# Cluster Analysis of 12 Chinese Native Chicken Populations Using Microsatellite Markers*

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**ABSTRACT :** The genomes of Chinese native chicken populations were screened using microsatellites as molecular markers. A total of, 528 individuals comprisede12 Chinese native chicken populations were typed for 7 microsatellite markers covering 5 linkage groups and genetic variations and genetic distances were also determined. In the 7 microsatellite loci, the number of alleles ranged from 2 to 7 per locus and the mean number of alleles was 4.6 per locus. By using fuzzy cluster, 12 Chinese native chicken populations were divided into three clusters. The first cluster comprised Taihe Silkies, Henan Game Chicken, Langshan Chicken, Dagu Chicken, Xiaoshan Chicken, Beijing Fatty Chicken and Luyuan Chicken. The second cluster included Chahua Chicken, Tibetan Chicken, Xianju Chicken and Baier Chicken. Gushi Chicken formed a separate cluster and demonstrated a long distance when comparing with other chicken populations. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 8 : 1047-1052)***

**Key Words** : Chinese Native Chicken Populations, Microsatellite Makers, Genetic Relationship, Cluster Analysis

## INTRODUCTION

Current breeding strategies for commercial poultry concentrate on specialized production lines derived by intense selection from a raw breeds and very large populations with a great genetic uniformity of traits under selection (Notter, 1999). However, people's requirements for animal production tend to become more and more varied. China has a vast territory and its ecological environments are complex and varied. Many kinds of native characteristic poultry resources were formed through natural and artificial selection over a long period of time. The genetic diversity of Chinese native chicken breeds is abundant, and much contribution to world poultry industry. It can be assumed that Chinese native chicken breeds contained the genes and alleles relevant to their adaptation to the particular environments and local breeding goals. Those native chicken breeds are needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and a source of research material.

In the process of evaluating genetic diversity to develop conservation measures in chickens, it is of special interest to assess genetic variation between different chicken breeds by utilizing modern molecular tools (Groene et al., 1998). Monolocus microsatellites have been shown to be suitable markers for this purpose and may resolve genetic relationships between closely related populations (Tautz, 1989).

The objective of the paper was to investigate and compare genetic variance and homozygosity with 7 microsatellite loci in 12 Chinese native chicken populations. Based on that information, genetic relationships among 12 Chinese native chicken populations were estimated using fuzzy cluster analysis.

## MATERIALS AND METHODS

### Experimental populations

A total of 528 chickens comprising of 12 Chinese native chicken populations were examined. These Chinese native chicken populations in this study were as follows: Luyuan Chickens (LY), Gushi Chickens (GS), Tibetan Chickens (TC), Baier Chickens (BE), Xianju Chickens (XJ), Chahua Chickens (CH), Dagu Chickens (DG), Beijing Fatty Chickens (BFO), Langshan Chickens (LS), Henan Game Chickens (HG), Taihe Silkies (TS) and Xiaoshan Chickens (XS). These chickens from different regions in China have been long domesticed under different environmental conditions. The information on origin of 12 Chinese native chicken populations and number of individuals examined per breed are as presented in Table 1.

### DNA isolation

Exactly 0.4 ml of venous blood was collected from the ulnar vein of each individual with heparin as anticoagulant, then added 4 ml splitting liquid lysate to tubes, sample stored at 4°C. DNA was isolated from the whole blood according to the method described by Sambrook (1998).

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**Table 1.** Origin and number of sample of 12 Chinese native chicken populations

| Populations | Origins | No. of individuals |
|---|---|---|
| LY | Jiangsu province | 44 |
| GS | Henan province | 44 |
| TC | Tibetan autonomous region | 44 |
| BE | Jiangxi province | 44 |
| XJ | Zhejiang province | 44 |
| CH | Yunnan province | 44 |
| DG | Liaoning province | 44 |
| BF | Beijing | 44 |
| LS | Jiangsu province | 44 |
| HG | Henan province | 44 |
| TS | Jiangxi province | 44 |
| XS | Zhejiang province | 44 |

TS: Taihe silkies; HG: Henan game chickens; LS: Langshan chickens; DG: Dagu chickens; XS: Xiaoshan chickens; BF: Beijing fatty chickens; LY: Luyuan chickens; CH: Chahua chickens; TC: Tibetan chickens, XJ: Xianju chickens; BE: Baier chickens; GS: Gushi chickens.

