

Genetic diversity and relationship between genetic distance and geographical distance in 14 Chinese indigenous chicken breeds and red jungle fowl

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ABSTRACT: Genetic diversity and the relationship between genetic distance and geographical distance in red jungle fowl and 14 Chinese indigenous chicken breeds were evaluated using 29 microsatellite loci. The number of alleles per locus ranged from 2 to 25 and the average expected heterozygosity and PIC of all loci were 0.6683 and 0.50, respectively. The average number of alleles per locus ranged from 3.41 in Gushi chicken breed to 6.28 in Wannan Three-yellow chicken breed. The overall expected heterozygosity of 15 Chinese chicken breeds was 0.6686 ± 0.0254 and all breeds showed relatively large heterozygosity. The average of genetic differentiation among populations was 16.4% ($P < 0.001$). Red jungle fowl and Gushi chicken had distant genetic relationship from other breeds, while Huainan Partridge and Tibetan chicken were more closely related with other breeds. The results did not provide enough support for a significant correlation between the genetic and geographical pair-wise distances.

Keywords: genetic diversity; microsatellite; genetic distance; geographical distance; chicken

China has a wide variety of indigenous poultry resources with its long history of animal husbandry and diversified geographical conditions. There are 108 native chicken breeds in China (Chen et al., 2004a). Many local varieties have valuable genetic features. For instance, Taihe Silkies in the Taihe county of Jiangxi province is an important source for traditional Chinese medicine (Li, 1983). However, as a result of the introduction of modern commercial chicken breeds and the limitation for conservation measures, some populations have decreased rapidly in population sizes. Some chicken breeds, such as Beijing Fatty chickens, Lingkun chickens, Pudong chickens, Ningjing chickens, Zhangmu chickens, are even facing extinction, according to the statistics published by the Ministry of Agriculture of China (The State of Animal Genetics Resource in China, 2004).

Though decisions on conservation rely upon a range of information including the degree of en-

dangerment, adaptation to a specific environment, possession of traits of current or future economic importance, possession of unique traits of scientific interest, and the cultural or historical value of the breed, accurate assessment of populations with regard to their contribution to national and overall genetic diversity is an important step in determining priorities for conservation. Some centres of poultry resources in China were set up according to their geographical distribution in the last decades, however, in the process of developing strategies to conserve genetic diversity in domestic chickens, it is important to assess quantitatively the genetic uniqueness of a given population, which may be deduced from genetic distances, and molecular markers may serve as an important initial guide to evaluate breeds as genetic resources (Barker, 1994; Ruane, 1999; Weigend and Romanov, 2001).

With the characteristics of high polymorphism, locus specificity, abundance and random distribution

over the genome, and their co-dominant inheritance, microsatellites are currently used most commonly to assess the population structure and diversity (Romanov and Weigend, 2001; Chen et al., 2004b; Du et al., 2004). According to FAO recommendations, determining classic genetic distances using neutral, highly polymorphic microsatellite markers is the method of choice for investigating genetic relationships and breed differentiation. This methodology also provides information for establishing preservation priorities for livestock breeds (Barker, 1999).

The aim of this article was to evaluate genetic diversity in red jungle fowl and 14 Chinese indigenous chicken breeds with 29 microsatellite mark-

ers and to analyze the relationship between all pairs of geographical distance and genetic distance. The results may be useful to understand genetic differentiation of these important local breeds and contribute to a more efficient conservation.

