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Genetic Variation of Nine Chicken Breeds Collected from Different Altitudes Revealed by Microsatellites

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Genetic polymorphisms of 19 microsatellites were investigated in nine local chicken breeds collected from low, middle and high altitudes areas in China (total number was 256) and their population genetic diversity and population structure were analyzed. All breeds were assigned into three groups, including the high (Tibetan chicken (T) and Grey chicken (G), their altitudes were above 1000 m); middle (Chengkou mountainous chicken (CK), Jiuyuan chicken (JY) and Pengxian yellow chicken (PY), their altitudes were between 500 and 1000 m), and low groups (Da ninghe chicken (DH), Tassel first chicken (TF), Gushi chicken (GS) and Wenchang chicken (WC), their altitudes were below 500 m). We found 780 genotypes and 324 alleles via the 19 microsatellites primers, and the results showed that the mean number of alleles (N_a) was 17.05; the average polymorphism information content (*PIC*) was 0.767; the mean expected heterozygosity (*He*) was 0.662; as for observed heterozygosity (H_o), it was 0.647. The AMOVA results indicated the genetic variation mainly existed within individuals among populations (80%). There was no genetic variation among the three altitude groups (0%). The mean inbreeding coefficient among individuals within population (F_{IS}) was 0.031 and the mean gene flow (N_m) was 1.790. The mean inbreeding coefficient among populations within a group (F_{ST}) was 0.157. All loci deviated Hardy-Weinberg equilibrium. The genetic distance ranged from 0.090 to 0.704. Generally, genetic variations were mainly made up of the variations among populations and within individuals. There were rich gene diversities in the populations for the detected loci. Meanwhile, frequent genes exchange existed among the populations. This can lead to extinction of the peripheral species, such as the Tibetan chicken breed.

Key words: altitude, chicken, genetic variation, microsatellite, population structure

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Introduction

Chicken riches human civilization and promotes social development. China is one of the countries having the richest chicken genetic resources in the world, but has low resource utilization. Evaluating germplasm resources of excellent properties and making unique conservation strategies for each population, will cater for the future needs not only for China, but also for the whole world.

Studies indicated that the level of genetic variation within populations decreased with the environment altitudinal gradients increased (Premoli, 1997). However, there are also reports showed opposite results (Wen and Hsiao, 2001; Gämperle and Schneller, 2003), genetic variation wasn't affected by altitude at all (Saenz-Romero and Tapia-Olivares, 2003). Thus, more investigations for the relationship between the altitude and genetic diversities are needed.

Meanwhile, local breeds in China are facing the blow of increasing gene exchange resulted by the convenient transportation. It's important to strengthen the protection of genetic resources by detecting their genetic diversity to provide applicable preserve strategies. Microsatellites were used in diversity studies due to their dominant, highly polymorphic nature and availability throughout the genome. They are reliable markers for genetic diversity evaluation in both wild and domestic animal populations (Tadano *et al*., 2007). Studies have carried out via microsatellite scanning to study genetic diversity of different chicken breeds (Wimmers *et al*., 2000). In the current study, we collected nine chicken breeds originally raised in Southwest China and located at the high (＞1000 m), middle (500−1000 m) and low (＜500 m) altitudes farms to make clear whether the genetic structures of the chicken breeds were affected by the farm altitude and whether there is gene flow among these native

