3	F: GGATTACAGCACCTGAAGCCACTA R: AAACCAGCCATGGGTGCAGATTGG	64	c, f
LEI0094	4	F: CAGGATGGCTGTTATGCTTCCA R: CACAGTGCAGAGTGGTGCGA	63	e, d
MCW0295	4	F: ATCACTACAGAACACCCTCTC R: TATGTATGCACGCAGATATCC	62	a, d
MCW0014	6	F: AAAATATTGGCTCTAGGAACTGTC R: ACCGGAAATGAAGGTAAGACTAGC	63	c, d
MCW0067	10	F: GCACTACTGTGTGCTGCAGTTT R: GAGATGTAGTTGCCACATTCCGAC	65	a, d
MCW0081	5	F: GTTGCTGAGAGCCTGGTGCAG R: CCTGTATGTGGAATTACTTCTC	63	a, d
MCW0183	7	F: ATCCCAGTGTCGAGTATCCGA R: TGAGATTTACTGGAGCCTGCC	64	a, d
MCW0294	Z	F: ACTGAACAGAAACAGTCTTCC R: CTTCTCTAGATGTCCACTACC	63	d
MCW0330	17	F: TGGACCTCATCAGTCTGACAG R: AATGTTCTCATAGAGTTCCTGC	63	a, d
ADL123	11	F: GCTGTGTCAAGATTAGAATCAC R: AACAATGAAAAACACTACCTGA	53	g, d
ADL201	Z	F: GCTGAGGATTCAGATAAGAC R: AATGGCTGACGTTTCACAGC	53	b, e
MCW120	7	F: CTATGTAAAGCTTGAATCTTCA R: ATTCCTGGGTGCTAATTTACC	57	e, f
MCW174	8	F: TGGACTTAACACTGCTATTGC R: CTCTCTACCTTGGAGGGCTGA	54	d
ADL176	2	F: TTGTGGATTCTGGTGGTAGC R: TTCTCCCGTAACACTCGTCA	52	h, d
LEI0166	3	F: AAGCAAGTGCTGGCTGTGCTC R: TCCTGCCCTTAGCTACGCAC	58	d
LEI0066	14	F: GATCAGATGCATCCAAAGTTC R: GAAGCAGGAAAATAGAAAAGGC	56	e, d
MCW0085	4	F: GTGCAGTTATATGAAGTCTCTC R: GGTATACAGGGCTTCTGAAACA	57	e, d
MCW0264	2	F: AGACTGAGTCACACTCGTAAG R: CTTACTTTTCACGACAGAAGC	56	f, d
MCW134	9	F: GGAGACTTCATTGTGTAGCAC R: ACCAAAAGACTGGAGGTCAAC	58	g, d

TA= Optimal annealing temperature

Reference: a = (Jossi *et al*., 2003), b = (Zhu *et al*., 2001), c = (Romanov *et al*., 1996), d = (Chen *et al*., 2004b), e = (Olowofeso *et al*., 2005), f = (Zhu and Li 2003), g = (Du *et al*., 2004), h = (Chen *et al*., 2003).

