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Assessment of Population Structure and Genetic Diversity of 15 Chinese Indigenous Chicken Breeds Using Microsatellite Markers

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ABSTRACT : The genetic structure and diversity of 15 Chinese indigenous chicken breeds was investigated using 29 microsatellite markers. The total number of birds examined was 542, on average 36 birds per breed. A total of 277 alleles (mean number 9.55 alleles per locus, ranging from 2 to 25) was observed. All populations showed high levels of heterozygosity with the lowest estimate of 0.440 for the Gushi chickens, and the highest one of 0.644 observed for Wannan Three-yellow chickens. The global heterozygote deficit across all populations (F_{IT}) amounted to 0.180 ($p<0.001$). About 16% of the total genetic variability originated from differences between breeds, with all loci contributing significantly to this differentiation. An unrooted consensus tree was constructed using the Neighbour-Joining method and pair-wise distances based on marker estimated kinships. Two main groups were found. The heavy-body type populations grouped together in one cluster while the light-body type populations formed the second cluster. The STRUCTURE software was used to assess genetic clustering of these chicken breeds. Similar to the phylogenetic analysis, the heavy-body type and light-body type populations separated first. Clustering analysis provided an accurate representation of the current genetic relations among the breeds. Remarkably similar breed rankings were obtained with all methods. (**Key Words :** Chicken, Microsatellites, Genetic Differentiation, Genetic Structure)

INTRODUCTION

With its long history of animal husbandry and diversified geographical conditions, China has a wide variety of indigenous poultry resources. There are 108 native chicken breeds recorded in China (Chen et al., 2004a). The majority of these chickens are local and fancy breeds characterized by medium to low performances. They are usually maintained in small populations. Many of these local chicken varieties have valuable genetic features. Taihe Silkies in Taihe county of Jiangxi province, for instance, are not only used for entertainment, but are also used as an important source of traditional Chinese medicine (Li, 1983). However, the population sizes of some indigenous chicken breeds have been rapidly decreasing. According to the report of the Ministry of Agriculture, Beijing Fatty chickens, Lingkun chickens, Pudong chickens, Ningjing chickens and Zhangmu chickens are even facing extinction (The State of Animal Genetics Resource in China, Ministry of Agriculture of China, 2004). The decrease in population sizes of indigenous chickens is mainly attributed to the introduction of modern commercial chicken breeds and the limited resources available for conservation measures.

Genetic variation is the basic material for animal breeding and influences the viability of populations. Further loss of local chicken breeds will reduce the overall chicken diversity. Conservation measures are however expensive to implement and as a result not all breeds or populations will be included. Unique and genetically diverse populations should therefore be identified in order to cover the widest range of genetic variability. The accurate evaluation of populations with regard to their contribution to national and overall genetic diversity is an important step in determining priorities for conservation (Weigend et al., 1995).

In the process of developing strategies to conserve genetic diversity in domestic chickens, it is important to assess the genetic uniqueness of a given population, which may be deduced from genetic distances (Hillel et al., 2003).

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Table 1. Description of the 15 indigenous Chinese chicken breeds

Breed (Abbreviation)	Main original area	Specific features	Number of animals studied
Xianju chicken (XIA)	Xianju county, Zhejiang	Three yellow*,light-sized, layer breed	38
Chahua chicken (CHA)	Xishuangbanna, Yunnan	Light-sized, meat and egg dual-purpose breed	38
Luyuan chicken (LUY)	Zhangjiagang city, Jiangsu	Heavy-sized, meat and egg dual-purpose breed	34
Gushi chicken (GUS)	Gushi county, Henan	Three yellow*, medium-sized, meat and egg dual-purpose breed	40
Tibetan chicken (TIB)	Ganzi and Aba Tibetan autonomous region	Light-sized, selected for yellow plumage, meat and egg dual-purpose breed	38
Baier chicken (BAI)	Shangrao city, Jiangxi	Three yellow*, light-sized, layer breed, white earlobe	34
Dagu chicken (DAG)	Zhuanghe county, Liaoning	Heavy-sized, meat and egg dual-purpose breed	35
Henan game (DOU)	Zhengzhou city, Henan	Heavy-sized, fancy breed	33
Langshan chicken (LAN)	Rudong county, Jiangsu	Heavy-sized, meat and egg dual-purpose breed	40
Taihe silkies (WUG)	Taihe county, J iangxi	Light-sized, medicine and entertainment breed	40
Xiaoshan chicken (XIS)	Xiaoshan county, Zhejiang	Heavy-sized, meat and egg dual-purpose breed	40
Beijing fatty chicken (YOU)	Chaoyang, Beijing	Heavy-sized, meat and egg dual-purpose breed	38
Huainan partridge (HP)	Huainan 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 (WTY)	Qinyan county, Anhui	Medium-sized, egg purpose breed	32

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

According to FAO recommendations (FAO, 2004), determination of genetic distances using neutral, highly polymorphic microsatellite markers is currently 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).

