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Analysis of Pairwise Genetic Distance and its Relation with Geographical Distance of 15 Chinese Chicken Breeds

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Abstract: Genetic distance and gene flow of all pairwises of 14 Chinese indigenous chicken breeds and one jungle fowl and their relations with geographical distances were evaluated in the present study. The pairwise $F_{ST}/(1-F_{ST})$ of 15 Chinese chicken breeds was evaluated by 29 microsatellite loci. The results showed that the number of alleles per locus was ranged from 2-25 and the average of expected heterozygosity and PIC of all loci were 0.6683 and 0.50, respectively. The average of genetic differentiation among population measured as FST value, was 16.4% ($p<0.001$), all loci were contributed significantly ($p<0.001$) to this differentiation. Red jungle fowl and Gushi chickens were observed distant genetic relationship with other breeds, whereas Huainan Partridge and Tibetan chickens were observed close relationship with other breeds. The geographical elements may own the close relationship for particular population pairs. However, the equation $F_{ST}/ (1-F_{ST}) = -0.0162 + 0.0313\ln (d)$ and the result of Mantel's test ($p = 0.054$) did not provide enough support for a significant correlation between the genetic and geographical pairwise distances.

Key words: Indigenous chicken breeds, red jungle fowl, genetic distance, gene flow, geographical distance

INTRODUCTION

China has a wide variety of indigenous poultry resources, is a result of long history of animal husbandry and diversified geographical conditions. There are 108 native chicken breeds in China (Chen *et al*., 2004a) most of them have valuable genetic features. For instance, Taihe Silkies in Jiangxi province is an important source of traditional Chinese medicine (Li, 1983). Some populations have decreased rapidly while some populations are even facing extinction, due to the introduction of modern commercial chicken breeds and the limitation of conservation measures. Therefore, decisions on conservation rely upon the degree of endangerment, adaptation to a specific environment, traits of economic importance, cultural or historical value of the breed, the contribution of populations to national and overall genetic diversity is an important step in determining priorities for conservation. Some poultry resource centres in China were set up according to their geographical distribution in last decades. Molecular markers were an important guide to evaluate breeds as genetic resources (Barker, 1994; Ruane, 1999; Weigend and Romanov, 2001). Considering the charactesristics of high polymorphism, locus specificity, abundance and random distribution over the genome and their co-dominant inheritance, microsatellites are currently most commonly used to assess population structure and diversity (Romanov and Weigend, 2001; Chen *et al*., 2004b). In addition, 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 method also provides information for preservation priorities for livestock breeds (Barker, 1999).

The aim of this study, was to evaluate genetic distance and gene flow of 14 Chinese indigenous chicken breeds and one jungle fowl with 29 microsatellite markers and to analyze the relationship between 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 conversation.

MATERIALS AND METHODS

Experimental populations: A total 542 individuals from 15 Chinese chicken breeds were genotyped. All breeds except Wannan (three-yellow chickens), Huainan Partridges and red jungle fowls are from Poultry Institute,

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Table 1: Description of 15 indigenous Chinese chicken breeds

Breed	Origin of breeds	Specific features	Sample size
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, Sichuan	light-sized, selected for yellow plumage, meat and egg dual-purpose breed	38
Baier chicken (BE)	Shangrao city Jiangxi	three yellow, light-sized, layer breed, white earlobe	34
Dagu chicken (DG)	Zhuanghe county, Liaoning	heavy-sized, meat and egg dual-purpose breed	35
Henan Game (HG)	Zhengzhou city, Henan	heavy-sized, fancy breed	33
Langshan chicken (LS)	Rudong county, Jiangsu	heavy-sized, meat and egg dual-purpose breed	40
Taihe Silkies(TS)	Taihe county Jiangxi	light-sized, medicine and entertainment breed	40
Xiaoshan chicken (XS)	Xiaoshan county, Zhejiang	heavy-sized, meat and egg dual-purpose breed	40
Beijing Fatty chicken (BF)	Chaoyang, Beijing	heavy-sized, meat and egg dual-purpose breed	38
Huainan Partridge(HP)	Huainai city, Anhui	heavy-sized, meat and egg dual-purpose breed	32
Gallus gallus spadiceus (RJF-SC)	Shimao county, Yunnan	Red jungle fowl(wild)	30
Wannan Three-yellow chicken(WTY)	Qinyan county, Anhui	medium-sized, egg purpose breed	32

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

Academy of Chinese Agricultural Sciences, Yangzhou, P.R. China; Wannan (three-yellow Chickens) are from Poultry Resource Centre in Qinyan County, Anhui Province; Huainan Partridges are from Poultry Resource Centre, Institute of Agricultural Science, Huainan city, Anhui Province; and Red jungle fowls are from in Wild Animal Conservation Centre, Yunnan Province P. R. China. The information of breeds, origin, specific features and number of individuals studied were presented in Table 1.

