RESEARCH ARTICLE

# Analysis of genetic structure and relationship among nine indigenous Chinese chicken populations by the *Structure* program

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## Abstract

The multi-locus model-based clustering method *Structure* program was used to infer the genetic structure of nine indigenous Chinese chicken (*Gallus gallus*) populations based on 16 microsatellite markers. Twenty runs were carried out at each chosen value of predefined cluster numbers ($K$) under admixture model. The *Structure* program properly inferred the presence of genetic structure with 0.999 probabilities. The genetic structure not only indicated that the nine kinds of chicken populations were defined actually by their locations, phenotypes or culture, but also reflected the underlying genetic variations. At $K$ = 2, nine chicken populations were divided into two main clusters, one light-body type, including Chahua chicken (CHA), Tibet chicken (TIB), Xianju chicken (XIA), Gushi chicken (GUS) and Baier chicken (BAI); and the other heavy-body type, including Beijing You chicken (YOU), Xiaoshan chicken (XIA), Luyuan chicken (LUY) and Dagu chicken (DAG). GUS and DAG were divided into independent clusters respectively when $K$ equaled 4, 5, or 6. XIA and BIA chicken, XIA and LUY chicken, TIB and CHA chicken still clustered together when $K$ equaled 6, 7, and 8, respectively. These clustering results were consistent with the breeding directions of the nine chicken populations. The *Structure* program also identified migrants or admixed individuals. The admixed individuals were distributed in all the nine chicken populations, while migrants were only distributed in TIB, XIA and LUY populations. These results indicated that the clustering analysis using the *Structure* program might provide an accurate representation of the genetic relationship among the breeds.

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## Introduction

With its long history of animal husbandry and diversified geographical conditions, China has a wide variety of indigenous poultry resources. Majority of these chickens are local and fancy breeds characterized by medium-to-low performance. They are usually maintained in small populations. 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.

In the analysis of population genetics based on multilocus genotype data, it is common to adopt distance-based clustering methods to assign together individuals in analysing genetic structures and relationships (Romanov and Weigend 2001; Mateus *et al*. 2003; Wu *et al*. 2003, 2006; Amparo *et al*. 2006; Tu *et al*. 2006). The distance-based methods run by calculating a pair-wise distance matrix, whose entries provide the distance between every pair of individuals, which are then represented by a tree or a multidimensional scaling plot. However, these methods have a great dependence on distance measurements chosen, with concommitant difficulty in determining how much confidence we should focus on the clusters obtained in this way, and the difficulty of incorporating additional information (Jonathan *et al*. 2000).

The *Structure* program which implements a model-based clustering method for inferring population structure using genotype data was introduced by Pritchard (Jonathan *et al*. 2000; Anderson and Thompson 2002). It assumes a model in which there are $K$ populations (where $K$ may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals are assigned to populations according to their 'membership coefficients' for each cluster,

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which was interpreted as a probability of membership or as a fraction of the genome with membership in the cluster. The *Structure* program has been used in analysing 20 European chicken populations (Rosenberg *et al*. 2001), human populations (Noah *et al*. 2002; Rosenberg *et al*. 2005), Mongolian and Russian yak populations (Qie *et al*. 2005), Scottish wildcat populations (Beaumont *et al*. 2001), and west African cattle populations (Freeman *et al*. 2004).

This study aims to estimate the genetic structure of several 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

### *Experimental samples*

The nine selected populations are rare or endangered in China. In total, 360 unrelated birds of nine indigenous chicken populations were examined (BAI, Baier; CHA, Chahua; DAG, Dagu; GUS, Gushi; LUY, Luyuan; TIB, Tibetan; XIA, Xianju; XIS, Xiaosham; YOU, Beijing You). Forty birds per population were collected from private farms located at different parts of China (table 1), which is consistent with sample requirements for genetic diversity evaluation (Barker 1994). The Poultry Institute, Academy of Chinese Agricultural Sciences, Yangzhou, China also conserves these breeds.