Studies on chicken biodiversity based on microsatellite marker included estimation of genetic diversity in commercial broiler and layer lines (Crooijmans et al., 1996), assessment of conversation efficiency of Dagu chicken and Beijing Fatty chicken (Qu et al., 2004), and analysis of genetic relationships among highly inbred chicken lines (Zhou et al., 1999), among African, Asian and South American local chickens (Wimmers et al., 2000), between various populations of domestic and jungle fowl (Romanov and Weigend, 2001), in 52 chicken populations (Hillel et al., 2003), and in Chinese native chicken populations (Du et al., 2004; Qu et al., 2006).

Chen et al. (2004b) did a preliminary study on 12 of the 15 breeds in this study, using a panel of seven microsatellite markers. Since more markers and more sophisticated methods are available nowadays, this study aims to more reliably assess genetic diversity and estimate the genetic structure of these Chinese indigenous chicken breeds. The results may help to understand genetic differentiation of local breeds in China and contribute to more efficient conversation strategies.

MATERIALS AND METHODS

Chicken population

A total of 542 individuals originating from 15 Chinese indigenous chicken breeds were analysed in this study. Information about breeds, main original area of their distribution in China, specific features, and number of individuals sampled are presented in Table 1. All breeds except for Wannan Three-yellow chickens, Huainan Partridges and Red Jungle Fowls were kept at the Poultry Institute, Academy of Chinese Agricultural Sciences, Yangzhou, P. R. China. The Wannan Three-yellow chickens were kept at the Centre of Poultry Resource in Qinyan County, Anhui Province. The Huainan Partridges were maintained at the Institute of Agricultural Science in Huainan city, Anhui Province. The Red Jungle Fowl (*Gallus gallus spadiceus*) was collected from Wild Animal Conservation Centre, Yunnan Province P. R. China.

DNA isolation

Per individual, 0.4 ml whole blood was collected from the ulnar vein with heparin as anticoagulant. Then, 4 ml of DNA lysate solution (2 M urea, 100 mM Tris-HCl (pH 8.0), 1% SDS, 100 mM EDTA) was added, and the mixture was stored at 4°C. DNA was isolated by using a phenol/chloroform based method (Sambrook et al., 2001).

Genotyping

The DNA polymorphism was assessed at 29 microsatellite loci (Table 2). These markers are randomly distributed across the chicken genome, and 28 of these markers are part of the set of 30 microsatellites recommended by FAO (2004). Several multiplex PCR were carried out including two to five pairs of primers per reaction. Each PCR tube contained 20 ng of genomic DNA, 10 pmol of each forward primer labeled with either IRD700 or IRD800 (MWG-Biotech, Ebersberg, Germany), 10 pmol

Table 2. Number of alleles, range of allele sizes (bp), and F-statistics, for each of the 29 microsatellite markers in 15 Chinese chicken breeds