DNA isolation: Approximately 0.4 mL of blood was collected from each individual into 4 mL DNA lysate solution [2 M urea, 100 mM Tris-HCl (pH 8.0), 1% SDS, 100 M EDTA], stored at 4 °C. DNA was isolated from the whole blood according to the phenol/chloroform method (Sambrook *et al*., 2001).

Genotyping: Twenty nine microsatellite markers spread across the chicken genome were used for genotypes (Table 2). PCR products were obtained in 8 μL volume using thermal cycler (Master cycler, Eppendorf, Hamburg, Germany). Each PCR tube contained 20ng of genomic DNA, 10pmol of each forward primer labeled either IRD700 or IRD800 (MWG-Biotech, Ebensburg, Germany), 10pmol of unlabeled reverse primer and 1mM tetra-methyl-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). Special DNA fragments produced by amplification were visualized on 8% poly-acrylamide gel, which was performed with a LI-COR automated DNA analyzer (LI-COR Biotechnology Division, Lincoln, NE68504). Electrophoregram processing and allele-size scoring was performed with the RFLP scan package (Scanalytics, Division of CSP, Billerica, MA).

Statistical analysis

Genetic diversity: Allele frequency was obtained by direct counting of the number of different size. The observed and expected heterozygosity (Nei, 1987) for each locus across populations were estimated with Microsatellite-Toolkit for Excel (Park, 2001). Polymorphism Information Content (PIC) for each locus and each breed was obtained according to Botstein *et al*. (1980).

Genetic differentiation: F-statistics indices were estimated in the form of F, θ and f (Wright, 1978), sample-based respective estimators of these parameters were proposed by Weir and Cockerham (1984) and implemented in FSTAT program Version 2.9.3 (Goudet, 2002). Significance of F-statistics was determined from permutation tests with the sequential Benferroni procedure applied over loci (Hochberg, 1988). The F_{ST} values among pairs of breeds were calculated with 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 (Nm), was estimated based on the n island model of population structure. The estimate was based on the relationship $F_{ST} = 1/(4Nm+1)$, where N is the effective population size, m is the migration rate and F_{ST} is the mean

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

RESULTS

Genetic variability within breeds: A total of 277 alleles were detected in 15 Chinese indigenous chicken breeds with 29 microsatellite markers. Expected Heterozygosity (He) and mean Polymorphic Information Content (PIC) for each locus across 15 breeds were presented in (Table 2). The number of alleles per locus was ranged from 2 (MCW0103 and MCW 0098) to 25 (LEI0234) and the average number of observed alleles in 29 microsatellite loci was 9.55±5.82. Across breeds, MCW0098 locus had the lowest He and PIC, while, LEI0234 locus had the highest He and PIC.

The F_{IS} value was calculated as a measure of deviation from Hardy-Weinberg equilibrium. The negative F_{IS} values of some breeds indicated an excess of heterozygous genotypes with respect to the expected value. The values for the most breeds even if statistically significant were not far from 0, indicating that mating is close to panmixia.

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 15 Chinese chicken breeds were presented in (Table 2). The fixation coefficients of subpopulations within total population, measured as F_{ST} value for 29 loci were varied from 0.101 (MCW0020) to 0.319 (MCW0081), with mean 0.164 ($p<0.001$), all loci were contributed significantly to this differentiation. The global deficit of heterozygotes across populations (F_{IT}) was 18% ($p<0.001$) and overall significant deficit of heterozygotes (F_{IS}) was 2% ($p<0.001$) occurred in the analyzed loci because of inbreeding within populations. To some extent, nine loci were showed excess of heterozygotes with a negative value.

Estimated of gene flow (Nm) and Reynolds' genetic distances (D_R) between each population pair was presented in (Table 3). Reynolds' distance values were varied between 0.0478 (Xiaoshan chicken-Luyuan chicken pair) to 0.3353 (Red jungle fowl-Henan game chicken pair). The Nm value was ranged from 0.4967 (between Red jungle fowl and Gushi chicken pair) to 5.1033 (between Xiaoshan chicken and Luyuan chicken pair), most gene flow (Nm) values between pairs of breeds were below 2.0.