MATERIAL AND METHODS

Experimental populations

A total of 542 individuals from 14 Chinese indigenous chicken breeds and red jungle fowl were genotyped. All populations except for Wannan

Table 1. Description of 14 indigenous Chinese chicken breeds and red jungle fowl

Breed (abbreviation)	Main original area	Specific features	Number of animals studied
Xianju chicken (XJ)	Xianju county Zhejiang	three yellow*, light-sized, layer breed	38
Chahua chicken (CH)	Xishuangbanna, Yunnan	light-sized, meat and egg dual-purpose breed	38
Luyuan chicken (LY)	Zhangjiagang city, Jiangsu	heavy-sized, meat and egg dual-purpose breed	34
Gushi chicken (GS)	Gushi county, Henan	three yellow, medium-sized, meat and egg dual-purpose breed	40
Tibetan chicken (TC)	Ganzi and Aba Tibetan autonomous region	light-sized, selected for yellow plumage, meat and egg dual-purpose breed	38
Baier chicken (BE)	Sichuan	three yellow, light-sized, layer	34
Dagu chicken (DG)	Shangrao city Jiangxi	breed, white earlobe heavy-sized, meat and egg dual-	35
Henan game (HG)	Zhuanghe county, Liaoning	purpose breed heavy-sized, fancy breed	33
Langshan chicken (LS)	Zhengzhou city, Henan	heavy-sized, meat and egg dual-purpose breed	40
Taihe Silkies (TS)	Rudong county, Jiangsu	light-sized, medicine and entertainment breed	40
Xiaoshan chicken (XS)	Taihe county Jiangxi	heavy-sized, meat and egg dual-purpose breed	40
Beijing Fatty chicken (BF)	Xiaoshan county, Zhejiang	heavy-sized, meat and egg dual-purpose breed	38
Huainan Partridge (HP)	Chaoyang, Beijing	heavy-sized, meat and egg dual-purpose breed	32
Gallus gallus spadiceus (RJF-SC)	Huainai city, Anhui	Red jungle fowl (wild)	30
Wannan Three-yellow chicken (WTY)	Shimao county, Yunnan Qinyan county, Anhui	medium-sized, egg purpose breed	32

*three yellow features (plumage yellow, beak yellow and shank yellow)

Three-yellow chickens, Huainan Partridges and red jungle fowl were from the Poultry Institute, Academy of Chinese Agricultural Sciences, Yangzhou, P.R. China; Wannan Three-yellow Chickens were from the Centre of Poultry Resource in the Qinyan County, Anhui Province; Huainan Partridges were from the Centre of Poultry Resource in the Institute of Agricultural Science in the Huainan city, Anhui Province; the red jungle fowl was from the Wild Animal Conservation Centre, Yunnan Province, P.R. China. The information on breeds, main original areas in China, specific features, and number of individuals studied is presented in Table 1.

Genotyping

A total of 29 microsatellite markers (Table 2) spread across the chicken genome were used for genotyping. PCR products were obtained in an 8- μ l volume using a thermal cycler (Mastercycler; Eppendorf, Hamburg, Germany). Two pairs of microsatellite primers were run in one tube. Each PCR tube contained 20 ng of genomic DNA, 10 pmol of each forward primer labelled with either IRD700 or IRD800 (MWG-Biotech, Ebersberg, Germany), 10 pmol of each unlabelled reverse primer, 4 μ l HotStarTaq Master Mix (QIAGEN, Germany) and 1mM tetramethyl ammonium chloride. The amplification involved initial denaturation at 95°C (15 min), 35 cycles of denaturation at 95°C (1 min), annealing temperature varying between 48°C and 63°C (1 min), and extension at 72°C (1 min), followed by final extension at 72°C (10 min). DNA fragments were scored on 8% polyacrylamide gel using a LI-COR automated DNA analyzer (LI-COR Biotechnology Division, Lincoln, NE, USA). Electrophoregram processing and allele-size scoring were performed with the RFLP scan package (Scanalytics, Division of CSP, Billerica, MA, USA).