Markers	Total No. of alleles	Range of allele sizes (bp)	$F_{IT} = F$	$F_{ST} = \theta$	$F_{IS} = f$
MCW0103	2	266-270	0.323***	0.205***	0.148**
MCW0216	8	137-149	0.306***	0.190***	0.144***
MCW0295	12	88-110	0.178***	0.136***	0.049*
ADL0278	12	114-129	0.261***	0.255***	0.009
MCW0222	4	220-226	0.212***	0.130***	0.094***
MCW0037	6	154-159	0.301***	0.205***	0.120***
ADL0268	8	104-118	0.152**	0.218***	-0.085
MCW0183	14	296-324	0.217**	0.217***	-0.001
MCW0014	11	160-186	0.225***	0.172***	0.064*
MCW0067	6	178-186	0.071**	0.108***	-0.042
MCW0098	2	263-265	0.107**	0.116***	-0.010
LEI0166	6	356-376	0.230***	0.222***	0.010
MCW0069	9	158-176	0.137***	0.161***	-0.028
MCW0081	6	114-135	0.319***	0.319***	-0.000
ADL0112	4	124-132	0.145***	0.224***	-0.101
MCW0034	17	212-246	0.112***	0.138***	-0.030
MCW0111	12	96-120	0.117***	0.128***	-0.013
MCW0078	5	135-143	0.145***	0.160***	-0.018
MCW0206	11	221-247	0.133***	0.114***	0.021
LEI0094	20	247-289	0.232***	0.142***	0.105***
MCW0248	5	215-223	0.177***	0.137***	0.047
LEI0234	25	216-380	0.213***	0.163***	0.060***
MCW0330	7	258-290	0.204***	0.184***	0.025
MCW0016	11	162-188	0.164***	0.172***	-0.010
MCW0104	19	190-232	0.102***	0.160***	-0.069
MCW0020	4	179-185	0.125***	0.101***	0.027
MCW0165	3	114-118	0.226***	0.111***	0.129***
MCW0080	17	265-281	0.139***	0.120***	0.021
MCW0123	11	76-98	0.068***	0.107***	-0.044
Mean	9.55		0.180	0.164	0.020
(std. dev.)	(5.82)		(0.013)***	(0.009)***	(0.012)***

* p<0.05; ** p<0.01; *** p<0.001.

of each unlabeled reverse primer, and 1mM tetramethylammoniumchloride. The amplification protocol comprised of an initial denaturation and enzyme activation phase at 95°C (15 min), followed by 35 cycles of denaturation at 95°C (1 min), primer annealing at temperature varying between 58°C and 64°C (1 min), and extension at 72°C (1 min), and a final extension at 72°C for 10 minutes. DNA fragments were visualized as bands on 8% polyacrylamide gel performed on a LI-COR DNA analyzer (LI-COR Biotechnology Division, Lincoln, NE). Electrophoregram processing and allele-size scoring was performed with the RFLPscan software package (Scanalytics, Division of CSP, Billerica, USA).

Statistical analysis

Genetic diversity : Total number of alleles, allele frequencies, average number of alleles per locus, observed (Ho) and expected heterozygosity (He) for each population across the loci, were estimated with Microsatellite-Toolkit for Excel (Park, 2001).

Genetic differentiation : Population differentiation was estimated by Wright's (1978) fixation indices F_{IT}, F_{ST} and F_{IS} in the form of F, θ, and f, respectively, for each locus across populations according to the variance based method of Weir and Cockerham (1984) using FSTAT software (Version 2.9.3, Goudet, 2002). The significance of the F-statistics was determined by permutation tests with the sequential Bonferroni procedure applied over loci (Hochberg, 1988). The extent of inbreeding was further studied with GENEPOP software (Raymond and Rousset, 1995) by estimating the F_{IS} values and their significance level within each of the populations.

Pair-wise F_{ST} values were computed for all combinations of the 15 populations using GENEPOP. 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 (Slatkin and Barton, 1989). 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 calculated as mean over loci.

Clustering of breeds : The program STRUCTURE

Table 3. Mean number of alleles per locus, mean estimates of expected (He) and observed (Ho) heterozygosity and F_{IS} estimates of 15 Chinese chicken population

Breed	Alleles/locus±SD	F_{IS}	He±SE	Ho±SE
XIA	4.00±2.19	0.059***	0.533±0.035	0.501±0.015
CHA	4.62±2.27	0.083***	0.553±0.041	0.502±0.015
LUY	4.41±2.03	0.085***	0.574±0.032	0.527±0.016
GUS	3.41±1.45	0.015	0.440±0.041	0.434±0.015
TIB	5.52±2.77	0.019**	0.614±0.035	0.603±0.015
BAI	4.21±2.34	0.073***	0.537±0.032	0.498±0.016
DAG	5.17±2.27	-0.011	0.634±0.032	0.640±0.015
DOU	3.83±1.83	0.004	0.531±0.035	0.529±0.016
LAN	4.17±1.93	-0.134	0.542±0.031	0.613±0.014
WUG	4.59±1.99	0.022*	0.577±0.030	0.564±0.015
XIS	4.48±1.86	0.000*	0.608±0.023	0.608±0.014
YOU	4.41±1.76	-0.036	0.553±0.027	0.572±0.015
HP	5.55±2.86	0.076***	0.618±0.031	0.572±0.016
RJF-SC	3.79±1.37	0.004**	0.538±0.033	0.536±0.017
WTY	6.28±3.18	0.061***	0.644±0.027	0.605±0.016

* p<0.05; ** p<0.01; *** p<0.001.