### Microsatellite loci

The 7 pairs of microsatellite primer sequences were provided by Institute for Animal Science of Animal Behavior/FAL.

### PCR production

The PCR products were obtained in 25 μl by using thermal cycle. Each PCR reaction tube contained 50 ng of genomic DNA. 0.1 μM of forward primer. 200 μM of dNTP. 1.5-1.8 mM $Mg^{2+}$ and 0.5 IU of *TaqA* (Galloway et al., 1999).

*The amplification protocol was* : initial denaturation at 94°C for 10 min: 35 cycles of denaturation at 94°C for 1 min. primer annealing at the optimal temperature for each primer pair(48~55°C) for 1 min and at extension 72°C for 1min: final extension was at 72°C for 10 min (Georges M. et al., 1993).

**Table 2.** Microsatellite primer sequences selected for present study

| Marker name | Chromosome location | Size range (kb) | alleles number (n) |
|---|---|---|---|
| ADL268 | 1 | 104-116 | 5 |
| ADL278 | 8 | 114-122 | 4 |
| MCW103 | 3 | 266-270 | 2 |
| MCW183 | 7 | 296-318 | 7 |
| MCW222 | 3 | 220-226 | 3 |
| MCW248 | 1 | 215-223 | 5 |
| MCW67 | E29C28W13W27 | 178-186 | 6 |

### Genotyping

After PCR amplification. 6 μl of each reaction product was loaded onto an 8% denaturing polyacrylamide gel. A molecular size ladder pBR322/MspI marker was used for calibration as internal control. Electrophoregram processing and allele size scoring were performed with the software package.

### Allele frequency

By calculating the number of different size alleles outcome allele frequency.

*Heterozygosity* : population heterozygosity indicates the heterozygous frequency of loci. The heterozygosity was