### *DNA isolation*

Blood samples of 1.5 mL were collected aseptically by brachial venipuncture into haemotocrit tubes using EDTA and heparin as anticoagulant and stored at −20°C. DNA was extracted from the whole blood using the phenol/chloroform method previously described by Miller *et al*. (1988). DNA was quantified by spectrophotometry and the concentration was adjusted to 50 ng/$\mu$L.

### *Microsatellite loci and the PCR procedure*

Based on the primers used earlier in genetic diversity analysis of chicken breeds (Li *et al*. 2005; Chen *et al*. 2006), 16 pairs of highly polymorphic microsatellite markers were chosen based on their genomic location (table 2). The PCR products were obtained in a total reaction volume of 25 $\mu$L. The reaction contained 100 ng of genomic DNA, 5 pmol each of forward and reverse primers (Sangon Biotechnology, Shanghai, China), 2 mM $MgCl_2$, 5 mM dNTP, 1 unit *Taq* polymerase (Sangon Biotechnology, Shanghai, China). The amplification conditions were: 5 min initial denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 40 s, primer annealing at 52–64°C for 45 s (depending on the locus; table 2), and extension at 72°C for 1 min, followed by final extension at 72°C (5 min). Fluorescent end-labelled (fluorescent dye: FAM, TET, HEX; an external molecular size standard, Augct Biotechnology, Beijing, China) PCR primers were used, and size characterization of PCR product was performed by an ABI 310 DNA Genetic Analyzer (Applied Biosystems, Foster City, USA).

### *Cluster analysis*

There were 20,000 burn-in steps before 50,000 MCMC repeats. The number of clusters ($K$) ranged from 2 to 12, with 20 repeat runs for each $K$ value under admixture model and correlated frequencies model (Jonathan *et al*. 2007). The average pair-wise similarity ($H$) of runs was assessed by *CLUMPP* program (Mattias and Noah 2007) with greedy algorithm, 10,000 random input orders and 10,000 repeats.

**Table 1.** Description of the chicken populations.

| Population name | Abbreviation | Origin (province) | Specific features |
|---|---|---|---|
| Baier | BAI | Jiangxi | Three yellow*, light sized, layer breed, white earlobe |
| Chahua | CHA | Yunnan | Light sized, meat and egg dual-purpose breed |
| Dagu | DAG | Liaoning | Heavy sized, meat and egg dual-purpose breed |
| Gushi | GUS | Henan | Three yellow*, medium sized, meat and egg dual-purpose breed |
| Luyuan | LUY | Jiangsu | Heavy sized, meat and egg dual-purpose breed |
| Tibetan | TIB | Tibet | Light sized, selected for yellow plumage, meat and egg dual-purpose breed |
| Xianju | XIA | Zhejiang | Three yellow*, light sized, layer breed |
| Xiaoshan | XIS | Zhejiang | Heavy sized, meat and egg dual-purpose breed |
| Beijing You | YOU | Beijing | Heavy sized, meat and egg dual-purpose breed |

*Plumage yellow, beak yellow and shank yellow.

**Table 2.** The characterization of 16 microsatellite primers used in the final PCR.

| Microsatellite locus | Chromosome or linking group | Number of allele | Annealing temperature (°C) |
|---|---|---|---|
| MCW0295 | Chr. 4 | 5 | 58 |
| MCW0123 | E35c18w14 | 6 | 58 |
| MCW0165 | E27c36w25w26 | 2 | 58 |
| MCW0020 | Chr. 1 | 3 | 58 |
| MCW0104 | E48C28W13W27 | 8 | 58 |
| MCW0330 | E14W17 | 5 | 60 |
| LEI0234 | Chr. 2 | 10 | 55 |
| LEI0094 | Chr. 4 | 7 | 63 |
| MCW0206 | Chr. 2 | 5 | 60 |
| MCW0078 | Chr. 5 | 4 | 60 |
| MCW0111 | Chr. 1 | 5 | 62 |
| MCW0034 | Chr. 2 | 6 | 60 |
| MCW0069 | E60E04W23 | 5 | 60 |
| MCW0067 | E29C09W09 | 4 | 55 |
| MCW0014 | Chr. 6 | 4 | 55 |
| MCW0222 | Chr. 3 | 3 | 55 |

The run with the highest posterior probability was used to construct the dendrogram, visualized by the *Distruct* program (Noah 2007).