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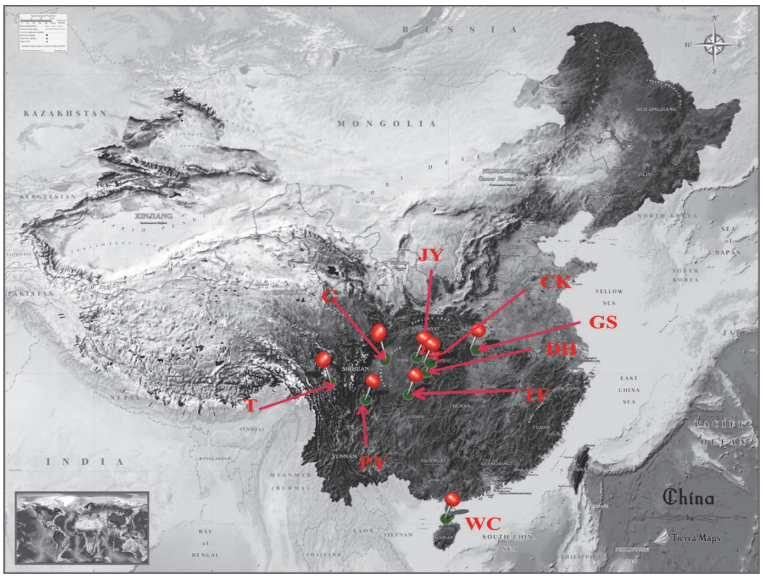

Fig. 1. **The locations of nine chicken populations.**

Table 1. **Nine chicken populations were surveyed in this study**

Breeds	Abbreviation	Location	Sample size	Altitude[1]	Group	Latitude[1]	Longitude[1]
Tibetan chicken	T	Ganzi, Sichuan	30	2930 m	High	28° 55′ 52.25′	99° 47′ 54.30′
Grey chicken	G	Guangyuan, Sichuan	30	1374 m	High	32° 38′ 52.48′	106° 05′ 46.36′
Chengkou mountainous chicken	CK	Chengkou, Chongqing	27	753 m	Middle	31° 56′ 51.92′	108° 39′ 50.76′
Jiuyuan chicken	JY	Taiping town, Sichuan	30	668 m	Middle	32° 04′ 01.40′	108° 02′ 13.92′
Pengxian yellow chicken	PY	Guihua town, Sichuan	30	643 m	Middle	31° 02′ 02.94′	103° 47′ 37.07′
Da ninghe chicken	DH	Wuxi, Chongqing	24	219 m	Low	31° 23′ 55.57′	109° 34′ 11.76′
Tassel first chicken	TF	Wulong, Chongqing	30	177 m	Low	29° 19′ 32.72′	107° 45′ 35.48′
Gushi chicken	GS	Gushi, Henan	26	54 m	Low	32° 11′ 22.25′	115° 40′ 18.79′
Wenchang chicken	WC	Wenchang, Hainan	29	17 m	Low	19° 44′ 27.63′	110° 46′ 21.40′

[1] Original locations of the breeds. The determination of altitude, latitude and longitude using the sampling points as the criterion.

breeds.

Materials and Methods

Populations

Nine populations, Tibetan chicken (T), Grey chicken (G), Chengkou mountainous chicken (CK), Jiuyuan chicken (JY), Pengxian yellow chicken (PY), Da ninghe chicken (DH), Tassel first chicken (TF), Gushi chicken (GS), and Wenchang chicken (WC) were sampled from their original environments in China (Fig. 1 and Table 1). Based on the altitudes of their farms, they were grouped into three categories, the high (breeds T and G, their altitudes were above 1000 m); middle (breeds CK, JY and PY, their altitudes were between 500 and 1000 m), and low (breeds DH, TF, GS and WC, their altitudes were below 500 m).

DNA Extraction

A total of 256 whole bloods were collected from wing veins and stored at −20℃. We extracted genomic DNA from whole blood using the standard phenol/chloroform method (Davoren *et al.*, 2007). The DNA concentrations were quantified by Spectrophotometer (Eppendorf Company, Germany) and samples were diluted to a final concentration of 100 ng/μL.

Polymerase Chain Reaction (PCR) and Microsatellite Genotyping

Nineteen microsatellite (MS) markers were referred to the procedures of Molecular Genetic characterization of Animal Genetic Resources (International Society for Animal Genetics–Food and Agriculture Organization, ISAG–FAO, 2011), as summarized in Table 2.

