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# Analysis of Genetic Diversity of Yangzhou Chicken by Microsatellite Markers

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**Abstract:** Genetic variation at 20 microsatellite loci and genetic diversity were examined for Yangzhou Chicken. Observed and effective number of alleles across the microsatellite loci varied from 2-6 with an overall mean of 3.778 and 2.404, respectively. Observed and effective heterozygosity varied from 0.129-0.755 with an average of 0.422 and 0.517, respectively. Average polymorphism information content was 0.464. The genetic structure indicated that Yangzhou Chickens have substantial genetic variation. Population showed fairly high level of inbreeding ($F_{is}$ = 0.184) and global heterozygote deficit. The allele frequency distribution displayed an L shape, suggesting that no recent bottleneck affecting the genetic variability occurred. The information generated in this study will greatly aid in the establishment of effective breeding strategies for Yangzhou Chicken and may further be utilized for studying differentiation and relationships among different chicken breeds.

**Key words:** Genetic diversity, chicken, microcatellite

## INTRODUCTION

Yangzhou Chicken is a dual-purpose chicken breed that mainly distributed in the southeastern region of China. This breed originated from the crossing of New Hampshires with the native chicken flock in Yangzhou. They were shipped to Zhejiang, Guangdong and Liaonin provinces to improve indigenous poultry production of these provinces and later was qualified a dual-purpose breed of Chinese poultry breed by authoritative Committee of The Breeds of Domestic Animal and Poultry in China in 1984. The number of this breed decreased after the meat-type and egg-type chickens were imported into China. Now this breed is one of the best preserved native breeds and is famous for its high meat quality. This breed has yellow skin and dark or light red feather and these appearances make it belong to yellow-feather chicken. At present, yellow-feather chicken production is prosperous in China. Because of this, Yangzhou Chicken could be bred into commercial strains and this may be the best choice to preserve and develop this native breed.

The genetic structure of a breed, reflecting the interaction of forces like selection, genetic drift and gene flow acting steadily throughout many generations, is basic information to preserve and construct breeding strategies. In recent years, microsatellite marker has been widely used in revealing genetic variation and population structure in chicken because of its advantages of high variability, high mutation rate, large number, distribution through out the genome, co-dominant inheritance, neutrality with respect to selection and easy genotyping (Hillel *et al*., 2003; Chen *et al*., 2004; Pandey *et al*., 2005; Tu *et al*., 2006). The present study used 20 microsatellite markers to estimate genetic structure of the Yangzhou Chicken in order to provide genetic information to be used in breeding the commercial strains and the improvement of this breed.

## MATERIALS AND METHODS

**Microsatellite genotyping:** Blood samples were collected from a total of 360 randomly selected Yangzhou chickens in the Experimental Farm of Yangzhou University. Genomic DNA was extracted from blood samples using a standard phenol/chloroform protocol. A battery of 20 microsatellite markers was selected based on consistent and reproducible amplification under standard conditions to generate data in a panel of 360 chickens (Liu *et al*., 2006). Each of 25 μL PCR reaction contained 1.0 μL of template DNA (100 ng), 0.5 μL of Taq polymerase (2 U $\mu L^{-1}$), 0.5-1.0 μL dNTPs (10 mM), 0.5 μL of each primer (100 ng $\mu L^{-1}$), 18-18.5 μL $ddH_2O$ and 2.5 μL 10×reaction buffer (with 20 mM $MgCl_2$) provided by the enzyme supplier. An Eppendorf Thermal Cycler was programmed for an initial incubation at 94°C for 3 min; 35 cycles each with denaturing at 94°C for 50 s, annealing at 56-60°C for 45 s and extension at 72°C for 50 s; and a final cycle at 72°C for 5 min. The PCR products were electrophoresed with polyacrylamide gel and silver stained (Bassam *et al*., 1991).

**Data analyses:** Allele frequencies, percentage of polymorphic loci, the mean number of alleles per locus (A), observed heterozygosity (Ho), expected

heterozygosity (He) and test of genotypic frequencies to Hardy-Weinberg (HW) equilibrium for each locus were computed using the Popgen 3.2 software package. Paired t-test was used to evaluate statistical significance of the deviations between observed and expected heterozygosity. Inbreeding coefficient index within a population (also called fixation index of population, $F_{is}$) based on Weir and Cockerham (1984) were calculated using the program FSTAT (Goudet, 2001), the significance was obtained by 5000 permutations.