Analysis of genetic diversity

Allele frequencies were estimated by direct allele counting. The observed and expected heterozygosities (Nei, 1987) for each population across the loci and those for each locus across the populations were estimated with Microsatellite-Toolkit for Excel (Park, 2001). Polymorphism information

content (PIC) for each locus and each breed was calculated according to Botstein et al. (1980):

$$PIC = 1 - \sum_{i=1}^n p_i^2 - 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^n p_i^2 p_j^2$$

where:

n = the number of alleles

$p_i p_j$ = frequencies of corresponding alleles

Genetic differentiation

The F -statistics indices (Wright, 1978) were estimated in the form of F , θ , and f , the sample-based, respective estimators of these parameters proposed by Weir and Cockerham (1984), as implemented in FSTAT program (Version 2.9.3, Goudet, 2002). Significance of the F -statistics was determined from permutation tests by the sequential Bonferroni procedure applied across loci (Hochberg, 1988). As a measure of deviation from the Hardy-Weinberg equilibrium, the F_{IS} value was calculated and type-I error probability was computed.

The F_{ST} values among pairs of breeds were calculated by GENEPOP program (Raymond and Rousset, 1995). Rousset's (1997) isolation by distance was applied to these chicken breeds. A linear regression was used to estimate the coefficients

$$F_{ST}/(1 - F_{ST}) = \alpha + \beta \ln(d)$$

where:

d = represents the pair-wise geographical distance between breeds

Gene flow between populations, defined as the number of reproductively successful migrants per generation (N_m), was estimated by the methods based on the n island model of population structure. The estimate was based on the relationship

$$F_{ST} = 1/(4N_m + 1)$$

where:

N = the effective population size, m is the migration rate

F_{ST} = the mean F_{ST} value calculated across all loci (Slatkin and Barton, 1989)

The Reynolds' genetic distance (Reynolds et al., 1983) between breeds was calculated, based on F_{ST} values.

Table 2. Number of alleles, range of allele sizes, F -statistics, expected heterozygosity (He), and mean polymorphic information content (PIC) for each of the 29 microsatellite markers in 14 Chinese chicken breeds and red jungle fowl

Markers	Chromosome	No. of alleles	Range of allele sizes (bp)	$F_{IT} = F$	F_{ST}	$F_{IS} = f$	He	Mean PIC
MCW0103	3	2	266–270	0.323***	0.205***	0.148**	0.3945	0.25
MCW0216	13	8	137–149	0.178***	0.136***	0.049*	0.5622	0.41
MCW0295	4	12	88–110	0.212***	0.130***	0.094***	0.7168	0.57
ADL0278	8	12	114–129	0.152***	0.218***	–0.085	0.6617	0.45
MCW0222	3	4	220–226	0.217***	0.217***	–0.001	0.6085	0.46
MCW0037	3	6	154–159	0.225***	0.172***	0.064*	0.6703	0.52
ADL0268	1	8	104–118	0.071*	0.108***	–0.042	0.7245	0.52
MCW0183	7	14	296–324	0.107**	0.116***	–0.010	0.7297	0.53
MCW0014	6	11	160–186	0.230***	0.222***	0.010	0.6707	0.50
MCW0067	10	6	178–186	0.137***	0.161***	–0.028	0.6420	0.50
MCW0098	4	2	263–265	0.319***	0.319***	–0.000	0.3032	0.22
LEI0166	3	6	356–376	0.145***	0.224***	–0.101	0.6081	0.42
MCW0069	26	9	158–176	0.112***	0.138***	–0.030	0.7646	0.60
MCW0081	5	6	114–135	0.117***	0.128***	–0.013	0.4524	0.26
ADL0112	10	4	124–132	0.145***	0.160***	–0.018	0.5043	0.32
MCW0034	2	17	212–246	0.133***	0.114***	0.021	0.8259	0.66
MCW0111	1	12	96–120	0.232***	0.142***	0.105***	0.7280	0.57
MCW0078	5	5	135–143	0.177***	0.137***	0.047	0.6553	0.48
MCW0206	2	11	221–247	0.213***	0.163***	0.060***	0.7019	0.57
LEI0094	4	20	247–289	0.204***	0.184***	0.025	0.8852	0.72
MCW0248	1	5	215–223	0.164***	0.172***	–0.010	0.6973	0.44
LEI0234	2	25	216–380	0.102***	0.160***	–0.069	0.9111	0.74
MCW0330	17	7	258–290	0.125***	0.101***	0.027	0.7346	0.54
MCW0016	3	11	162–188	0.226***	0.111***	0.129***	0.7280	0.55
MCW0104	13	19	190–232	0.068***	0.107***	–0.044	0.8171	0.65
MCW0020	1	4	179–185	0.261***	0.255***	0.009	0.6190	0.49
MCW0165	23	3	114–118	0.301***	0.205***	0.120***	0.5696	0.42
MCW0080	15	17	265–281	0.139***	0.120***	0.021	0.7151	0.61
MCW0123	14	11	76–98	0.306***	0.190***	0.144***	0.7803	0.65
Mean		9.55		0.180	0.164	0.020	0.6683	0.50
		(5.82)		(0.013)***	(0.009)***	(0.012)***	(0.1354)	(0.13)