## Results

### *Dendrograms constructed by Structure*

In the estimation of the value of $K$ or clustering reliability, it was necessary to run several independent runs for each $K$ value. Using the same initial conditions, independent repeats at the same inferred value $K$ may result in different individual membership coefficients matrix (Q-matrix). To facilitate interpretation, *CLUMPP* program was used for aligning multiple replicate analyses of the same data set. Twenty *Structure* runs at each $K$ (from two to nine) produced nearly identical individual membership coefficients, and the average pairwise similarity coefficients were above 0.90 (table 3). Each colour in the figures represents a different cluster, and the individuals of different populations are separated by black lines. Figure 1 shows a given $K$, which is based on the highest probability run results. Figure 2 shows the independent distribution of YOU, GUS and DAG populations. Figure 3 shows the estimated population structures of subpopulation 1 (including CHA, TIB, XIA, GUS and BAI) and subpopulation 2 (including YOU, XIS, LUY and DAG). Each individual is represented by a vertical line, which is partitioned into $K$ coloured segments, which corresponded to its membership coefficients in $K$ clusters.

### *Distribution of estimated membership probablities*

The result of a single cluster analysis was typically given as a matrix, summing to one across $K$ clusters, where each individual was given a 'membership coefficient' for each cluster. For each population, membership coefficients of each cluster created a population Q-matrix, in which individuals were averaged. Table 4 shows the distribution of average estimated membership probabilities of 360 individuals. DAG, CHA, XIA, GUS, BAI and YOU chicken populations accounted for more than 80 per cent in their own clusters. XIS and LUY accounted for 75.4 and 76.8 per cent, respectively but TIB only accounted for 39.9 per cent.

### *Individual's identification*

According to their membership coefficients, individuals in the sample were assigned to populations (Corander and Marttinen 2006). If membership coefficient was more than 0.800, it would be assigned to the cluster completely. If the value was lower than 0.800, it indicated that they were admixed and would be assigned to two or more population clusters. The membership coefficient could also identify the migrants who had infiltrated into other chicken population clusters. Table 5 shows the distribution of admixed individuals and migrants in nine populations.

## Discussion

### *Effectiveness of inferring genetic structure*

When running the *Structure* program, if the variability across runs for a given $K$ value was high upon comparing with the variability of estimations obtained for different $K$, then it needed to use more runs or more burn-in steps. If $ln\mathbf{P}(D)$ appeared to be bimodal or multimodal, the MCMC scheme might find different solutions (Jasra *et al*. 2005; Jonathan *et al*. 2007). In this study, if the crucial parametres for 20 runs under each inferred $K$ value remained constant and stable, that indicated the running models and burn-in length chosen in advance were effective to infer the genetic structures of the nine populations (Rosenberg *et al*. 2001; Jonathan *et al*. 2007). The results of inferring the presence of genetic structure with 0.999 probabilities showed the nine chicken populations defined by locations, phenotypes or cultures of origin