PCR with two fluorescence labeling primers pairs were carried out with a total volume of 12.5 μL (Beijing Tianwei Biology Technique Corporation, Beijing, China), which containing ddH_2O 4.75 μL, mix 6.25 μL, DNA 0.5 μL, and 0.5 μL of each primer (10 pmol/μL). Nine primers pairs were labeled fluorescence FAM, and the others were labeled

Table 2. **Detail of Microsatellite markers with fluorescence labeling at the end of 5′ fragment of forward primers**

Locus	Chromo-some	Forward primer (5′ −3′)	Reverse primer (5′ −3′)	Tm (℃)	Size range (*bp*)	Fluorescence labeling at the end of 5′-fragment
LEI0166	3	CTCCTGCCCTTAGCTACGCA	TATCCCCTGGCTGGGAGTTT	57.5	354−370	HEX
MCW0014	6	TATTGGCTCTAGGAACTGTC	GAAATGAAGGTAAGACTAGC	57.7	164−182	FAM
MCW0069	E60C04W23	GCACTCGAGAAAACTTCCTGCG	ATTGCTTCAGCAAGCATGGGAGGA	59	158−176	FAM
MCW0103	3	AACTGCGTTGAGAGTGAATGC	TTTCCTAACTGGATGCTTCTG	61	266−270	HEX
MCW0037	3	ACCGGTGCCATCAATTACCTATTA	GAAAGCTCACATGACACTGCGAAA	63.4	154−160	FAM
MCW0330	17	TGGACCTCATCAGTCTGACAG	AATGTTCTCATAGAGTTCCTGC	61	256−300	HEX
LEI0094	4	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	57.7	247−287	HEX
MCW0216	13	GGGTTTTACAGGATGGGACG	AGTTTCACTCCCAGGGCTCG	59	139−149	FAM
LEI0234	2	ATGCATCAGATTGGTATTCAA	CGTGGCTGTGAACAAATATG	59	216−364	HEX
MCW0078	5	CCACACGGAGAGGAGAAGGTCT	TAGCATATGAGTGTACTGAGCTTC	57.7	135−147	FAM
MCW0206	2	CTTGACAGTGATGCATTAAATG	ACATCTAGAATTGACTGTTCAC	57	221−249	HEX
ADL0112	10	GGCTTAAGCTGACCCATTAT	ATCTCAAATGTAATGCGTGC	57.7	120−134	FAM
LEI0192	6	TGCCAGAGCTTCAGTCTGT	GTCATTACTGTTATGTTTATTGC	57.7	244−370	HEX
ALD0268	1	CTCCACCCCTCTCAGAACTA	CAACTTCCCATCTACCTACT	61	102−116	FAM
MCW0222	3	GCAGTTACATTGAAATGATTCC	TTCTCAAAACACCTAGAAGAC	61	220−226	HEX
MCW0034	2	TGCACGCACTTACATACTTAGAA	TGTCCTTCCAATTACATTCATGGG	57.7	212−246	HEX
MCW0111	1	GCTCCATGTGAAGTGGTTTA	ATGTCCACTTGTCAATGATG	61	96−120	FAM
MCW0067	10	GCACTACTGTGTGCTGCAGTTT	GAGATGTAGTTGCCACATTCCGAC	63.4	176−186	HEX
MCW0295	4	ATCACTACAGAACACCCTCTC	TATGTATGCACGCAGATATCC	64	88−106	FAM

Tm, annealing temperature.

fluorescence HEX (Table 2). The reaction steps were as following: an initial step of 5 min at 94℃, followed by 35 cycles of 40 s at 94℃, Tm (annealing temperature) for 30 s, then 30 s at 72℃ and ended with a full extension cycle at 72℃ for 5 min.

ABI 3730 xl Genetic Analyzer (Applied Biosystems, USA) was used for the capillary electrophoresis of the PCR product with the following volume: HI-DI 8 μL, ROX 500 0.3 μL, and the product of PCR 0.5 μL. The estimation of allele size was determined with Gene marker Software (Soft Genetics, USA). The allele data was subjected to further genetic analysis.