F = total inbreeding estimate; F_{ST} = measure of population differentiation; f = within-population inbreeding estimate
 mean jack-knife estimates across loci; standard deviations are given in parentheses
 significance of F -statistics was done using Bonferroni permutations based on 1 000 resamplings

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Clustering of populations

An unrooted neighbour-joining cladogram (Saitou and Nei, 1987) was obtained based on the pair-wise kinship distance matrix between populations using the neighbour-joining program implemented in PHYLIP (Felsenstein, 1995). A consensus tree, evaluated by 1 000 bootstraps across the set of loci, was constructed.

RESULTS

Genetic variability within populations

A total of 277 alleles were detected in 14 Chinese indigenous chicken breeds and red jungle fowl at 29 microsatellite loci. Expected heterozygosity (H_e) and mean polymorphic information content (PIC) for each locus across 15 populations are listed in Table 2.

The number of alleles per locus ranged from 2 (*MCW0103* and *MCW0098*) to 25 (*LEI0234*) and the average number of the alleles observed at 29 microsatellite loci was 9.55 ± 5.82 . Across breeds, locus *MCW0098* had the lowest H_e (0.3032) and

the lowest PIC (0.22), while locus *LEI0234* had the highest H_e and PIC values (0.9111 and 0.74, respectively).

The average number of alleles per locus, expected and observed heterozygosity and F_{IS} for each breed across 29 loci are shown in Table 3.

The average number of alleles per locus ranged from 3.41 in Gushi chicken breed to 6.28 in Wannan Three-yellow chicken breed. All breeds showed relatively large heterozygosity. Across 29 loci, the lowest value 0.4402 of heterozygosity was obtained for Gushi chicken breed, and the highest 0.6441 was found for Wannan Three-yellow chickens. The overall expected heterozygosity of 14 Chinese indigenous chicken breeds and red jungle fowl was 0.6686 ± 0.0254 .

In the exact test for deviation from the Hardy-Weinberg equilibrium, more or less populations showed significant deviation for all loci, except *MCW0183*, *MCW0081*, *MCW0098*, *LEI0166*, *MCW0248* and *MCW0080* (data not shown). The negative F_{IS} values of some breeds indicated an excess of heterozygotes. The values for the most of the breeds, even if statistically significant, were not far from 0, indicating that mating was close to panmixia.