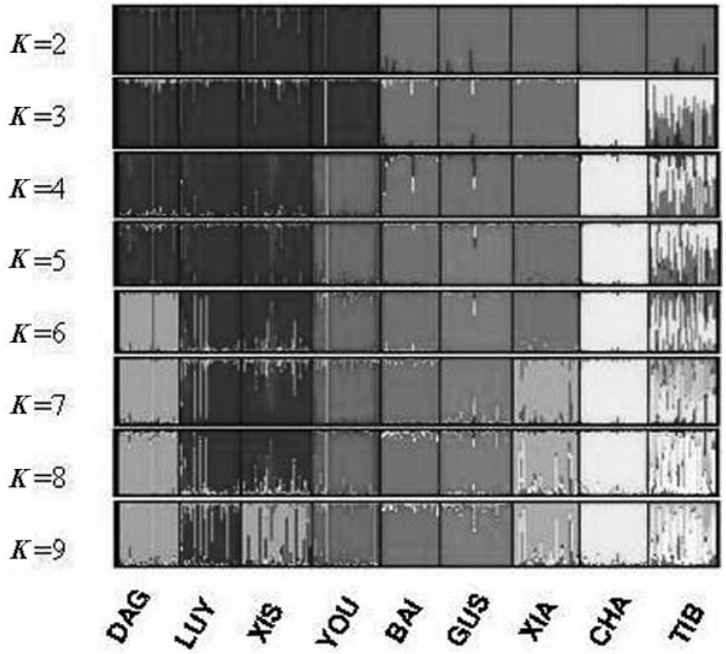


**Figure 1.** Estimated population structure for the nine chicken populations. Populations are labelled below the figure, and their locations above it.