Data Analysis

Genetic information of nine chicken breeds was assessed by calculating the total number of alleles (N_a), gene diversity (D_g), number of genotype (N_g), the main allele frequency (*MAF*), observed (H_o) and expected (H_e) heterozygosities. Polymorphism information content (*PIC*) was analyzed by PowerMarker V3.25. Hardy-Weinberg equilibrium and effective alleles (N_e) was calculated using the GenALEx version 6.4. Molecular variance (AMOVA) was performed to estimate the hierarchical structure of genetic diversity using the program GenALEx (version 6.4). The pair-wise comparisons between populations and regions, the F_{ST}-values and the estimated pairwise N_m were also using the program GenALEx version 6.4 (Peakall and Smouse, 2012). Multilocus pairwise F_{ST} was quantified by ARLEQUIN software, version 3.1 (Schneider *et al*., 2000; Cortellini *et al*., 2011). To further analyze the Nei's standard genetic distance analysis (DA, Nei *et al*., 1983) among populations, we used the program PowerMarker V3.25 and MEGA5.1.

Results

Genetic Diversity of Chicken Breeds

The microsatellite polymorphism, evaluated by the N_a per locus, D_g, N_g, *MAF*, H_o, H_e and *PIC* for each breed were summarized in Table 3. A total of 324 alleles identified via the 19 microsatellite primers distributed in 256 individuals from 9 populations. All the microsatellite loci were polymorphic. The number of alleles per locus ranged from 7 (for primers MCW0222) to 54 (for primers LEI0234) (Table 3), with a mean of 17.05; the average *PIC* was 0.767, which ranged from 0.470 to 0.928. The mean H_e was 0.662, which ranged from 0.489 (for primers MCW0103) to 0.848 (for primers LEI0234). For H_o, its mean value was 0.647, and ranged from 0.461 to 0.816; the average main allele frequency was 0.320 (Table 3).

Diversity parameters in nine breeds from low, middle and high groups were shown in Table 4. The mean N_a in each breed ranged from 4.79 (breed PY) to 7.05 (breed DH). The DH breed had the highest diversity, with the highest D_g (0.70), H_e (0.70), N_e (3.95), N_a (7.05), N_g (10.84) and *PIC* (0.67), respectively. Breed TF had the highest *MAF* (0.49), and breed CK had the highest H_o. PY breed had the lowest H_o (0.57), *PIC* (0.58), D_g (0.63), H_e (0.63), N_e (2.95), N_a (4.79) and N_g (8.05).

Population Structure

Partitioning of genetic variability by analysis of molecular variance indicated that 80% of the total genetic variation was distributed within individual among populations, within

Table 3. **Descriptive statistics of the 19 microsatellite loci across nine chicken breeds**

Marker	*MAF*	N_g	N_a	D_g	H_o	H_e	*PIC*
LEI0166	0.428	21	9	0.684	0.648	0.640	0.631
MCW0014	0.252	45	18	0.843	0.531	0.653	0.825
MCW0069	0.193	57	20	0.883	0.816	0.766	0.873
MCW0103	0.525	10	8	0.560	0.461	0.489	0.470
MCW0037	0.350	14	8	0.726	0.582	0.644	0.679
MCW0330	0.281	38	16	0.842	0.691	0.718	0.825
LEI0094	0.201	84	29	0.909	0.746	0.784	0.902
MCW0216	0.279	20	10	0.802	0.547	0.540	0.774
LEI0234	0.193	135	54	0.931	0.688	0.848	0.928
MCW0078	0.398	18	10	0.755	0.461	0.531	0.723
MCW0206	0.389	28	11	0.777	0.508	0.530	0.752
ADL0112	0.346	20	11	0.769	0.711	0.565	0.735
LEI0192	0.332	79	40	0.857	0.582	0.714	0.849
ADL0268	0.412	18	9	0.710	0.758	0.675	0.662
MCW0222	0.383	20	7	0.717	0.606	0.652	0.671
MCW0034	0.252	83	27	0.890	0.738	0.769	0.883
MCW0111	0.270	29	14	0.822	0.785	0.651	0.799
MCW0067	0.236	26	11	0.841	0.766	0.671	0.822
MCW0295	0.361	35	12	0.801	0.664	0.735	0.780
Mean±SD	0.320±0.091	41.05±32.68	17.05±12.43	0.796±0.091	0.647±0.111	0.662±0.098	0.767±0.112

MAF, main allele frequency; N_g, number of genotype; N_a , number of alleles per locus; D_g, gene diversity; H_o, observed heterozygosity; H_e, expected heterozygosity; *PIC*, polymorphism information content. SD, standard deviation.