(Pritchard et al., 2000) which implements a model-based clustering method for inferring population structure using multilocus genotypes was utilized. This program uses a Monte Carlo Markov chain (MCMC) algorithm to assess the presence of a structure underlying the genetic information provided by the genetic markers. We ran STRUCTURE 100 times with 50,000 iterations, after a burn-in period of 20,000 iterations, for each number of genetic clusters (K) chosen a priori. Thereby, we analysed population structure for K values ranging from two to seven. A pair-wise comparison of the hundred solutions for each K value was done using SIMCOEFF software (Rosenberg et al., 2002). Solutions with over 95% similarity were considered as identical. The most frequent solution for each K was taken as the most probable clustering and visualized using DISTRUCT software (Rosenberg, 2004).

Additional sub-clustering were carried out in those subsets of the populations which did show population differentiation at level K = 7. The three new subsets analysed comprised Chahua chicken, Tibetan chicken, Xianju chicken, Gushi chicken and Baier chicken as the first one, Wannan Three-yellow chicken, Huainan Partridge chicken, Henan Game chicken and Dagu chicken as the second one, and Luyuan chicken, Xiaoshan chicken and Beijing Fatty chicken as the third subset. We ran STRUCTURE and SIMCOEFF as described above for each subset separately up to K = 5 for first subset, K = 4 and K = 3 for second and third subsets respectively.

Marker estimated kinships : Similarity indices between and within populations were calculated from allele frequencies using the Malecot's definition of similarity (Eding and Meuwissen, 2001):

$$S_{ij} = \sum_x \left(p_{i,x} p_{j,x}\right)$$

where $p_{i,x}$ is the x^{th} allele frequency in population i and $p_{j,x}$ is the x^{th} allele frequency in population j. These similarity indices were subsequently used to estimate Marker Estimated Kinships (MEK) among populations using a weighted log-linear model (Eding and Meuwissen, 2003). In this model, similarity estimates are decomposed in a mean coefficient of kinship f and the probability of alleles being alike in state and not identical by descent. Per locus similarities are weighted with the inverse of the expected error variance to account for variation in informativeness of different loci.

In order to construct a phylogenetic tree, the MEK were then converted to kinship distance using the formula:

$$D(i,j) = \hat{f}_{ii} + \hat{f}_{jj} - 2\hat{f}_{ij}$$

where $\hat{f}_{ii}$ and $\hat{f}_{jj}$ are the within kinship estimates of populations i and j, and $\hat{f}_{ij}$ is the between population i and population j kinship estimate (Mateus et al., 2004). We obtained an unrooted Neighbor-Joining cladogram (Saitou and Nei, 1987) based on pair-wise kinship distance matrix between populations using the Neighbor-Joining program implemented in PHYLIP (Felsenstein, 1995). A consensus tree, evaluated by 1,000 bootstraps across the set of loci, was constructed.

RESULTS

Genetic diversity within and among chicken breeds

A total of 277 alleles were observed in the 15 Chinese indigenous chicken breeds. All microsatellite loci typed were polymorphic (Table 2). The number of alleles per locus ranged from two (MCW0103 and MCW0098) to 25

Table 4. Matrix of gene flow (*Nm*) between breeds (below the diagonal) and marker estimated kinship within (diagonal) and between populations (above the diagonal) using the weighted log-linear model method of estimation