Table 2: Number of alleles, allele sizes, F-statistics, expected Heterozygositiy (He) and mean Polymorphic Information Content (PIC) in 29 microsatellite markers in 15 Chinese chicken breeds

Markers	No. of alleles	Allele size (bp)	F_{IT}=*F*	F_{ST}	F_{IS}=*f*	He	Mean PIC
MCW0103	2	266-270	0.323***	0.205***	0.148**	0.3945	0.25
MCW0216	8	137-149	0.178***	0.136***	0.049*	0.5622	0.41
MCW0295	12	88-110	0.212***	0.130***	0.094***	0.7168	0.57
ADL0278	12	114-129	0.152***	0.218***	-0.085	0.6617	0.45
MCW0222	4	220-226	0.217***	0.217***	-0.001	0.6085	0.46
MCW0037	6	154-159	0.225***	0.172***	0.064*	0.6703	0.52
ADL0268	8	104-118	0.071**	0.108***	-0.042	0.7245	0.52
MCW0183	14	296-324	0.107**	0.116***	-0.01	0.7297	0.53
MCW0014	11	160-186	0.230***	0.222***	0.01	0.6707	0.5
MCW0067	6	178-186	0.137***	0.161***	-0.028	0.642	0.5
MCW0098	2	263-265	0.319***	0.319***	0	0.3032	0.22
LEI0166	6	356-376	0.145***	0.224***	-0.101	0.6081	0.42
MCW0069	9	158-176	0.112***	0.138***	-0.03	0.7646	0.6
MCW0081	6	114-135	0.117***	0.128***	-0.013	0.4524	0.26
ADL0112	4	124-132	0.145***	0.160***	-0.018	0.5043	0.32
MCW0034	17	212-246	0.133***	0.114***	0.021	0.8259	0.66
MCW0111	12	96-120	0.232***	0.142***	0.105***	0.728	0.57
MCW0078	5	135-143	0.177***	0.137***	0.047	0.6553	0.48
MCW0206	11	221-247	0.213***	0.163***	0.060***	0.7019	0.57
LEI0094	20	247-289	0.204***	0.184***	0.025	0.8852	0.72
MCW0248	5	215-223	0.164***	0.172***	-0.01	0.6973	0.44
LEI0234	25	216-380	0.102***	0.160***	-0.069	0.9111	0.74
MCW0330	7	258-290	0.125***	0.101***	0.027	0.7346	0.54
MCW0016	11	162-188	0.226***	0.111***	0.129***	0.728	0.55
MCW0104	19	190-232	0.068***	0.107***	-0.044	0.8171	0.65
MCW0020	4	179-185	0.261***	0.255***	0.009	0.619	0.49
MCW0165	3	114-118	0.301***	0.205***	0.120***	0.5696	0.42
MCW0080	17	265-281	0.139***	0.120***	0.021	0.7151	0.61
MCW0123	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)

Note: F, total inbreeding; F_{ST}, population differentiation; f, within-population inbreeding, Mean jack-knife estimates over loci; standard deviations are given in parentheses, Significance of F statistics was done using Bonferroni permutations based on 1000 resamplings, * $p<0.05$; **$p<0.01$; *** $p<0.001$

Table 3: Reynolds' genetic distances and the gene flow

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	0.271	0.2649	0.1983	0.2448	0.2663	0.186	0.3	0.1691
LY	1.4612	0.9133		0.2441	0.147	0.1729	0.1317	0.2155	0.1845	0.196	0.0478	0.1455	0.1223	0.3235	0.1151
GS	1.7232	0.6499	0.9042		0.1871	0.253	0.2013	0.2515	0.2755	0.212	0.2375	0.2771	0.1987	0.4077	0.1488
TC	4.76	4.3625	1.5788	1.2154		0.1055	0.0988	0.185	0.1636	0.1186	0.1485	0.1764	0.0829	0.209	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	1.231	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	1.149	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	1.366	1.5383	1.0948		0.1875	0.1796	0.208	0.1187	0.308	0.1341
TS	1.6835	1.1397	1.1545	1.0589	1.9861	1.2725	2.093	1.0307	1.212		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	0.284	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	1.922	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	0.677	1.0152	0.6275	0.693	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	1.742	2.4039	2.6981	1.8093	4.76	1.1236	