Table 4. **Diversity parameters in nine chicken breeds**

Parameters	High group		Middle group			Low group			
	T	G	CK	JY	PY	DH	TF	GS	WC
MAF	0.49±0.15	0.44±0.13	0.44±0.13	0.41±0.12	0.49±0.13	0.43±0.14	0.49±0.16	0.47±0.16	0.49±0.15
N_g	9.11±4.56	9.84±5.80	9.68±4.96	9.84±5.10	8.05±2.92	10.84±4.19	9.95±5.62	9.90±5.08	8.95±5.30
N_a	5.58±2.39	6.53±4.25	6.63±4.04	6.42±3.60	4.79±1.55	7.05±3.44	6.42±4.11	6.95±3.95	5.74±2.85
N_e	3.21±0.34	3.68±0.47	3.59±0.35	3.79±0.44	2.95±0.40	3.95±0.40	3.49±0.40	3.63±0.50	3.23±0.32
D_g	0.63±0.14	0.67±0.12	0.68±0.12	0.69±0.11	0.63±0.11	0.70±0.12	0.64±0.15	0.66±0.13	0.64±0.13
H_o	0.61±0.19	0.68±0.18	0.71±0.18	0.71±0.17	0.57±0.14	0.64±0.20	0.58±0.17	0.65±0.16	0.68±0.21
H_e	0.63±0.03	0.67±0.03	0.68±0.03	0.69±0.03	0.63±0.02	0.70±0.03	0.64±0.03	0.66±0.03	0.64±0.03
PIC	0.58±0.15	0.62±0.14	0.63±0.13	0.63±0.13	0.58±0.12	0.67±0.13	0.60±0.16	0.62±0.14	0.59±0.15
F_{IS}	0.03±0.13	0.05±0.23	−0.03±0.22	−0.02±0.18	0.10±0.23	0.13±0.21	0.12±0.22	0.03±0.18	−0.06±0.33

MAF, major allele frequency; N_g, number of genotype; N_a, number of alleles; N_e, number of effective alleles; D_g, gene diversity; H_o, observed heterozygosity; H_e, expected heterozygosity; *PIC*, polymorphism information content; F_{IS}, Between individuals within population inbreeding coefficient

regions relative to the total (0%) and among populations within regions (16%), with the remaining split within populations relative to the total (3%, Table 5).

The mean observed heterozygosity (H_o) was lower than the mean expected heterozygosity (H_e), which leaded in positive overall heterozygote deficiency (F_{IS}) and inbreeding coefficient (F_{IT}) (Table 6). However, the levels of inbreeding within individual populations and among all individuals were not significant, as indicated by jackknifed F_{IS} and F_{IT} estimates (0.031 and 0.184, respectively; Table 6). The F_{IS} values varied greatly across loci, which ranged from −0.216 to 0.199. The gene flow ranged from 0.565 to 4.772 with a mean of 1.790 (Table 6).

F_{ST} values between paired populations ranged from 0.022 to 0.267. The highest level of genetic differentiation was between breed T and breed TF (F_{ST}=0.267), and the lowest was between breed CK and breed JY (F_{ST}=0.022, Table 7).

Hardy-Weinberg Equilibrium

Date summarized in Table 8 indicated that no marker matched Hardy-Weinberg equilibrium. The Jiuyuan chicken (JY) only had five loci deviated Hardy-Weinberg equilibrium. In contrast, Pengxian yellow chicken (PY) had sixteen loci deviated Hard-Weinberg equilibrium.