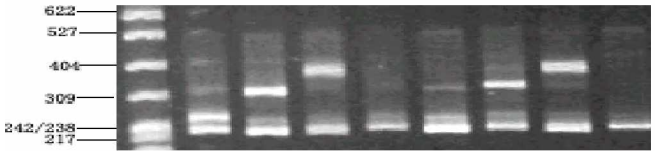


**Figure 1.** A portion of PCR results of MCW248 in Henan Game chicken.

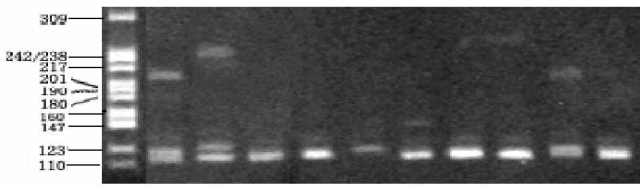


**Figure 3.** A portion of PCR results of MCW222 in Beijing Fatty chicken.

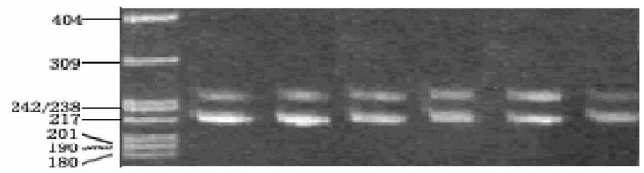


**Figure 5.** A portion of PCR results of ADL278 in Xianju chicken.

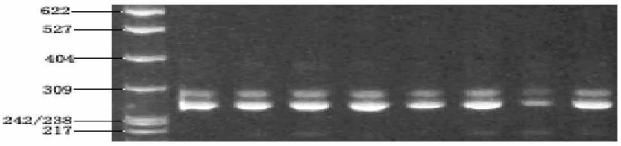


**Figure 2.** A portion of PCR results of MCW103 in Baier chicken.

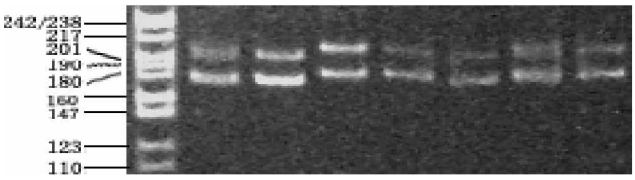


**Figure 4.** A portion of PCR results of MCW183 in Baier chicken.

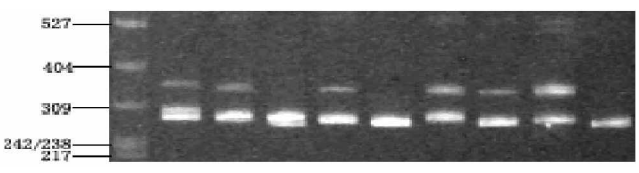


**Figure 6.** A portion of PCR results of MCW67 in Xianju chicken.

obtained by using Botstein (1980) defined as:

$$H = 1 - \sum_{i=1}^{n} p_i^2$$

n= the number of alleles
$p_i$= gene frequency of the allele i

*Polymorphism information content (PIC)* : PIC was obtained by:

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

n= the number of alleles
$p_i$= gene frequency of the allele i
$p_j$= gene frequency of the allele j

**Clustering analysis**

The genetic relationships among 12 Chinese native chicken populations were estimated using the fuzzy clustering method. Based on the allele frequency of different populations to construct fuzzy-consistency relation matrix. then transformed it to fuzzy-equilibrium relationship for clustering population genetic relationship by formula as follows:

$$\mu_{\underline{R}}(x, y) = \frac{1}{2} \ln(J_{xy} / \sqrt{J_x J_y}) + 1$$

$J_x$ and $J_y$=the mean of probability of the same alleles among locus obtained randomly in population x and y.