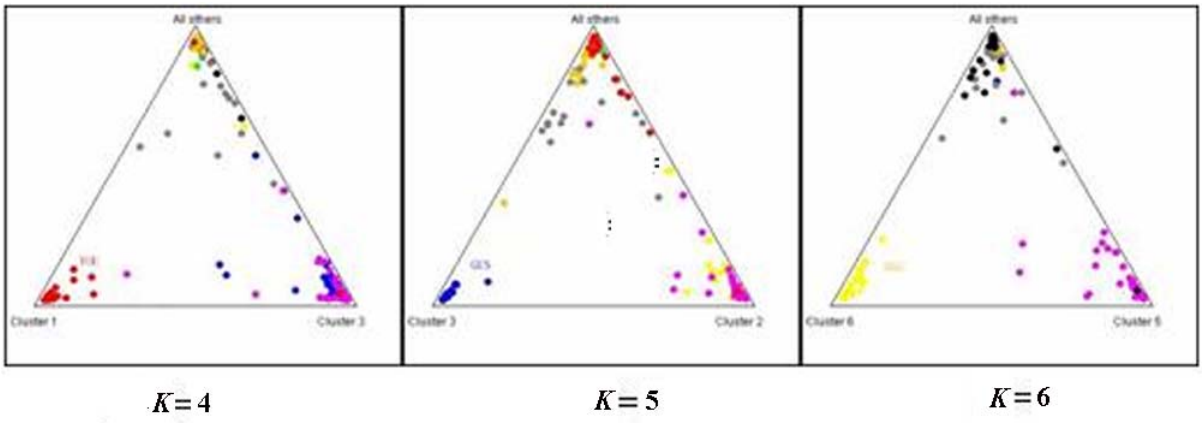


**Figure 2.** Independent distributions of YOU, GUS and DAG populations.

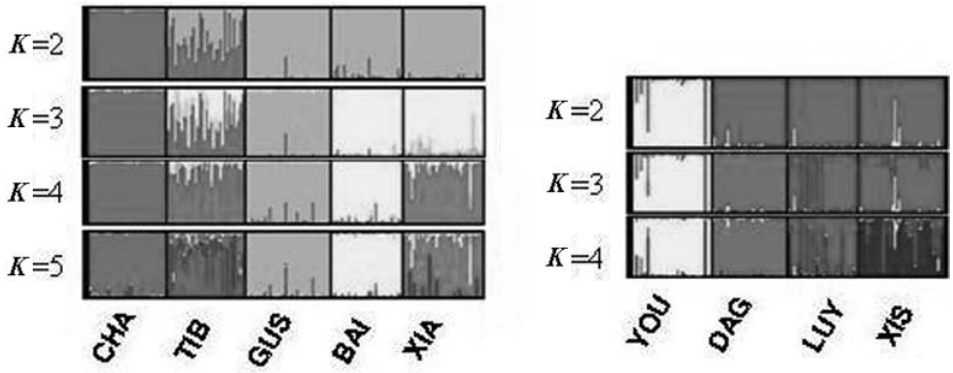


**Figure 3.** Estimated population structure for subpopulations 1 and 2. Populations are labelled below the figure.

**Table 3.** Estimated posterior probabilities of $K$ and average pair-wise similarity ($H$) of $Q$-matrix.

| $K$ | $ln\mathbf{P}(X\|K)$ | $\mathbf{P}(K\|X)$ | $H$ |
|---|---|---|---|
| 2 | −15663.6 | ~ 0 | 0.9996 |
| 3 | −14861.3 | ~ 0 | 0.9886 |
| 4 | −14447.8 | ~ 0 | 0.9633 |
| 5 | −14066.7 | ~ 0 | 0.9045 |
| 6 | −13769.8 | ~ 0 | 0.9163 |
| 7 | −13571.3 | ~ 0 | 0.9239 |
| 8 | −13464.1 | ~ 0 | 0.9390 |
| 9 | −13415.1 | ~ 0.999 | 0.9110 |
| 10 | −13425.7 | ~ 0 | 0.7862 |
| 11 | −13446.0 | ~ 0 | 0.6934 |
| 12 | −13587.9 | ~ 0 | 0.6845 |

$ln\mathbf{P}(X/K)$, estimated natural logarithm of the probability of data; $\mathbf{P}(K/X)$, estimated posterior probability of $K$.

**Table 4.** Average estimated membership probabilities of 360 individuals to different inferred clusters.

| | Inferred clusters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Pops | 1BAI | 2CHA | 3TIB | 4DAG | 5GUS | 6LUY | 7XIS | 8XIA | 9YOU |
| BAI | **0.911** | 0.006 | 0.029 | 0.007 | 0.011 | 0.006 | 0.009 | 0.016 | 0.005 |
| CHA | 0.007 | **0.938** | 0.017 | 0.008 | 0.006 | 0.006 | 0.006 | 0.005 | 0.007 |
| TIB | 0.025 | 0.320 | **0.399** | 0.022 | 0.031 | 0.012 | 0.027 | 0.143 | 0.021 |
| DAG | 0.012 | 0.008 | 0.014 | **0.872** | 0.012 | 0.011 | 0.015 | 0.040 | 0.016 |
| GUS | 0.006 | 0.006 | 0.011 | 0.007 | **0.929** | 0.008 | 0.009 | 0.018 | 0.006 |
| LUY | 0.012 | 0.008 | 0.004 | 0.029 | 0.016 | **0.768** | 0.138 | 0.010 | 0.015 |
| XIS | 0.010 | 0.008 | 0.013 | 0.015 | 0.013 | 0.163 | **0.754** | 0.011 | 0.013 |
| XIA | 0.106 | 0.006 | 0.017 | 0.007 | 0.033 | 0.009 | 0.013 | **0.803** | 0.006 |
| YOU | 0.014 | 0.029 | 0.007 | 0.024 | 0.010 | 0.012 | 0.043 | 0.006 | **0.855** |

**Table 5.** Admixed individuals and migrants in the inferred clusters.

| Populations | Admixed individuals* | Migrant individuals |
|---|---|---|
| XIS | 10 (0.757 ~ 0.789) | 2 (LUY) |
| LUY | 8 (0.312 ~ 0.765) | 2 (XIS) |
| TIB | 20 (0.108 ~ 0.772) | 3 (CHA) |
| XIA | 6 (0.406 ~ 0.757) | – |
| BAI | 1 (0.402) | – |
| CHA | 1 (0.757 ~ 0.791) | – |
| DAG | 2 (0.741 ~ 0.765) | – |
| GUS | 2 (0.707 ~ 0.782) | – |
| YOU | 3 (0.553 ~ 0.643) | – |

*The number and letter in the bracket indicates genome fraction of complicated individuals and cluster type for migrants.

reflected their underlying genetic variations. The same results were acquired in subpopulations 1 and 2, which further underscored the effectiveness of the procedure.