Table 3. Mean no. of alleles per locus, mean heterozygosity (H_e and H_o) and F_{IS} per population

Breed	Mean No. of alleles per locus (mean \pm SD)	F_{IS}	Expected heterozygosity H_e (mean \pm SD)	Observed heterozygosity H_o (mean \pm SD)
Xianju	4.00 \pm 2.19	0.1477	0.5334 \pm 0.0349	0.5006 \pm 0.0152
Chahua	4.62 \pm 2.27	0.0438	0.5531 \pm 0.0413	0.5024 \pm 0.0153
Luyuan	4.41 \pm 2.03	0.1113	0.5742 \pm 0.0324	0.5265 \pm 0.0161
Gushi	3.41 \pm 1.45	-0.0288	0.4402 \pm 0.0412	0.4339 \pm 0.0146
Tibetan	5.52 \pm 2.77	0.0155	0.6138 \pm 0.0347	0.6030 \pm 0.0149
Baier	4.21 \pm 2.34	0.0914	0.5372 \pm 0.0316	0.4979 \pm 0.0160
Dagu	5.17 \pm 2.27	-0.0021	0.6339 \pm 0.0318	0.6404 \pm 0.0151
Henan game	3.83 \pm 1.83	-0.1103	0.5309 \pm 0.0349	0.5287 \pm 0.0162
Langshan	4.17 \pm 1.93	0.0645	0.5418 \pm 0.0315	0.6130 \pm 0.0143
Taihe Silkies	4.59 \pm 1.99	0.0234	0.5768 \pm 0.0303	0.5642 \pm 0.0146
Xiaoshan	4.48 \pm 1.86	-0.0236	0.6084 \pm 0.0225	0.6082 \pm 0.0143
Beijing Fatty	4.41 \pm 1.76	-0.0216	0.5529 \pm 0.0273	0.5725 \pm 0.0149
Huainan Partridge	5.55 \pm 2.86	-0.0101	0.6181 \pm 0.0315	0.5715 \pm 0.0163
Gallus gallus spadiceus	3.79 \pm 1.37	0.1732	0.5379 \pm 0.0335	0.5356 \pm 0.0169
Wannan Three-yellow	6.28 \pm 3.18	0.0922	0.6441 \pm 0.0268	0.6053 \pm 0.0161

Genetic differentiation

Genetic differentiation was examined by fixation indices F_{IT} , F_{ST} , F_{IS} for each locus. Results of the F -statistics analysis for 29 microsatellite markers in 14 Chinese indigenous chicken breeds and red jungle fowl are presented in Table 2.

The fixation coefficients of subpopulations within the total population, measured as F_{ST} value, for the 29 loci varied from 0.101 (*MCW0020*) to 0.319 (*MCW0081*), with a mean of 0.164 ($P < 0.001$). All loci contributed to this differentiation significantly. The global deficit of heterozygotes across populations (F_{IT}) amounted to 18% ($P < 0.001$). An overall significant deficit of heterozygotes (F_{IS}) of 2% ($P < 0.001$) occurred at the analyzed loci because of inbreeding within populations. Nine loci showed a significant deficit of heterozygotes. Thirteen markers, to some extent, showed an excess of heterozygotes (negative value).

Estimated gene flow (Nm) and Reynolds' genetic distances (D_R) between each population pair are presented in Table 4. Reynolds' distance values varied between 0.0478 (Xiaoshan chicken – Luyuan chicken pair) and 0.3353 (red jungle fowl – Henan game chicken pair). The Nm value ranged from 0.4967 (between red jungle fowl and Gushi chicken pair) to 5.1033 (between Xiaoshan chicken and Luyuan chicken pair). Most of Nm values between pairs of breeds were below 2.0.

The application of Rousset's isolation by distance method, as implemented in GENEPOP program,

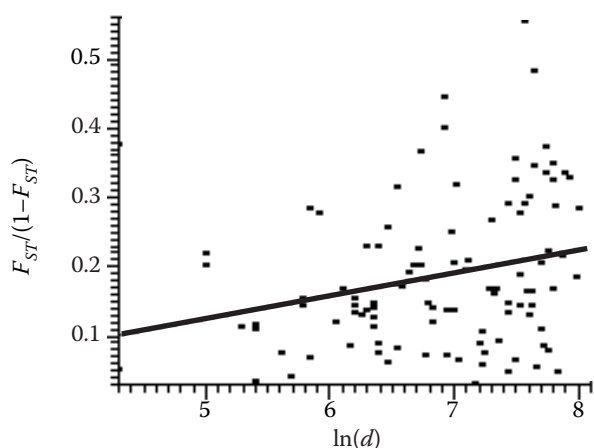


Figure 1. Plot of relationship between geographical distance, $\ln(d)$, and pairwise $F_{ST}/(1-F_{ST})$ for all pairs of Chinese indigenous chicken breeds. The fitted line correspond to the equation $F_{ST}/(1-F_{ST}) = -0.0162 + 0.0313\ln(d)$