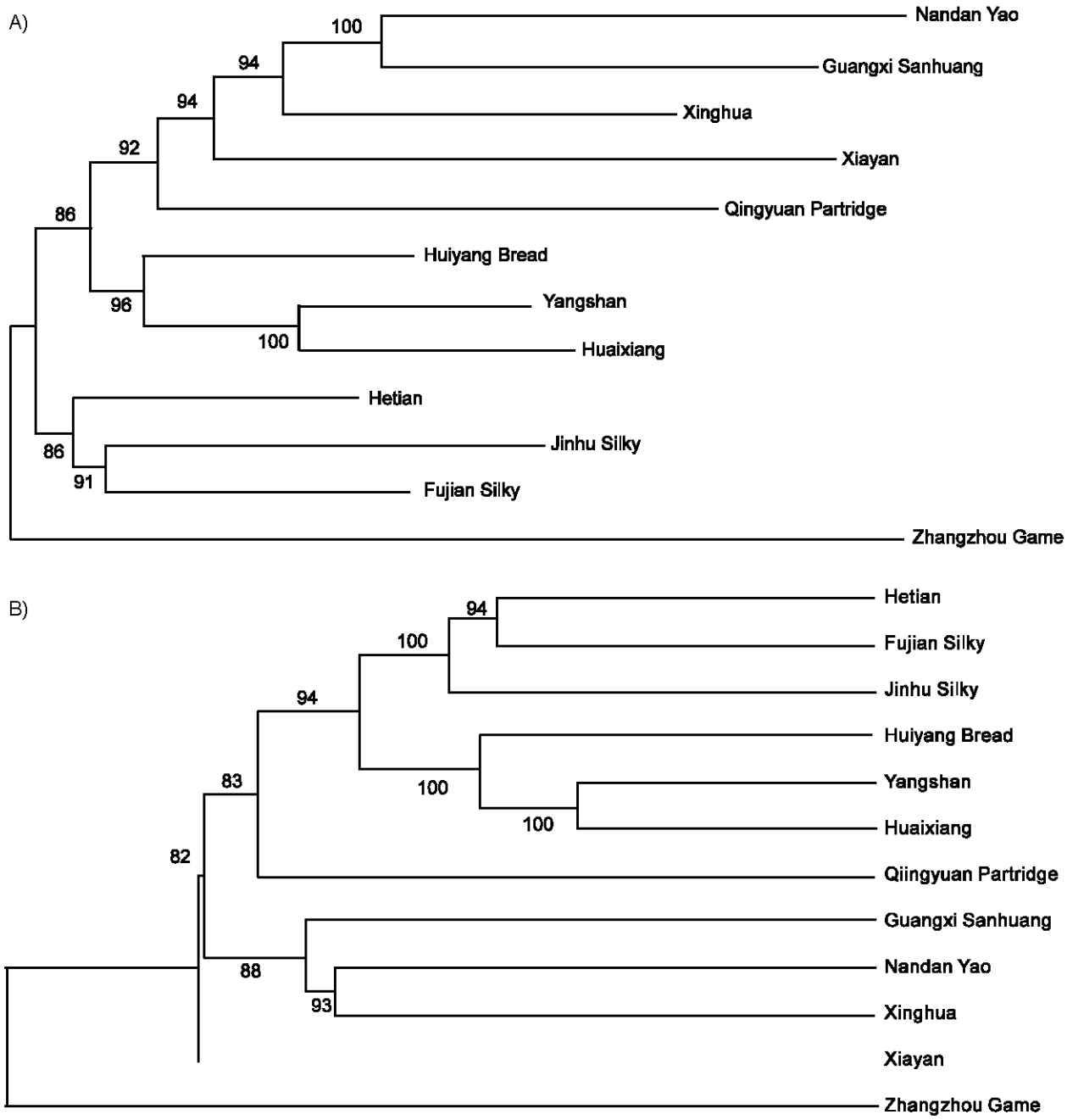

Fig 1: Topology trees showing the genetic relationship among 12 Chinese indigenous chicken populations using genetic distance for 30 microsatellite loci. The numbers at the nodes indicate the percentage of group's occurrence in a bootstrap resampling of 1000 times. A: Neighbor-Joining dendrogram; B: Unweighted Pair-Group method with arithmetic means dendrogram.

populations. However, the result was nearly similar to that reported by Romanov and Weigend (2001). The variation in results could be adduced to differences in location, sample sizes, experimental chicken and sources of the microsatellite markers used. Huiyang Beared has the highest genetic diversity (0.703) of all the 12 chicken populations, their large population size and the breed have not been subjected to intense selection (Chen *et al*., 2004a) could be the reason. The low genetic diversity in Zhangzhou Game (0.641) could be attributed to its improvement breeding history for some traits. Zhangzhou Game is a game-purpose breed in Fujian province with small population size; the breed was thought to be established for fighting, and low

Table 3: Observed (HO) and expected (HE) heterozygosities, number of alleles per locus and polymorphic information content (PIC) in 12 indigenous chicken populations