***Clustering analysis***

Cluster analysis can effectively resolve the genetic similarity of a group of highly diverged breeds and has great potential for identifying individuals with different or similar multi-locus genotypes (Ibeagha-Awemu and Erhardt 2005). At $K = 2$, the nine chicken populations were divided into two main clusters, one light-weighted (including CHA, TIB, XIA, GUS and BAI) and the other heavy-weighted (includes YOU, XIS, LUY and DAG). This result suggests that the body weights were probably an important selection factor in breeding histories of Chinese indigenous chicken breeds. This might be due to the difference of their distributions and limited geographical conditions. Beijing fatty chicken had been reared in the Qing dynasty, about 200 years ago, was only distributed in Beijing, and supplied as tributes because of its special external appearance (topknot, feather leg and bearded beak) and high quality meat. DAG chicken was

mainly distributed in southern Liaodong peninsula in Liaoning province. GUS chicken originated in the Henan province which is surrounded by mountains. The geographic peculiarities may act as barriers to external exchange of genes. These breeds, therefore, probably had less opportunity for genetic exchanges and gene flows with other populations.

CHA chicken, mainly distributed along the border of southern Yunnan province, was an original native breed between red jungle fowl and modern breeds, and had retained many primitive features (Liu *et al*. 1996). Tibetan chickens were distributed across a wide geographic area in the Tibet autonomous region of China. Its external appearance and living behaviours were also similar to red jungle fowl. Little selection has been performed on the two breeds. Moreover, Yunnan province is geographically close to Tibet, hence raising the possibility of interbreeding, this may explain why the Tibetan chicken cluster together with the Chahua chickens at lower $K$ values. XIA and BAI chicken which were famous layer breeds with light size. Both of them had three yellow characteristics (yellow plumage, beak and shanks). BAI chicken was not only distributed in Jiangxi province but also in the region the XIA chicken originated, Zhejiang province. The overlap of geography probably facilitated the genetic exchanges between the two breeds. The main original area, Xiaoshan and Zhangjiagang city for XIS and LUY chicken, respectively, are located very close to each other. Similar culture between these two places and similar selection probably led to similar genetic basis of these two breeds. These facts could explain why XIA and BAI, TIB and CHA, XIS and LUY were still divided into one cluster even at $K = 6$, 7 or 8. These results were basically consistent with gene flows of the nine populations and relationships in previous studies (Wu *et al*. 2003; Li *et al*. 2005; Chen *et al*. 2006).

***Identifying individuals***

According to their membership coefficients, the *Structure* program identified the migrants and admixed individuals (Freeman *et al*. 2004; Corander and Marttinen 2006). This special function is not possessed by distance-based clustering methods. In this study, DAG, CHA, XIA, GUS, BAI and YOU chicken populations had more than 80 per cent membership coefficients in their respective inferred clusters. However, TIB populations had complicated genetic background, its membership coefficients were distributed in almost all the nine inferred clusters and consisted of 20 admixed individuals, and three migrants into the CHA inferred cluster. This was consistent with TIB's great gene flow, highly differentiated production performances and diversiform phenotypic characters (Du *et al*. 2004). These genetic characteristics of TIB may result from 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. XIS and LUY populations were also identified as having complicated genetic backgrounds, with 76.8 and 75.4 per cent membership coefficients, as well as ten and eight admixed individuals and two migrants, respectively. However, the distributions of their membership coefficients differed in TIB admixed individuals; XIS and LUY individuals were mainly distributed in clusters of each which together summed to 0.917 and 0.906 respectively, while having a lower percentage in each of the other seven inferred clusters. This showed that XIS and LUY populations had more distant relationships with the other seven chicken populations. These two populations can be considered as genetically very similar. The main area of origin, Xiaoshan city and Zhangjiagang city for XIS and LUY chicken respectively, are located very close to each other. Further, the similar culture between these two places makes interbreeding of the XIS and LUY breed likely as confirmed by the high estimates of gene flow (Chen *et al*. 2008). XIA populations were identified having six admixed individuals whose extra membership coefficients were mostly distributed in BAI populations, but with no migrants. So when TIB, XIS, LUY and XIA chicken populations were selected for researches, it was necessary to identify the admixed individuals and migrants in addition to random selection.

These results indicated that clustering analysis of the *Structure* program might provide an accurate representation of the current genetic relations among the breeds, and contribute to devising more efficient conversation strategies.

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