$J_{xy}$= the mean of probability of the same alleles among

**Table 3.** Alleles frequencies of 7 microsatellite loci in 12 Chinese native chicken populations

| Locus allele (bp) | LY | GS | TC | BE | XJ | CH | DG | BF | LS | HG | TS | XS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MCW67 | | | | | | | | | | | | |
| 178 | 0.2444 | 0.0000 | 0.0333 | 0.0000 | 0.0000 | 0.2889 | 0.1500 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0227 |
| 180 | 0.4556 | 0.4205 | 0.4667 | 0.3444 | 0.3095 | 0.4667 | 0.5000 | 0.5556 | 0.4432 | 0.5135 | 0.4146 | 0.3636 |
| 182 | 0.0000 | 0.0000 | 0.0111 | 0.0000 | 0.0000 | 0.1778 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.5854 | 0.6137 |
| 183 | 0.0222 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 184 | 0.2778 | 0.5795 | 0.3889 | 0.6556 | 0.6667 | 0.0666 | 0.3500 | 0.4444 | 0.5568 | 0.4865 | 0.0000 | 0.0000 |
| 186 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0238 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| MCW248 | | | | | | | | | | | | |
| 215 | 0.5556 | 0.4205 | 0.1556 | 0.5444 | 0.4286 | 0.0667 | 0.7625 | 0.7667 | 0.6818 | 0.6351 | 0.6220 | 0.6477 |
| 217 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0610 | 0.0000 |
| 219 | 0.444 | 0.2841 | 0.8111 | 0.4556 | 0.5714 | 0.9000 | 0.2500 | 0.2222 | 0.2386 | 0.2703 | 0.2805 | 0.3182 |
| 221 | 0.0000 | 0.0000 | 0.0111 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 223 | 0.0000 | 0.2954 | 0.0222 | 0.0000 | 0.0000 | 0.0333 | 0.0375 | 0.0111 | 0.0796 | 0.0946 | 0.0365 | 0.0341 |
| MCW22 | | | | | | | | | | | | |
| 220 | 0.3667 | 0.1136 | 0.2778 | 0.5000 | 0.3928 | 0.2889 | 0.3000 | 0.2444 | 0.5227 | 0.1892 | 0.3049 | 0.3523 |
| 222 | 0.2111 | 0.6705 | 0.5222 | 0.4000 | 0.4286 | 0.6444 | 0.2000 | 0.2556 | 0.1136 | 0.3108 | 0.1951 | 0.1704 |
| 226 | 0.4222 | 0.2159 | 0.2000 | 0.1000 | 0.1786 | 0.0667 | 0.5000 | 0.5000 | 0.3637 | 0.5000 | 0.5000 | 0.4773 |
| MCW183 | | | | | | | | | | | | |
| 296 | 0.0778 | 0.0568 | 0.5227 | 0.5568 | 0.4250 | 0.5976 | 0.3875 | 0.2778 | 0.6023 | 0.5811 | 0.3780 | 0.1477 |
| 298 | 0.2667 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0135 | 0.0000 | 0.0000 |
| 302 | 0.2667 | 0.9432 | 0.1932 | 0.1136 | 0.2625 | 0.2805 | 0.3875 | 0.2889 | 0.3977 | 0.3784 | 0.4512 | 0.5455 |
| 304 | 0.0000 | 0.0000 | 0.1250 | 0.2614 | 0.2625 | 0.1219 | 0.0875 | 0.3444 | 0.0000 | 0.0270 | 0.1098 | 0.1250 |
| 310 | 0.0000 | 0.0000 | 0.0114 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 312 | 0.0555 | 0.0000 | 0.0114 | 0.0682 | 0.0375 | 0.0000 | 0.1000 | 0.0111 | 0.0000 | 0.0000 | 0.0000 | 0.1023 |
| 318 | 0.3333 | 0.0000 | 0.1363 | 0.0000 | 0.0125 | 0.0000 | 0.0375 | 0.0778 | 0.0000 | 0.0000 | 0.0610 | 0.0795 |
| MCW103 | | | | | | | | | | | | |
| 266 | 0.3214 | 0.4091 | 0.4773 | 0.0444 | 0.3780 | 0.1585 | 0.4875 | 0.4889 | 0.4886 | 0.4595 | 0.5244 | 0.5227 |
| 270 | 0.6786 | 0.5909 | 0.5227 | 0.9556 | 0.6220 | 0.8415 | 0.5125 | 0.5111 | 0.5114 | 0.5405 | 0.4756 | 0.4773 |
| ADL278 | | | | | | | | | | | | |
| 114 | 0.2333 | 0.1364 | 0.8864 | 0.7333 | 0.7738 | 0.9432 | 0.4744 | 0.2976 | 0.4419 | 0.3108 | 0.4512 | 0.3583 |
| 119 | 0.6000 | 0.6704 | 0.0568 | 0.2222 | 0.1667 | 0.0000 | 0.4487 | 0.4881 | 0.4186 | 0.4730 | 0.3903 | 0.4773 |
| 120 | 0.0000 | 0.0000 | 0.0114 | 0.0334 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 122 | 0.1667 | 0.1932 | 0.0454 | 0.0111 | 0.0595 | 0.0568 | 0.0769 | 0.2143 | 0.1395 | 0.2162 | 0.1585 | 0.1704 |
| ADL268 | | | | | | | | | | | | |
| 104 | 0.0889 | 0.0000 | 0.0000 | 0.0444 | 0.0238 | 0.0116 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 110 | 0.3778 | 0.0000 | 0.0333 | 0.1445 | 0.1071 | 0.0116 | 0.1026 | 0.4000 | 0.1250 | 0.2838 | 0.3049 | 0.1364 |
| 122 | 0.0000 | 0.0000 | 0.0111 | 0.0000 | 0.0000 | 0.0116 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 114 | 0.4667 | 0.3068 | 0.2222 | 0.6222 | 0.2976 | 0.0698 | 0.7051 | 0.5111 | 0.6023 | 0.5405 | 0.4146 | 0.7045 |
| 116 | 0.0666 | 0.6932 | 0.7334 | 0.1889 | 0.5715 | 0.8954 | 0.1923 | 0.0889 | 0.2727 | 0.1757 | 0.2805 | 0.1591 |

locus obtained randomly in population x and y.

μR (x,y)=the subordinate function to fuzzy-consistency relation R between population x and y.