Population name	Abbreviation	H_O	H_E	PIC	No. of alleles/locus
Hetian	HT	0.778	0.696	0.632	5.400
Zhangzhou Game	ZZG	0.769	0.641	0.560	4.633
Jinhu Silky	JHS	0.747	0.649	0.570	4.800
Fujian Silky	FJS	0.780	0.665	0.589	4.667
Huiyang Beard	HYB	0.776	0.703	0.641	5.467
Qingyuan Partridge	QYP	0.767	0.685	0.618	5.167
Xinghua	XH	0.773	0.678	0.608	5.200
Yangshan	YS	0.765	0.654	0.579	4.667
Huaixiang	HX	0.757	0.656	0.579	4.967
Xiayan	XY	0.767	0.696	0.629	5.100
Nandan Yao	NDY	0.761	0.653	0.575	4.633
Guangxi Sanhuang	GXS	0.766	0.651	0.569	4.200
Mean		0.767	0.669	0.596	4.908

HO, HE and PIC are observed heterozygosity, expected heterozygosity and polymorphism information content, respectively.

Table 4: Genetic parameters in 12 Chinese chicken populations with 30 microsatellite loci

Locus	H_T	H_S*	Na**	Ne**	Fis
ADL136	0.903	0.877	11	8.079	-0.141
ADL166	0.898	0.843	11	6.331	-0.186
ADL185	0.900	0.764	9	4.225	-0.310
ADL195	0.876	0.837	9	6.104	-0.167
ADL210	0.900	0.683	7	3.152	-0.465
ADL212	0.900	0.610	10	2.562	-0.640
ADL225	0.981	0.790	9	4.756	-0.242
ADL123	0.899	0.670	9	3.035	-0.491
ADL201	0.859	0.549	4	2.216	-0.822
ADL176	0.895	0.809	10	5.229	-0.231
LEI0166	0.748	0.571	6	2.330	-0.752
LEI0066	0.897	0.687	6	3.187	-0.453
LEI0094	0.799	0.756	11	4.081	-0.323
MCW0295	0.896	0.801	9	5.005	-0.244
MCW0014	0.894	0.778	9	4.489	-0.278
MCW67	0.852	0.778	6	4.482	-0.287
MCW0081	0.795	0.691	6	3.232	-0.441
MCW183	0.792	0.743	9	3.877	-0.067
MCW294	0.656	0.623	7	2.651	-0.606
MCW330	0.620	0.791	10	4.772	0.216
MCW0085	0.667	0.577	6	2.360	-0.735
MCW264	0.882	0.742	6	3.8636	-0.349
MCW134	0.691	0.681	7	3.131	-0.469
MCW104	0.844	0.682	8	3.144	-0.237
MCW145	0.834	0.777	8	4.470	-0.288
MCW150	0.842	0.743	4	3.889	-0.346
MCW32	0.995	0.839	8	6.169	-0.188
MCW4	0.857	0.767	8	4.283	-0.119
MCW120	0.895	0.811	9	5.259	-0.229
MCW147	0.682	0.537	6	2.157	-0.831
Mean	0.838	0.727	7.933	4.084	-0.357

*Expected heterozygosity was computed using Levene (1949).

**Na = Observed number of alleles; Ne = Effective number of alleles (Kimura and Crow, 1964).

***Fis = Wright's fixation index as a measure of heterozygote deficiency or excess (Wright, 1978).

diversity resultant directly from intense selection for low fat deposition (Chen *et al*., 2004a). Genetic changes in fancy breeds may occur rather rapidly in these relatively small populations because of intense selection for exhibition traits, inbreeding, crossbreeding genetic drift, bottleneck and founder effects (Obata *et al*., 1994 and Ponsuksili *et al*., 1999).

In total, 238 alleles were found at 30 loci across the 12 chicken populations in this study. The average number of alleles found per locus, considering all populations, was 7.933 (the mean effective number of alleles was 4.084). Barker (1994) suggested that microsatellite loci used in studies of genetic distance should have more than four alleles in order to reduce the standard errors of distance estimates; thus, the microsatellite markers used in this study were suitable for genetic diversity analysis.