Breed	XIA	CHA	LUY	GUS	TIB	BAI	DAG	DOU	LAN	WUG	XIS	YOU	HP	RJF-SC	WTY
XIA	0.309	0.091	0.112	0.273	0.163	0.187	0.086	0.108	0.137	0.122	0.088	0.089	0.112	0.051	0.102
CHA	1.210	0.298	0.023	0.071	0.168	0.077	0.010	0.003	0.017	0.045	0.011	0.016	0.033	0.000	0.031
LUY	1.461	0.913	0.243	0.126	0.058	0.103	0.062	0.071	0.084	0.053	0.157	0.108	0.073	0.013	0.067
GUS	1.723	0.650	0.904	0.511	0.128	0.148	0.098	0.165	0.127	0.124	0.116	0.114	0.109	0.066	0.134
TIB	4.760	4.363	1.579	1.215	0.174	0.116	0.047	0.036	0.069	0.075	0.044	0.051	0.070	0.044	0.061
BAI	2.187	1.051	1.324	0.869	2.248	0.289	0.077	0.082	0.137	0.068	0.104	0.061	0.097	0.008	0.094
DAG	1.909	1.231	1.776	1.121	2.407	1.898	0.141	0.072	0.071	0.067	0.065	0.067	0.070	0.018	0.073
DOU	1.149	0.803	1.039	0.874	1.230	1.055	1.628	0.302	0.089	0.043	0.063	0.074	0.071	0.004	0.069
LAN	1.465	0.824	1.234	0.788	1.407	1.366	1.538	1.095	0.309	0.109	0.070	0.087	0.114	0.035	0.079
WUG	1.684	1.140	1.155	1.059	1.986	1.273	2.093	1.031	1.212	0.226	0.059	0.055	0.074	0.030	0.069
XIS	1.395	0.902	5.103	0.933	1.562	1.594	2.163	1.140	1.271	1.308	0.186	0.088	0.064	0.013	0.080
YOU	1.095	0.819	1.596	0.783	1.296	0.965	1.699	1.008	1.081	1.093	1.633	0.273	0.092	0.035	0.081
HP	2.396	1.223	1.922	1.137	2.891	2.220	3.296	1.574	1.984	1.887	2.017	1.779	0.156	0.016	0.082
RJF-SC	0.710	0.715	0.655	0.497	1.076	0.677	1.015	0.628	0.693	0.805	0.761	0.712	0.933	0.307	0.034
WTY	2.712	1.357	2.050	1.558	3.106	2.467	4.811	1.644	1.742	2.404	2.698	1.809	4.760	1.124	0.131

(LEI0234), and the average number of the alleles observed was 9.55.

The fixation indices (F_{IT}, F_{ST}, F_{IS}) for each locus across all populations are also shown 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 significantly to this differentiation. The global deficit of heterozygotes across populations (F_{IT}) amounted to 0.180 ($p<0.001$). Mean F_{IS} was found to be 0.020 ($p<0.001$) within populations. Nine loci showed significant deficit of heterozygotes, while thirteen markers showed excess of heterozygotes.

Average number of alleles per locus ranged from 3.41 in Gushi chicken breed to 6.28 in Wannan Three-yellow chicken breed (Table 3). The lowest estimate of expected heterozygosity (0.440) was obtained for Gushi breed, while the highest one (0.644) was found in Wannan Three-yellow breed. Furthermore, ten breeds showed an overall significant deficit of heterozygotes, while three breeds showed an excess of heterozygous genotypes with respect to the expected value.

Genetic distances and clustering of breeds

Estimated gene flow (*Nm*) between each population pair is presented in Table 4. The *Nm* value ranged from 0.497 (between Red Jungle Fowl and Gushi chicken) to 5.103 (between Xiaoshan and Luyuan chicken). Most *Nm* values were below 2.0. Table 4 also gives the matrix of Marker Estimated Kinships (MEK) within and between the populations under study. The highest value of within population MEK was 0.511 observed in Gushi population. The lowest estimates were 0.131 and 0.141, respectively, in the Wannan Three-yellow and Dagu breeds. High between population kinships were observed between Xianju and Gushi breeds (0.273), and a very low level of coancestry was found between the Red Jungle Fowl and Chahua breeds (0.000).

The results of the clustering analysis using STRUCTURE are displayed in Figure 1. At K = 2, two main groups that generally corresponded to light-body type and heavy-body type chickens were formed. At this K value, the two medium-sized chicken breeds (Gushi and Wannan Three-yellow) grouped into different clusters. Gushi chickens clustered with the light-body type breeds while Wannan Three-yellow breed clustered in the group of heavy-body type chickens. At K = 3, the most frequent (N = 13) solution split Red Jungle Fowl, Chahua chicken and Tibetan chicken from the rest of the light-body type cluster, while the heavy-body type cluster maintained its structure as formed as K = 2. At K = 4, the heavy-body type populations clustered into two distinct clusters, separating the Luyuan, Xiaoshan, and Beijing Fatty from the rest. At K = 5, Red Jungle Fowl made up their own separate cluster. The Langshan chicken split off to form its own cluster at K = 6. Subsequently the Taihe Silkies split off from the light-body type populations at K = 7.