## RESULTS

### The results of PCR microsatellite primer and polymorphism

The amplified results of the 7 pairs of microsatellite primers are as shown in Figures 1-6 respectively.

### Microsatellite alleles frequency distribution

All microsatellite primers gave PCR products, which were polymorphic in the 12 chicken populations. The total number of alleles was 32 across 12 chicken populations. The number of alleles per locus ranged from 2 (MCW103) to 7 (MCW183) and the mean number of alleles across all microsatellite loci and individuals typed was 4.6 alleles in 12 chicken populations. However, some alleles (MCW67's 183 and 186; MCW248's 217 and 221; MCW183's 298 and 310; ADL278's 120; ADL268's 122) where not observed in some populations analyzed (XJ,LY; TC,TS; LY,HG, TC; TC,BE; TC, respectively). Table 3 showed that the number of alleles and alleles frequency in 12 chicken populations revealed significant difference in same locus.

The maximum size difference between the alleles observed within the loci ranged from 4 (in MCW103) to 22 (in MCW183), with an average 9.71 bp per locus. Two markers (MCW67 and ADL278) displayed size differences of 1bp between some alleles, i.e. ADL278 locus showed two alleles differing in size by 1 bp across both Baier and Tibetan Chicken populations. MCW67 locus showed a series of three alleles differing in size by 1 bp. ADL268 locus showed two alleles differing in size by 2 bp across12 chicken populations. MCW222 locus showed a series of three alleles differing in size by 2-6 bp across12 chicken populations. MCW183 locus showed a series of five alleles differing in size by 2-16 bp across Tibetan Chicken populations.

We observed the maximum allele frequency in MCW183 locus (0.9432) in Gushi chicken population. MCW103 locus (0.9556) in Baier chicken population. ALD278 locus and MCW248 (0.9432 and 0.9000) in Chahua chicken population. Those alleles frequency were approximate to 1, which showed some loci were homozygous among those populations (Table 3).

### Mean gene heterozygosity and mean polymorphism information content (PIC)

The population heterozygosity and mean polymorphism information content in 12 Chinese native chicken populations were obtained by calculating the gene frequency of different genes. The results are as shown in Table 4.

Table 4 showed that the heterozygosity of LY, DG, BT, LS, Hg, XS were high, the degree of variance in population was greater, the genetic diversity was abundant; the other populations showed lower heterozygosity indicating the degree of variance was small and uniform.

### Matrix of fuzzy similarity relationship

Based on frequencies of 32 alleles in 12 Chinese native chicken populations, the matrix of fuzzy similarity relationship was obtained by using fuzzy clustering software and results are as shown in Table 5.

### Genetic relationship analyzed

Using fuzzy clustering method, a genetic distance (Figure 7) was reconstructed for 12 Chinese chicken populations. In this paper, Taihe Silkies and Henan

**Table 4.** Mean heterozygosity and mean PIC values in 12 Chinese native chicken populations

| | LY | GS | TC | BE | XJ | CH | DG | BF | LS | HG | TS | XS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean H | 0.5929 | 0.4491 | 0.4354 | 0.4547 | 0.5246 | 0.3514 | 0.5436 | 0.5563 | 0.5261 | 0.5537 | 0.578 | 0.5448 |
| Mean PIC | 0.5159 | 0.3761 | 0.4147 | 0.3869 | 0.4445 | 0.3143 | 0.4668 | 0.4717 | 0.4327 | 0.4627 | 0.4918 | 0.4671 |