Genetic Difference and Distance among Breeds

Table 9 showed D_A genetic distance among nine breeds. The genetic distance ranged from 0.090 (G from high group

Table 5. **Analysis of molecular variance (AMOVA) based on microsatellites**

Source of variation	*df*	Variance components	%Total
Among regions	2	0.000	0
Among pops	6	1.255	16
Among Individuals	247	4.000	3
Within Individual	256	6.148	80
Total	511	7.731	100

df, degrees of freedom; %Total, percentage of the total variance.

Table 6. ***F* statistics for 19 polymorphic loci across nine chicken breeds**

Locus	F_{IS}	F_{IT}	F_{ST}	N_m
LEI0166	−0.008	0.060	0.068	3.433
MCW0014	0.193	0.364	0.212	0.932
MCW0069	−0.068	0.067	0.127	1.724
MCW0103	0.078	0.185	0.116	1.896
MCW0037	0.101	0.193	0.103	2.180
MCW0330	0.060	0.187	0.135	1.605
LEI0094	0.059	0.178	0.127	1.718
MCW0216	0.005	0.310	0.307	0.565
LEI0234	0.188	0.259	0.087	2.616
MCW0078	0.160	0.387	0.270	0.675
MCW0206	0.069	0.344	0.296	0.596
ADL0112	−0.216	0.085	0.247	0.761
LEI0192	0.199	0.320	0.151	1.403
ADL0268	−0.130	−0.074	0.050	4.772
MCW0222	0.082	0.166	0.092	2.482
MCW0034	0.044	0.167	0.128	1.704
MCW0111	−0.185	0.049	0.198	1.013
MCW0067	−0.127	0.083	0.186	1.095
MCW0295	0.083	0.158	0.081	2.838
Mean±SD	0.031±0.13	0.184±0.124	0.157±0.078	1.790±1.087

F_{IS}, deficiency of heterozygosity relative to the Hardy-Weinberg expectation; F_{IT}, the overall inbreeding coefficient; F_{ST}, differentiation among populations; N_m, gene flow. SD, standard deviation.

and WC from low group) to 0.704 (G from high group and TF from low group). Fig. 2 displayed a phylogenetic tree constructed with D_A genetic distances. The average genetic diversity was 0.418. Nine local breeds clustered into 2 groups. Tassel first chicken, Da ninghe chicken and Penxian yellow chicken were clustered in one group. The others were clustered in another group, and formed a close group with Tibetan chicken. There was no geographic specificity in the phylogenetic tree.

Discussions

Our results revealed that a total of 324 alleles were identified in 256 individuals from 9 chicken populations via the 19 microsatellites scanning method. The loci (primers MCW0103) had the poorest polymorphism. Ninety-five present of *PIC* was more than 0.5. We can draw a conclusion that 19 microsatellites loci were highly polymorphic (Schumm *et al*., 1988). The population we determined in the current study did not suffer severe genetic bottlenecks. LEI0234 locus had the highest *PIC* (0.928), which had the larger heterozygous proportion and more genetic information than other microsatellites loci (Wu *et al*., 2004). All of the nine chicken breeds had high heterozygosity ($H_e > 0.5$, $H_o > 0.5$), which meant that genetic variation was large and inbreeding degree was weak (Pariset *et al*., 2003).

AMOVA results revealed there's no genetic variation among the groups from high, middle and low altitude regions (0% of the total variation) and high level of variation with individuals among populations (80%). These results suggested that geographic isolation among these groups has been broken, and it could not play critical roles in the genetic differentiation among populations. Among the nine groups, there was no significant genetic structure difference; because the local breeds were facing the blow of increasing gene exchange frequency resulted by the convenient transportation.