Since the clustering algorithm implemented in STRUCTURE is very computer intensive, we did not proceed with higher K values in the total set of populations. Instead, we analyzed subsets of populations which did not show population separation at level K = 7. In the first subset encompassing breeds Chahua, Tibetan, Xianju, Gushi and Baier, the Gushi breed separated from the remaining populations first. In contrast, Chahua and Tibetan did not split until K = 5. In the second subset including Wannan Three-yellow, Huainan Partridge, Henan Game and Dagu, Henan Game birds formed a distinct cluster first (K = 2) followed by Dagu chicken (K = 3). In the third subset encompassing Luyuan, Xiaoshan and Beijing Fatty, Beijing

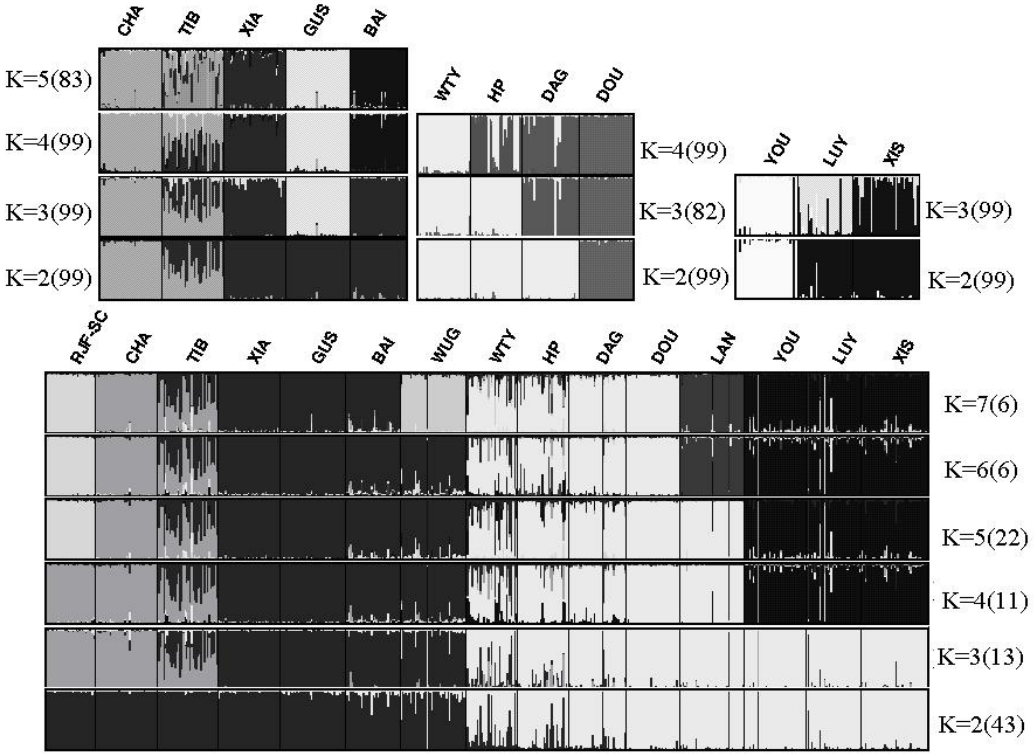

Figure 1. STRUCTURE clustering of 15 Chinese indigenous chicken breeds. Numbers in parenthesis indicate the number of identical solutions at 95% threshold. RJF-SC = Red Jungle Fowl; CHA = Chahua; TIB = Tibetan; XIA = Xianju; GUS = Gushi; BAI = Baier; WUG = Taihe silkies; WTY = Wannan Three-yellow; HP = Huainan Partridge; DAG = Dagu; DOU = Henan game; LAN = Langshan; YOU = Beijing Fatty; LUY = Luyuan; XIS = Xiaoshan.

Fatty chicken formed its own cluster first, followed by Luyuan chicken. Tibetan always appeared as a mixture population.

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, Dagu, 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.

DISCUSSION

The mean number of alleles observed in these 15 Chinese native populations (9.55) was greater than that observed in 11 Chinese native chicken breeds using 20 microsatellite markers (Gao et al., 2004), or in 12 Chinese native chicken breeds, using seven microsatellite markers (Chen et al., 2004b), but lower than that observed in 78 Chinese native chicken breeds using 27 microsatellite markers (Qu et al., 2006). Such difference could be attributed to the number of breeds studied, the variance in sample size and number of loci used. 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).