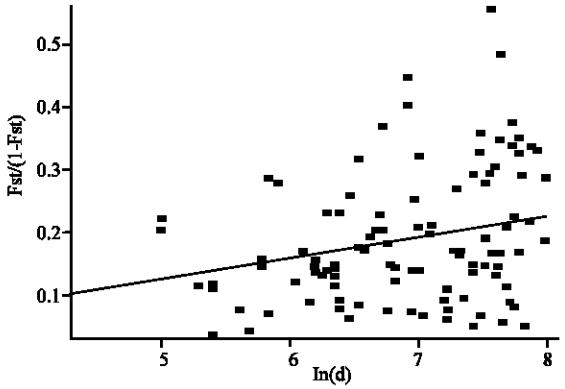

Fig. 1: The relationship between geographical distance ln(d) and pairwise $F_{ST}/(1-F_{ST})$ for all pairs of Chinese indigenous chicken breeds. The fitted line was correspond to the equation $F_{ST}/(1-F_{ST})$ = -0.0162+ 0.0313ln (d)

The application of Rousset's isolation by distance method, as implemented in GENEPOP program, allowed the parameters α and β in the regression, $F_{ST}/(1-F_{ST})$ = -0.0162+ 0.0313ln(d) (Fig. 1). However, the regression was 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 diagonal in Table 3 is Reynolds' genetic distances and below the diagonal is gene flow, Nm between breeds.

Numbers in bold face are highest and lowest values of D_R and Nm.

DISCUSSION

Genetic diversity within breeds: Fairly the high PIC values for majority of markers employed are suggestive of their utility in biodiversity evaluation of native Chinese chicken breeds. The average gene diversity (expected heterozygosity) within populations exceeds the value reported for 52 European chicken breeds (Hillel *et al*., 2003) and commercial chicken breeds (Crooijmans *et al*., 1996). The number of observed alleles in 15Chinese native chickens (9.55) was greater than that observed in 11 Chinese native chicken breeds using 20 microsatellite markers (Gao *et al*., 2004) and 12 Chinese native chicken breeds using 7 microsatellite markers (Wu *et al*., 2004), whereas it was lower than that observed in 78 Chinese native chicken breeds using 27 microsatellite markers (Qu *et al*., 2006). In the exact test of deviation from Hardy-Weinberg equilibrium, more or less populations showed significant deviation for all loci, except MCW0183, MCW0081, MCW0098, LEI0166, MCW0248 and MCW0080(data not shown). Departures from HWE, maybe due to small population size, assortative mating system (including inbreeding and out breeding), selection and existence of 'null alleles'.

Genetic differentiation among populations: In our study, the genetic differentiation (F_{ST}) among breeds was 16.4% (Table 2), is relatively high value and extremely significant ($p<0.001$), which indicated that there is a 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 was corresponds to differences of breeds and the remaining 84% is a result of differences among individuals and all loci were contribute significantly to this differentiation.

The coefficient F_{IS} was indicate the degree of departure from random mating, the positive F_{IS} value mean a significant deficit of heterozygotes, while the negative F_{IS} value was indicate an excess of heterozygous genotypes with respect to the expected value. In this study high average of F_{IS} was 0.020. In addition, nine loci MCW0103, MCW0295, MCW0222, MCW0014, LEI0094, LEI0234, MCW0165, MCW0037 and MCW0216 were showed significant deficit of heterozygotes. Two reasons maybe contribute to the deficit of heterozygotes for these nine loci: first, the locus may be under selection (genetic

hitchhiking effect) with some morphological or productive traits of selective interest; secondly, 'null alleles' may be present (Nei, 1987).

Chickens relationship: Tibetan chicken and Chahua, Xiaoshan chicken and Luyuan chicken had a close genetic relationship. From geographical locations, Yunnan province (Chahua chicken), is neighbour to Tibetan and this was facilitate migration these two chicken breeds. The high gene flow, Nm (4.3625) between Chahua chicken and Tibetan chicken, also supported that there may be genetic migration between these two chicken breeds.

Xiaoshan chickens and Luyuan chickens were genetically similar (Rosenberg *et al*., 2001), their origin were Xiaoshan city and Zhangjiagang city, respectively. Have the second nearest geographical distance among all pairwises of chicken breeds and the gene flow between these two breeds is very high, 5.1033.

Geographical elements may owe to close relationship for particular population pairs. Huainan Partridge and Wannan Three Yellow chicken had the nearest geographical distance (in the neighbour cities of Anhui Province) among all pairs of chicken breeds, these two breeds had not showed close genetic relationship. The result of Mantel's test was 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 cultured history.

In the history of animal domestication and breeding, most original areas of livestock were relative separated regions without the convenient transportation. Therefore, many local breeds were developed because of diversified geographical conditions and lack of gene flow. For poultry, the gene flow was more convenient accomplish by carrying eggs from one area to other area, compared to other livestock. 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. Therefore, the geographical condition was only a reference when we set up the program of chicken conservation, the genetic distance should be served as the most important guide in determining priorities for conservation of Chinese indigenous chicken breeds.

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