With the 19 loci examined the mean inbreeding coefficient within individual populations and among all individuals

Table 7. **Estimated pairwise F_{ST} values for nine chicken breeds and between three regions**

Population		High group		Middle group			Low group			
		T	G	CK	JY	PY	DH	TF	GS	WC
High group	T	—								
	G	0.093***								
Middle	CK	0.129***	0.057***							
group	JY	0.124***	0.060***	0.022***						
	PY	0.262***	0.249***	0.234***	0.233***					
Low group	DH	0.229***	0.217***	0.199***	0.192***	0.064***				
	TF	0.267***	0.254***	0.242***	0.236***	0.067***	0.053***			
	GS	0.119***	0.054***	0.028***	0.025***	0.220***	0.178***	0.225***		
	WC	0.114***	0.034***	0.081***	0.086***	0.249***	0.218***	0.258***	0.074***	

*** $P<0.001$.

Table 8. **Results of Hardy-Weinberg tests**

Marker	High group		Middle group			Low group			
	T	G	CK	JY	PY	DH	TF	GS	WC
LEI0166	1.89	6.33	23.71**	23.47	15.28	31.47	5.32	0.83	4.79
MCW0014	75.07**	15.89*	11.64	4.36	71.76***	32.06	92.78***	62.06***	26.83*
MCW0069	23.55	47.28	46.93***	18.25	67.66***	74.38*	39.53	81.81*	107.48***
MCW0103	3.10	62.13***	27.65***	0.37	3.45	74.37***	3.88	26.68***	32.39***
MCW0037	6.79	13.26*	5.35	63.49***	37.10***	3.75	8.43	3.06	3.13
MCW0330	69.26***	21.75	42.35*	63.45**	73.71***	37.94*	11.05	68.99***	36.98**
LEI0094	17.34**	90.00*	61.07	67.23	93.13***	88.43	72.13	112.28	79.737
MCW0216	5.97	2.11	3.15	1.60	1.54	50.79***	5.51	2.17	15.37
LEI0234	17.11	212.25**	131.52	185.52**	146.55***	187.76**	309.53***	226.96	59.51
MCW0078	1.88	6.65	4.31	5.44	30.46***	36.31***	40.08***	33.47**	96.47***
MCW0206	10.30	3.33	10.34	20.46**	41.19**	60.91***	34.64***	67.74***	63.51***
ADL0112	18.57*	30.00***	2.67	38.01***	39.71**	60.25***	63.23***	60.36***	58.00***
LEI0192	95.56***	43.60	145.49***	50.49	83.48***	72.55**	237.30***	59.96	38.35
ADL0268	18.63**	18.34*	11.61	9.96	19.36*	34.87**	41.76**	29.37*	27.65**
MCW0222	8.13	32.65***	6.86	0.95	34.99*	26.33*	39.23***	6.43	1.344
MCW0034	63.90*	122.40***	92.37*	16.81	72.61***	78.38	108.38*	182.72***	51.74
MCW0111	10.11	26.03***	11.64	14.06	62.50***	65.16***	37.21**	39.55**	51.13***
MCW0067	13.88	18.64*	30.02***	23.51	60.84***	57.77***	62.12***	44.79**	36.65**
MCW0295	30.72	44.30***	15.50	19.79	79.61***	6.54	83.82***	56.48**	24.54

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table 9. **Nei's DA genetic distance (Nei *et al*., 1983) among populations**

Population		High group		Middle group			Low group			
		T	G	CK	JY	PY	DH	TF	GS	WC
High group	T	—								
	G	0.181	—							
Middle group	CK	0.274	0.171	—						
	JY	0.242	0.171	0.091	—					
	PY	0.682	0.696	0.658	0.670	—				
Low group	DH	0.665	0.680	0.649	0.653	0.154	—			
	TF	0.679	0.704	0.663	0.677	0.156	0.167	—		
	GS	0.214	0.155	0.106	0.092	0.641	0.626	0.651	—	
	WC	0.204	0.090	0.184	0.190	0.687	0.679	0.693	0.170	—