On average, the genetic differentiation index, F_{ST}, among breeds was 0.164 (Table 2). About 16% of the total genetic variation corresponds to differences between breeds and the remaining 84% was the result of variation among individuals within breeds. All loci contributed to this differentiation significantly. This level of differentiation value is very similar to the values reported in Swiss goat breeds, F_{ST} = 0.170 (Saitbekova et al., 1999), in European wild rabbits, F_{ST} = 0.150 (Surridge et al., 1999), but higher than that reported among 78 Chinese indigenous chicken breeds (F_{ST} = 0.106, Qu et al., 2006), in African cattle breeds (F_{ST} = 0.060, Ibeagha-Awemu et al., 2005), and human populations (F_{ST} = 0.054, Rosenberg et al., 2002).

The overall F_{IS} value (0.020), estimated at the marker level (Table 2), was significantly higher than zero. Nine loci, MCW0103, MCW0295, MCW0222, MCW0014, LEI0094, LEI0234, MCW0165, MCW0037 and MCW0216 showed significant deficit of heterozygotes. A possible explanation of this observation might be genetic drift or that these nine

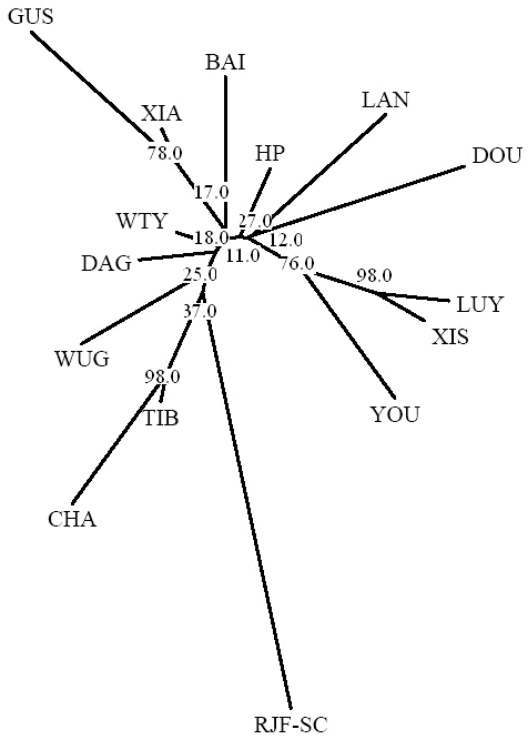

Figure 2. Neighbour-Joining tree of 15 Chinese indigenous chicken breeds based on Marker Estimated Kinships. RJF-SC = Red Jungle Fowl; CHA = Chahua; TIB = Tibetan; XIA = Xianju; GUS = Gushi; BAI = Baier; WUG = Taihe silkies; WTY = Wannan Three-yellow; HP = Huainan Partridge; DAG = Dagu; DOU = Henan game; LAN = Langshan; YOU = Beijing Fatty; LUY = Luyuan; XIS = Xiaoshan.

loci are linked to loci affecting morphological, productive or adaptive traits of selective interest and have undergone selection (Ibeagha-Awemu et al., 2005). Three breeds, Dagu, Langshan and Beijing Fatty, showed negative F_{IS} values. Breeding strategies to avoid inbreeding have been applied in the conservation of these breeds. The avoidance of mating between closely related animals might be one reason why a slight excess of heterozygotes was found in these populations.

Wannan Three-yellow chicken had the highest genetic variability in terms of expected heterozygosity and number of alleles (Table 3). This might due to the fact that the Wannan Three-yellow has just been founded in recent years with a large number of individuals and broad distribution area. The genetic basis of the founder population of this breed is complicated. Some gene flow between Wannan Three-yellow and other breeds found in neighbouring regions possibly exist. This would explain the generally high *Nm* values of the Wannan Three-yellow and all other breeds (Table 4).

Tibetan chickens are also distributed across a wide geographic area in Tibet autonomous region of China. Little selection has been performed on this breed. In contrast, the Huainan Partridge has just been founded in recent years with low level of selection. Any of these factors might explain why the Huainan Partridge and Tibetan breeds had higher gene diversity and higher numbers of alleles.