yielded the parameters α and β in the regression, $F_{ST}/(1-F_{ST}) = -0.0162 + 0.0313\ln(d)$ (Figure 1). However, regression failed to provide enough support for a significant correlation between the genetic and geographical pair-wise distances, as indicated by Mantel's test ($P = 0.052$).

The neighbour-joining (NJ) tree derived from the kinship distances is given in Figure 2. The tree topology revealed two main clusters, although the relationships between breeds were not always supported by high bootstrap values. The heavy-body sized chicken breeds, Luyuan, Xiaoshan, Beijing Fatty, Daggu, Henan Game, Langshan and Huainan Partridge formed one cluster; and the light-body sized chicken breeds, including Xianju, Baier, Taihe Silkies, Tibetan, Chahua, and Red Jungle Fowl, formed the second main cluster. The two medium-sized chicken breeds, Gushi and Wannan Three-yellow, clustered with the light-body sized chicken breeds.

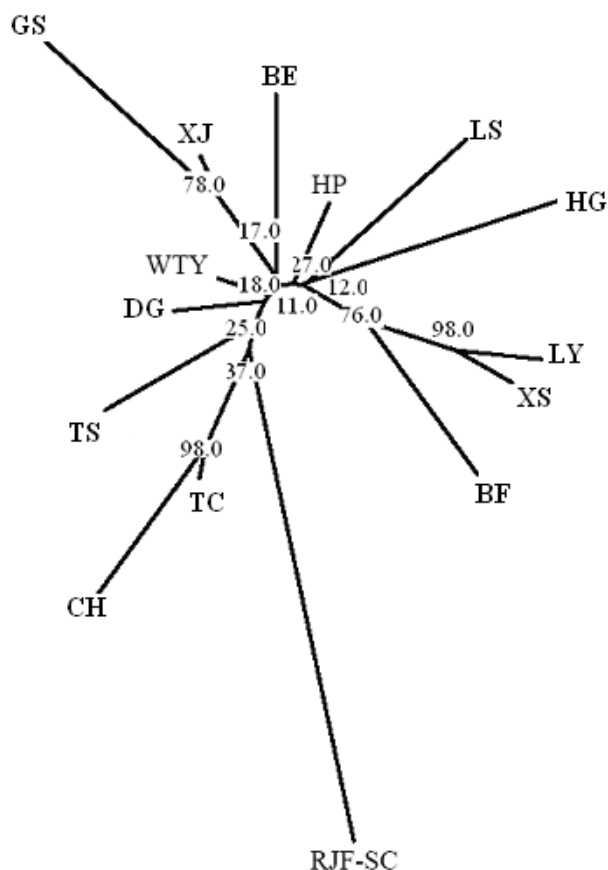


Figure 2. Neighbour-Joining tree of 15 chicken populations based on Marker Estimated Kinships

DISCUSSION

Genetic diversity within populations

The average PIC was 0.50. Fairly high PIC values for the majority of the markers employed are suggestive of their use in biodiversity evaluation of native Chinese chicken breeds. The average expected heterozygosity within populations exceeded the value reported for the 52 European chicken breeds using DNA pools typed at 22 microsatellite loci (Hillel et al., 2003), and was also higher than the values estimated for commercial breeds (Crooijmans et al., 1996).