**Table 5.** Matrix of fuzzy similarity relation for 12 Chinese native chicken populations

| | LY | GS | TC | BE | XJ | CH | DG | BF | LS | HG | TS | XS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LY | 1.0000 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9480 | 0.9480 | 0.9480 | 0.9480 | 0.9480 | 0.9480 |
| GS | 0.9030 | 1.0000 | 0.9030 | 0.9030 | 0.9030 | 0.9030 | 0.9030 | 0.9030 | 0.9030 | 0.9030 | 0.9030 | 0.9030 |
| TC | 0.9400 | 0.9030 | 1.0000 | 0.9590 | 0.9710 | 0.9720 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 |
| BE | 0.9400 | 0.9030 | 0.9590 | 1.0000 | 0.9590 | 0.9590 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 |
| XJ | 0.9400 | 0.9030 | 0.9710 | 0.9590 | 1.0000 | 0.9710 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 |
| CH | 0.9400 | 0.9030 | 0.9720 | 0.9590 | 0.9710 | 1.0000 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9400 |
| DG | 0.9480 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 1.0000 | 0.9780 | 0.9810 | 0.9810 | 0.9810 | 0.9810 |
| BF | 0.9480 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9780 | 1.0000 | 0.9780 | 0.9780 | 0.9780 | 0.9780 |
| LS | 0.9480 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9810 | 0.9780 | 1.0000 | 0.9830 | 0.9830 | 0.9810 |
| HG | 0.9480 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9810 | 0.9780 | 0.9830 | 1.0000 | 0.9850 | 0.9810 |
| TS | 0.9480 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9810 | 0.9780 | 0.9830 | 0.9850 | 1.0000 | 0.9810 |
| XS | 0.9480 | 0.9030 | 0.9400 | 0.9400 | 0.9400 | 0.9400 | 0.9810 | 0.9780 | 0.9810 | 0.9810 | 0.9810 | 1.0000 |

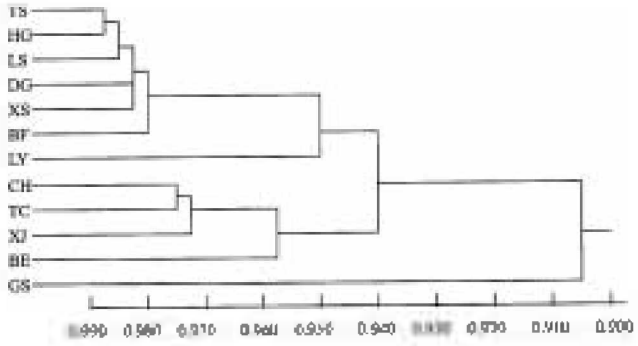


**Figure 7.** The figure of fuzzy cluster in 12 Chinese native chicken populations.

Game Chicken firstly got together in a cluster on the level of $\lambda$=0.9850, indicating the genetic relationship was the closest between them; while Gushi Chicken and other chickens masked on the level of $\lambda$=0.9030, showing the genetic relationship between Gushi Chicken and other chickens was further. 12 Chinese chicken populations were subdivided into three major clusters. The first cluster comprised Taihe Silkies, Henan Game Chicken, Langshan Chicken, Dagu Chicken, Xiaoshan Chicken, Beijing Fatty Chicken and Luyuan chicken. The second cluster included Chahua Chicken, Tibetan Chicken, Xianju Chicken and Baier Chicken. Gushi Chicken formed a separate cluster. This result has identical on the breeding history and natural distribution, while it did not complete conformed to the classification of native populations with morphological characteristics.

**Microsatellite alleles distribution**

Microsatellite analysis is a well-established method for measuring the genetic relationships between and within breeds or populations. This research was to characterize and compare 12 Chinese native chicken populations typed with 7 microsatellite loci and analysis of genetic relationships among 12 Chinese native chicken populations. The results revealed much greater microsatellite allele variation in Chinese chicken and 32 alleles were obtained. The alleles frequencies were between 0.0111 and 0.9556, 14 of 32 alleles existed in the 12 Chinese native chicken populations. The PIC of MCW183 locus was abundant, about 7 alleles, while the locus of MCW103 only had 2 alleles. The mean number of alleles across all microsatellite loci and individuals typed was 4.6 in the 12 Chinese native chicken populations. Relative to the chicken breeds studied, we found that genetic relationship is conformed with their breeding origin and evolution. However, studies based on microsatellite loci are needed to confirm this findings, because due to small number of microsatellite loci analyzed there is also a likelihood that the data have enough information content.

By calculating allele frequency of all locus in each population indicated that some specific alleles existed in some populations and the allele frequency for all locus also showed significant difference between different populations (Table 3). Similarly, individual clustering based on the proportion of shared alleles (Kim et al., 2002). Using the fuzzy results the dendrogram (Figure 7) was generated.