Polymorphism information content (PIC) and Reliability (β) of allele frequency were calculated with the following formula (Liu *et al*., 2002).

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

$$\beta = \int_0^{\lambda} \frac{2e^{-\frac{\lambda^2}{2}}}{\sqrt{2\pi}} d\lambda$$

where,

p = Is the gene frequency.

λ = Is the standardized deviation of gene frequency.

## RESULTS AND DISCUSSION

**Gene frequency:** Gene frequencies of microsatellite loci were shown in Table 1. The reliability of estimation for all the allele frequencies accept ADL183$^{E}$, ADL185$^{E}$, ADL217$^{D}$, ADL217$^{E}$, LEI94$^{C}$, MCW154$^{D}$, MCW154$^{E}$, MCW154$^{F}$, MCW180$^{B}$, MCW294$^{B}$ was above 99%, which showed that gene frequencies in the population were reliable and the data could be used to estimate the population genetic diversity. Few loci had alleles with small frequency and small allele frequency accompanied with low reliability of estimation. Small sample size and low allele frequency may lead to low reliability of estimation for the allele frequency (Liu *et al*., 2002). Sample size was large in this study, so the small allele frequency estimation was the reason for the low reliability of the alleles.

Table 1: Gene frequencies of microsatellite loci in yangzhou chickens

| Locus | Allele | Gene frequency | Reliability | Locus | Allele | Gene frequency | Reliability (β) |
|---|---|---|---|---|---|---|---|
| ADL155 | A | 0.920 | 1.000 | LEI166 | A | 0.724 | 1.000 |
| | B | 0.080 | 1.000 | | B | 0.198 | 1.000 |
| ADL183 | A | 0.323 | 1.000 | | C | 0.079 | 1.000 |
| | B | 0.265 | 1.000 | MCW85 | A | 0.522 | 1.000 |
| | C | 0.242 | 1.000 | | B | 0.208 | 1.000 |
| | D | 0.057 | 0.999 | | C | 0.270 | 1.000 |
| | E | 0.012 | 0.861 | MCW120 | A | 0.057 | 0.999 |
| | F | 0.101 | 1.000 | | B | 0.064 | 1.000 |
| A185 | A | 0.587 | 1.000 | | C | 0.498 | 1.000 |
| | B | 0.093 | 1.000 | | D | 0.331 | 1.000 |
| | C | 0.100 | 1.000 | | E | 0.050 | 0.998 |
| | D | 0.215 | 1.000 | MCW154 | A | 0.854 | 1.000 |
| | E | 0.005 | 0.658 | | B | 0.078 | 1.000 |
| ADL201 | A | 0.540 | 1.000 | | C | 0.043 | 0.996 |
| | B | 0.460 | 1.000 | | D | 0.012 | 0.861 |
| ADL217 | A | 0.802 | 1.000 | | E | 0.003 | 0.538 |
| | B | 0.035 | 0.989 | | F | 0.010 | 0.799 |
| | C | 0.138 | 1.000 | MCW170 | A | 0.176 | 1.000 |
| | D | 0.012 | 0.861 | | B | 0.288 | 1.000 |
| | E | 0.012 | 0.861 | | C | 0.377 | 1.000 |
| ADL273 | A | 0.837 | 1.000 | | D | 0.159 | 1.000 |
| | B | 0.163 | 1.000 | MCW180 | A | 0.377 | 1.000 |
| ADL292 | A | 0.463 | 1.000 | | B | 0.012 | 0.861 |
| | B | 0.228 | 1.000 | | C | 0.232 | 1.000 |
| | C | 0.262 | 1.000 | | D | 0.245 | 1.000 |
| | D | 0.047 | 0.997 | | E | 0.134 | 1.000 |
| LEI66 | A | 0.404 | 1.000 | MCW258 | A | 0.310 | 1.000 |
| | B | 0.243 | 1.000 | | B | 0.495 | 1.000 |
| | C | 0.353 | 1.000 | | C | 0.195 | 1.000 |
| LEI94 | A | 0.911 | 1.000 | MCW264 | A | 0.895 | 1.000 |
| | B | 0.072 | 1.000 | | B | 0.105 | 1.000 |
| | C | 0.017 | 0.922 | MCW294 | A | 0.988 | 1.000 |
| MCW58 | A | 0.107 | 1.000 | | B | 0.012 | 0.861 |
| | B | 0.441 | 1.000 | MCW330 | A | 0.385 | 1.000 |
| | C | 0.315 | 1.000 | | B | 0.486 | 1.000 |
| | D | 0.137 | 1.000 | | C | 0.129 | 1.000 |