As for special breeds, the Wannan Three-yellow chicken had the highest genetic variability, and the Gushi chicken had the lowest one. The reason may be that the Wannan Three-yellow has a large number of individuals and a broad distribution area, whereas special geographical conditions limited the red jungle fowl and Gushi in a relatively isolated region and they had a smaller opportunity for genetic exchange with other populations, which can also be demonstrated by the lower N_m values for red jungle fowl (0.4907 to 1.0756) and Gushi chicken (0.4967 to 1.7232), compared with other chicken breeds. Tibetan chicken, on the other hand, has a large area in the Tibet autonomous region of China. Huainan Partridge has just been founded in recent years. Little selection was performed on these chicken breeds. Any of these factors might explain why Huainan Partridge and Tibetan chicken had the higher gene diversity and next to the largest number of mean alleles. And this may also be related to their highly varying production performance and morphological variation within the populations (Du et al., 2004). In Tibetan chicken and Huainan Partridge there has been a gene flow from other chicken breeds (the values for gene flow range from 1.0756 to 4.7600 and from 0.9332 to 4.7600, respectively; six of them are over 2.0 for each breed) and there was a lack of management during domestication. Thus, Tibetan chicken and Huainan Partridge appear as mixed breeds.

Departures from HWE may be due to a variety of causes: small population size, assortative mating system (including inbreeding and outbreeding), selection, and existence of 'null alleles'.

Genetic differentiation among populations

In our study, on average, the genetic differentiation (F_{ST}) among breeds was 16.4% (Table 2),

a highly significant value ($P < 0.001$) which indicates that there is great differentiation (Wright, 1978; Hartl and Clark, 1997) among 15 Chinese indigenous chicken breeds. It is clear that about 16% of the total genetic variation corresponds to differences among breeds and the remaining 84% is the result of differences among individuals. All loci contribute to this differentiation significantly.

As for the coefficient F_{IS} , which indicates the degree of departure from random mating, positive F_{IS} values mean a significant deficit of heterozygotes, while the negative F_{IS} values indicate an excess of heterozygous genotypes with respect to the expected values. In this study the high average of F_{IS} was 0.020. In addition, nine loci showed a significant deficit of heterozygotes. Two assumptions could explain the deficit of heterozygotes for these nine loci: firstly, the locus may be under selection due to association with some morphological or productive traits (genetic hitchhiking effect); secondly, 'null alleles' may be present (Nei, 1987).

Relationships among populations

Tibetan chicken and Chahua chicken, Xiaoshan chicken and Luyuan chicken are genetically closely related, respectively. In the NJ-tree, Tibetan and Chahua chickens clustered together and were supported by a high bootstrap value of 98.0 percent. Luyuan and Xiaoshan chicken clustered together with 98.0 percent bootstraps. From geographical locations, the Yunnan province (Chahua chicken) is a neighbour to the Tibetan province, so it was easy for these two chicken breeds to mix. This relationship is supported by a high value for gene flow, N_m (4.3625). Xiaoshan chicken and Luyuan chicken can be considered as genetically similar (Rosenberg et al., 2001). The main original locations Xiaoshan city and Zhangjiagang city, for Xiaoshan chicken and Luyuan chicken respectively, had the second nearest geographical distance among all pairs of chicken breeds. Furthermore, similar culture and living customs at these two places make it easy to communicate with each other. The gene flow between these two breeds is very high (5.1033). Environmental effects, historical process and life histories (e.g. mating system) may all constitute the genetic structure of populations to some extent (Balloux and Lugon-Moulin, 2002).