Genetic relationships: In this study, all populations were in hardy-weinberg equilibrium. The expected heterozygosity (H_E) is lower than that observed heterozygosity (H_O) for all populations. The level of genetic diversity obtained is slightly higher than values estimated in other Chinese chicken populations using microsatellites by Zhang *et al*. (2002) and Shen (2004). The source of chicken populations could be the main effect of these variations. At least 30 markers should be used for gaining reliable phylogeney according to Takezaki and Nei (1996) suggestions, The number of microsatellite markers used in this study and the divergence (D_A) varied from 0.088 to 0.495 (Table 5) were mostly explain the high reliability of the topology of the trees.

Both phylogenetic trees revealed that Zhangzhou Game formed one cluster, which may demonstrated a specific allele distribution as compared to other 11 chicken populations, in consistent with its breeding history selected for fighting (Chen *et al*., 2004a). As shown in Unweighted Pair-Group method tress (Fig. 1), Xiayan has its own branch, the possible reason could be due to intense selection the breed had been subjected for some production traits since 1974 (Chen *et al*., 2004a). In Neighbor-Joining trees, Hetian, Jinhu Silky and Fujian Silky in Fujian province were grouped together. The third cluster comprised the all populations of chicken sampled in Guangxi province and Guangdong province.

Table 5: Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal)

	HT	ZZ	JHS	FJS	HYB	QYP	XH	YS	HX	XY	NDY	GXS
HT		0.744	0.862	0.880	0.771	0.790	0.787	0.868	0.849	0.815	0.832	0.813
ZZ	0.296		0.729	0.759	0.610	0.657	0.654	0.750	0.698	0.689	0.708	0.768
JHS	0.148	0.316		0.856	0.716	0.758	0.733	0.833	0.803	0.760	0.799	0.806
FJS	0.128	0.276	0.155		0.792	0.834	0.832	0.851	0.786	0.784	0.862	0.831
HYB	0.260	0.495	0.335	0.233		0.765	0.800	0.761	0.766	0.756	0.765	0.774
QYP	0.236	0.420	0.277	0.182	0.268		0.832	0.813	0.733	0.843	0.754	0.724
XH	0.239	0.424	0.311	0.184	0.223	0.184		0.805	0.745	0.828	0.816	0.808
YS	0.141	0.287	0.182	0.162	0.273	0.207	0.217		0.816	0.848	0.898	0.871
HX	0.164	0.360	0.219	0.241	0.266	0.300	0.294	0.204		0.817	0.807	0.784
XY	0.205	0.372	0.275	0.244	0.280	0.171	0.188	0.165	0.202		0.830	0.833
NDY	0.184	0.345	0.225	0.148	0.267	0.282	0.203	0.108	0.214	0.187		0.916
GXS	0.208	0.264	0.216	0.185	0.256	0.324	0.213	0.138	0.244	0.183	0.088	

HT, ZZG, JHS, FJS, HYB, QYP, XH, YS, HX, XY, NDY and GXS are Chinese indigenous chicken populations Hetian, zhangzhou Game, Jinhu Silky, Fujian Silky, Huiyang Beard, Qingyuan Partridge, Xinhua, Yangshan, Huaixiang, Xiayan, Nandan Yao and Guangxi Sanhuang, respectively

Thus, the relatedness of current 12 chicken populations in Neighbor-Joining trees related to their geographical localization.

The topology trees from Unweighted Pair-Group method agreed with the traditional classification proposed earlier by some experts (Zhang, 1986), which indicated that breeds classification should relay on phenotype first, rather than geographical location. Conversely, Jossi *et al*. (2003) insisted that the approach of constructing evolutionary trees of indigenous populations is likely to give a misleading picture of their history. Once again, however, detailed scrutiny from chicken samples will be required to answer this issue.

In conclusion, the Chinese indigenous chicken breeds had high heterozygosity. These findings can be used as genetic information for the preservation and further improvement of the Chinese indigenous chicken breeds. The results of this study also confirmed the usefulness of microsatellite for the studying of genetic variation and divergence.

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