The Gushi breed showed the lowest genetic variability (Tables 3 and 4). The special geographical conditions limit the Gushi breed to a relatively isolated region. The region is surrounded by mountains and these may act as barriers to gene flow. The breed therefore has less opportunity for genetic exchange with other populations as was indicated by the highest within-breed MEK value and lower *Nm* values (from 0.497 to 1.723).

The results from MEK estimates further confirmed the results obtained from STRUCTURE based clustering. In the Neighbour-Joining tree derived from the kinship distances, Tibetan and Chahua chickens clustered together and were supported by high bootstrap value of 98.0 percent, indicating a close genetic relationship between the two populations. Yunnan province (Chahua chicken), is geographically close to Tibet, hence raising the possibility of interbreeding. Moreover, the Tibetan chicken has been bred recently, and some founder animals may have directly come from Chahua breed. The high gene flow (*Nm* = 4.363) and relatively high between populations kinship value (0.168), between Chahua and Tibetan chicken supported this close clustering of the two populations. STRUCTURE results further imply that there is migration of chickens from Chahua to Tibetan.

Chahua chickens, which are an original native breed between Red Jungle Fowl and modern breeds have had gene exchange with local Red Jungle Fowls and have retained many primitive features (Liu et al., 1996). This breed history explains why the Chahua chickens cluster together with the Red jungle fowl at lower K values.

In the Neighbour-Joining tree, Luyuan and Xiaoshan chicken clustered together with 98.0 percent bootstraps. During the STRUCTURE runs, they could not be distinguished until the number of clusters, K, equalled the number of breeds in the third subset. Thus, these two populations can be considered as genetically very similar. The main area of origin, Xiaoshan city and Zhangjiagang city for Xiaoshan chicken and Luyuan chicken respectively, are located very close to each other. Furthermore, the similar culture between these two places makes interbreeding of the Xiaoshan and Luyuan breed likely as confirmed by the high estimates of gene flow (*Nm* = 5.103; Table 4) and the high between-breed kinship estimates (0.157; Table 4).

It is noteworthy that three breeds, Xianju, Baier and Gushi chicken clustered together in the Neighbour-Joining tree. The three breeds did not separate during the STRUCTURE runs from K equals two to seven. This close genetic association may point to a common genetic

background. There are also similarities in morphological features among these three populations: All the three breeds have yellow plumage, beak and shanks (three yellow).

Cluster analysis can resolve effectively the genetic similarity of a group of highly diverged breeds and has great potential to help identify individuals with different or similar multilocus genotypes (Ibeagha-Awemu et al., 2005). In our study, the STRUCTURE analysis clustered individuals into separate populations or groups of closely related populations, and suggested that the Tibetan and Wannan Three-yellow breeds are mixture populations (Figure 1). The apparent mixed nature of both Tibetan and Wannan Three-yellow chicken is consistent with results from previous studies (Qu et al., 2004). The management practices for Tibetan chicken are characterized by no defined breeding goals and no controlled mating. Moreover, some gene flow between Tibetan chicken and other breeds may still be ongoing. This may be the reason why Tibetan chicken clustered as a mixture breed. Wannan Three-yellow chicken has been established only recently and may have intermixed origin, which can also be seen from the high estimates of gene flow with other chicken breeds. This population also appeared as a mixture population during STRUCTURE based clustering.

Chen et al. (2004b) applied a fuzzy clustering algorithm on a dataset comprising 12 of the 15 breeds in this study. However, the three clusters reported by Chen et al. (2004b) did not agree with the clustering of breeds obtained in the STRUCTURE analysis at K = 3 (Figure 1). Nor did the clustering agree with the consensus tree obtained from MEK estimates (Figure 2). Whereas breed history of geographical distribution cannot explain the clustering results reported by Chen et al. (2004b), the present results correspond to known breed history and geographical distribution. Thus, the differences in results are most probably attributable to the larger number of marker loci used (7 vs. 29) and the more sophisticated analysis methods. These have generated more accurate estimates of genetic diversity and structure of Chinese indigenous poultry breeds.

In conclusion, based on the various genetic diversity measures used in this study, high genetic diversity was observed in the 15 Chinese indigenous chicken breeds. The genetic relationships between these breeds were also clarified. Management of populations, in this study specifically tailored towards conservation, influences the genetic diversity within populations. Additionally, geographic distribution and geographic proximity seem to determine genetic relations between breeds as well as genetic diversity within breeds. Therefore, genetic diversity information, evaluated by integrating within and between population analyses may allow conservation priorities to be better established.

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