Table 2: Genetic variation in yangzhou chickens

| Locus | Chromosome | A | Ho | He | PIC | Fis |
|---|---|---|---|---|---|---|
| ADL155 | 3 | 1.172 | 0.129 | 0.147 | 0.136 | 0.124* |
| ADL183 | 1 | 4.058 | 0.745* | 0.755 | 0.712 | 0.013 |
| ADL185 | 2 | 2.443 | 0.551* | 0.592 | 0.544 | 0.069 |
| ADL201 | Z | 1.987 | 0.795* | 0.498 | 0.373 | -0.601++ |
| ADL217 | 2 | 1.508 | 0.345 | 0.337 | 0.310 | -0.022 |
| ADL292 | 5 | 2.963 | 0.907* | 0.665 | 0.603 | -0.367++ |
| LEI66 | - | 2.882 | 0.387* | 0.655 | 0.579 | 0.409** |
| LEI94 | 4 | 1.197 | 0.147 | 0.165 | 0.156 | 0.106* |
| LEI166 | 3 | 1.757 | 0.330 | 0.432 | 0.382 | 0.235** |
| MCW58 | 1 | 3.089 | 0.238* | 0.683 | 0.620 | 0.651** |
| MCW85 | 4 | 2.574 | 0.280* | 0.613 | 0.541 | 0.543** |
| MCW120 | 7 | 2.719 | 0.247* | 0.634 | 0.571 | 0.611** |
| MCW154 | Z | 1.356 | 0.267 | 0.263 | 0.251 | -0.018 |
| MCW170 | 4 | 3.555 | 0.581 | 0.721 | 0.668 | 0.194** |
| MCW180 | 4 | 3.650 | 0.609* | 0.727 | 0.678 | 0.162** |
| MCW258 | Z | 2.637 | 0.258* | 0.622 | 0.549 | 0.585** |
| MCW264 | 2 | 1.232 | 0.211 | 0.189 | 0.171 | -0.115 |
| MCW330 | 17 | 2.493 | 0.560 | 0.600 | 0.516 | 0.067 |
| Mean | | 2.404 | 0.422 | 0.517 | 0.464 | 0.184* |

*Loci showing significant deviation from Hardy-Weinberg equilibrium ($p<0.05$). *Loci showing significant levels of deficit in heterozygotes ($p<0.05$). **Loci showing significant levels of deficit in heterozygotes ($p<0.01$). ++Loci showing significant levels of excess in heterozygotes ($p<0.01$)

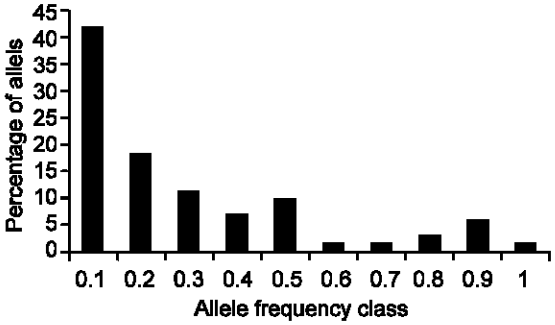


Fig. 1: Allele frequency distribution for the microsatellite loci examined in yangzhou chickens

The distribution of allele frequency was not uniform (Fig. 1). A majority (over 85%) of alleles showed a frequency lower than 0.5 (Fig. 1). The allele frequency distribution displayed an L shape, suggesting that no recent bottleneck affecting the genetic variability occurred (Yue *et al*., 2004; Pandey *et al*., 2005).