## DISCUSSION

**Genetic analysis within population**

The gene heterozygosity was a measurement unit for population heterozygosity, demonstrated that the heterozygotes frequency of tested locus in population. The level of mean population heterozygosity reflected the degree of population genetic consistency. The lower of population heterozygosity, the higher of the population genetic consistency and *vice versa*.

The present work showed the mean of heterozygosity of the 12 chicken populations in 7 microsatellite loci ranged from 0.3514 to 0.5929. Among them, the mean of heterozygosity of Luyuan chick population was the highest, 0.5929, showed abundant polymorphism, which indicated Luyuan chicken population should be further pure breeding to enhance the population performance and uniformity. However, the mean of heterozygosity of the Chahua chicken population was the lowest, 0.3415, indicated that the genotype of this population tended to consistency and the level of population diversity was lower.

The mean polymorphism information content (PIC) was an ideal index to measure the polymorphism of allele fragments. PIC>0.5, indicated the locus of high-polymorphism; 0.25<PIC<0.5, indicated the locus of medium-polymorphism; PIC<0.25, indicated the locus of low-polymorphism. In present study, only in Luyuan chicken, the PIC in 7 microsatellite loci were higher than 0.5, revealed genetic information contributed by Luyuan chicken was abundant than other chickens. However, the PIC was the lowest in Chahua. The reason might be related to geographical location and selection intensity. The PIC of the other native chicken populations ranged from 0.25 to 0.5. The results were identical on the former mean gene heterozygosity across all chicken populations examined.

**Genetic relationship analysis between populations**

Whether the affinity between species and breeds or not, which was quantitative measured by fuzzy cluster analysis (Chang, 1995). This analysis based on the breeds marker data gather and comparability between breeds, then transformation the comparability to fuzzy-consistency relation and ranked matrix based on the subordinate function. Initialization $\lambda$ indicated extent of the subordinate cluster. Therefore, classification formed a dynamic cluster

with changed of $\lambda$ value. All the methods revealed similar phylogenetic tree has support from the history and geographical location (Pandey et al., 2002). A phylogenetic tree was reconstructed. 12 Chinese chicken populations were divided to three clusters. Taihe Silkies and Henan Game Chicken firstly linked together in a cluster on the level of $\lambda$=0.9850, indicating the genetic relationship was the closest between them; Langshan chicken, Taihe Silkies and Henan game chicken masked in a cluster on the level of $\lambda$=0.9850; while Gushi Chicken and other chickens masked on the level of $\lambda$=0.9030, showing the genetic relationship between Gushi Chicken and other chickens was further. The cluster results in this work indicated that, credible level$\lambda$was lower, the number of populations comprised in $R_\lambda$ was more, the classification was inexact and *vice versa*.

The cluster could be confirmed from three aspects which maybe geographical, bodily form and economical purposes. The evidences can be ascribed as:

*To geography* : 7 chicken populations within the first cluster distributed the north or middle region of China and 4 chicken populations within the second cluster lied in south-western of China. Gushi chicken in the third cluster was upper reaches of Yellow river area of China.

As to bodily form: except for Taihe Silkies, chicken populations in the first cluster were all heavy-body. Chicken populations in the second cluster were small-body. Gushi Chicken in the third cluster was medium-size.

As to economical purpose: except for Taihe Silkies and Henan Game Chicken, other populations in the first cluster were dual-purpose with egg and meat chicken. Of the second cluster, Xianju Chicken and Baier Chicken belong to egg type chicken. Chuhua Chicken and Tibetan Chicken were other type chicken. Gushi Chicken was dual-purpose with meat and egg chicken.

The results of this classification conformed to the classification of blood type and protein type (Chen, 1991; Jian, 2000). This investigation demonstrated that the microsatellite DNA could reflect genetic diversity and differentiation between avian breeds. Because of microsatellites belonged to non-structural genes sequence and its specificity was not influenced by selection, so the diversity could be accumulated in large amount on the procession of evolution in populations. The genetic distance obtained by microsatellite markers was more suitable to reflect the poultry populations differentiation.

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