Geographical elements may owe to the close relationship for particular population pairs, for

Table 4. Reynolds' genetic distances (D_R) and the gene flow (Nm) between breeds, numbers in bold face are the highest and lowest values of D_R and Nm

Breed	XJ	CH	LY	GS	TC	BE	DG	HG	LS	TS	XS	BF	HP	RJF	WTY
XJ		0.1878	0.1579	0.1355	0.0512	0.1083	0.1231	0.1969	0.1576	0.1385	0.1649	0.2057	0.0993	0.3016	0.0882
CH	1.2103		0.2419	0.3255	0.0557	0.2134	0.1849	0.2710	0.2649	0.1983	0.2448	0.2663	0.1860	0.3000	0.1691
LY	1.4612	0.9133		0.2441	0.1470	0.1729	0.1317	0.2155	0.1845	0.1960	0.0478	0.1455	0.1223	0.3235	0.1151
GS	1.7232	0.6499	0.9042		0.1871	0.2530	0.2013	0.2515	0.2755	0.2120	0.2375	0.2771	0.1987	0.4077	0.1488
TC	4.7600	4.3625	1.5788	1.2154		0.1055	0.0988	0.1850	0.1636	0.1186	0.1485	0.1764	0.0829	0.2090	0.0774
BE	2.1866	1.0507	1.3243	0.8686	2.2475		0.1238	0.2127	0.1681	0.1794	0.1457	0.2304	0.1067	0.3143	0.0965
DG	1.9089	1.2310	1.7759	1.1214	2.4067	1.8978		0.1428	0.1506	0.1128	0.1094	0.1373	0.0731	0.2201	0.0507
HG	1.1490	0.8031	1.0393	0.8741	1.2302	1.0548	1.6283		0.2057	0.2172	0.1983	0.2215	0.1475	0.3353	0.1416
LS	1.4647	0.8243	1.2337	0.7882	1.4067	1.3660	1.5383	1.0948		0.1875	0.1796	0.2080	0.1187	0.3080	0.1341
TS	1.6835	1.1397	1.1545	1.0589	1.9861	1.2725	2.0930	1.0307	1.2120		0.1749	0.2059	0.1244	0.2704	0.0989
XS	1.3947	0.9015	5.1033	0.9326	1.5616	1.5937	2.1631	1.1397	1.2707	1.3076		0.1425	0.1169	0.2840	0.0886
BF	1.0948	0.8193	1.5964	0.7831	1.2961	0.9648	1.6986	1.0082	1.0812	1.0934	1.6325		0.1315	0.3008	0.1294
HP	2.3955	1.2232	1.9220	1.1373	2.8907	2.2204	3.2961	1.5735	1.9841	1.8868	2.0165	1.7792		0.2374	0.0512
RJF	0.7101	0.7145	0.6545	0.4967	1.0756	0.6770	1.0152	0.6275	0.6930	0.8053	0.7613	0.7123	0.9332		0.2009
WTY	2.7121	1.3567	2.0499	1.5577	3.1057	2.4674	4.8107	1.6439	1.7420	2.4039	2.6981	1.8093	4.7600	1.1236	

the data above and below the diagonal are Reynolds' genetic distances (D_R) and the gene flow (Nm) between breeds, respectively

instance Tibetan chicken and Chahua chicken, Xiaoshan chicken and Luyuan chicken. Though Huainan Partridge and Wannan Three-yellow chicken had the nearest geographical distance (in the neighbouring cities of Anhui Province) among all the pairs of chicken breeds, these two breeds did not show any close genetic relationship and clustered together in the NJ-tree. The result from Mantel's test failed to support a significant correlation between genetic and geographical pair-wise distances for the whole dataset. All these results indicated that the geographical distribution was not a decisive factor to influence the genetic structure of Chinese chicken populations during their cultural history.

In the long history of animal domestication and breeding, the majority of the main original areas of livestock were relatively isolated regions without convenient transportation. Many local breeds were developed because of diversified geographical conditions and lack of gene flow. For poultry, the gene flow was easier to accomplish by carrying eggs from one area to another. The results of this study also indicated that there was no significant correlation between the genetic and geographical pair-wise distances among Chinese chicken populations.

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- Received: 2007–01–19
Accepted after corrections: 2007–11–19

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