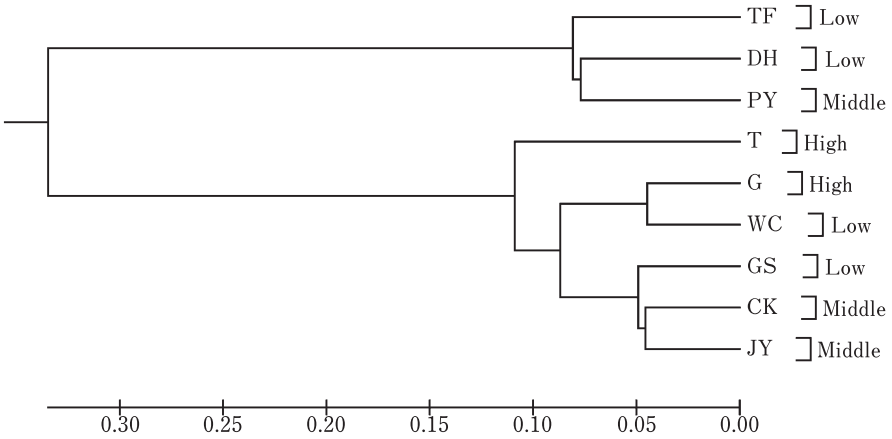

Fig. 2. **UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) dendrogram of genetic among nine chicken breeds based on D_A genetic distances estimated with 19 microsatellites.** Numbers on the nodes are bootstrap values of 1000 replications.

of the loci (except LEI0166, MCW0069, ADL0112, ADL 0268, MCW0111 and MCW0067) were positive, which indicated that the nine local breeds can't avoid heterozygosity loss and there existed inbreeding in these local populations. In all loci, the differentiation among populations (F_{ST}) were more than 0.05, the average of F_{ST} was 0.157. Our results suggested that among varieties there was a low degree of genetic differentiation. Meanwhile the pairwise F_{ST} value showed that altitude had little influence on F_{ST}. Wright's (1943) infinite-island approximation indicated that $F_{ST}=1/(1+4N_m)$, thus, the $N_m=1.790$. Wright pointed out that if $N_m<1$, genetic drift could lead to significant genetic differentiation between populations, and reducing the genetic variation. Kimura and Weiss (1964) had shown that when $N_m\geq 4$, the homogenizing effect of gene flow was sufficient to prevent stochastic differentiation of allele frequencies. Under such conditions, local adaptation may not be constrained by low levels of gene flow that produce a spatial averaging of fitness variation among different altitudes.

Heterozygote deficiency resulted in deviated Hardy-Weinberg equilibrium for all loci. Pengxian yellow chicken (PY) from middle group had sixteen loci deviate Hard-Weinberg equilibrium, which may be influenced by selection, migration, mutation or genetic drift.

The genetic distance was the basis for studying on genetic diversity; it reflected the genetic differentiation of investigated populations. The closer group clustered, the smaller genetic distance existed. The genetic distance between nine groups ranged from 0.090 to 0.704, which didn't completely range in the genetic distances ($0.2<D_A<0.8$) between species (Thorpe *et al*., 2003).The result of the cluster analysis demonstrated that there was no significant altitudinal effect on breeds, which was in accord with the earlier finding which reported altitude effect on microsatellite variation was limited (Zhang *et al*., 2006). The Tassel first chicken, Da ninghe chicken and Penxian yellow chicken had closer genetic relationship, which may be resulted by gene exchange due to close geographical distance. Tibetan chicken located in another group with relatively far distance with populations G, WC, CK, GS, and JY. Some studies indicate that Tibetan chicken has developed effective strategies through specific physiological and genetic adaptations to survive at high altitude, particularly to address the low-oxygen hypoxic environment (Storz *et al*., 2010). In addition, Wang *et al*. (2015) finding the size of their olfactory receptor gene family was reduced, which attributed possibly to adaptation to a highland environment where food sources and odorants are limited.

Our results indicate that genetic variations were mainly made up of the variations among populations and within individuals. There were rich gene diversities in the populations for the detected loci. Meanwhile, frequent genes exchange existed among the populations. This can lead to extinction of the peripheral species, such as the Tibetan chicken breed.

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