**Tests for neutrality and linkage disequilibria:** Population genetic analyses are based on several underlying assumptions. The most important of these is that the loci should be neutral and that the loci being genotyped are not in linkage disequilibrium. If either of these assumptions is consistently violated for any of the loci, then they should be excluded from further analyses of population genetic structure. The Ewens-Watterson Test for Neutrality showed that all of the twenty loci were neutral loci and they have not been infected by selection or other factors. Eighteen of the microsatellite loci used in the analysis was effectively independent since the pairs of microsatellite on the same chromosome did not show significant linkage disequilibrium. The microsatellite loci MCW 294 and ADL 273 on the Z chromosome were excluded in the following analysis because they showed significant linkage disequilibrium (data not shown, available on request). All the information indicated that the data from the eighteen microsatellite loci could be used to estimate genetic variation in this chicken population.

**Genetic diversity and levels of inbreeding:** Genetic variation of this sample is presented in Table 2. Among the eighteen microsatellites studied, all the loci were polymorphic. Ten of the 18 loci showed significant deviations from Hardy Weinberg equilibrium. Similar results have been reported from a wide range of chicken breeds (Chen *et al*., 2004; Qu *et al*., 2006). The number of alleles observed across the microsatellite loci varied from 2 (ADL155, ADL 201, ADL 273, MCW264 and MCW 294) to 6 (ADL183 and MCW 154) with an overall mean 3.778±1.309 (Table 1). The observed number of alleles across the loci was more than the effective number of alleles (1.232-4.058) as expected.

The PIC is an index reflecting genetic variation of microsatellite loci. The locus is highly polymorphic if PIC is larger than 0.5, medium polymorphic if between 0.25 and 0.5 and low polymorphic if smaller than 0.25. The values in this study showed that most of the loci were highly informative indicating the polymorphism across the loci with an overall mean of 0.464.

The observed heterozygosity ranged from 0.129-0.745 while, the expected heterozygosity (gene diversity) ranged from 0.147-0.755. Average Ho and He were 0.422±0.234, 0.517±0.207, respectively. Paired t-test indicated that the observed heterozygosity was slightly

lower than the expected heterozygosity (p=0.0625). Nine loci showed significant heterozygosity deficiency while two loci showed significant heterozygosity excess. The overall inbreeding coefficient of the population was positive value, which also indicated that Ho was lower than He.

The average genetic variation (0.517) observed in this study was higher than Gushi (0.453) and Baier (0.517) but lower than the values reported for 10 other Chinese breeds of chicken, viz. Xianju (0.532), Chahua (0.541), Luyuan (0.578), Tibetan (0.603), Dagu (0.627), Henan Game (0.518), Langshan (0.547), Taihe Silkies (0.569), Xiaoshan (0.609) and Beijing Fatty (0.541) (Chen *et al*., 2004; Qu *et al*., 2006). The result in this study indicated that Yangzhou Chicken have relatively lower genetic diversity among Chinese Chicken breeds and have moderate genetic diversity among the world breeds (Hillel *et al*., 2003) . The relative low genetic variations observed in this breed as compared to other Chinese breeds may be due to inbreeding in this breed.

$F_{IS}$, the inbreeding coefficient, measures the relative heterozygote deficit and non-random mating in the samples. Its value ranges between -1(all individuals heterozygote), 0 (random association of alleles) and 1 (all individuals homozygote). If inbreeding is avoided, F = 0; negative F indices are usually from selection in favor of the heterozygotes whereas positive values indicate that the considered population has an inbreeding system of mating. Ten $F_{IS}$ estimates across the loci were significantly positive (significant heterozygote deficit) ($p<0.05$) while two estimates were significantly negative (significant heterozygote excess) ($p<0.01$). The estimates ranged from -0.601-0.611 with an average of 0.184. Significant heterozygote deficiencies have been reported in other chicken breeds (Qu *et al*., 2006). The heterozygote deficiency could be due to the following reasons: segregation of non-amplifying (null) alleles, Wahlund effects (presence of population substructure), locus under selection (genetic hitchhiking), scoring biases (heterozygotes scored incorrectly as homozygotes) or inbreeding (Foltz, 1986). Null alleles are most unlikely to be segregating at all the loci. Nor do Wahlund effects (localities with subpopulations) account for the heterozygote deficiency because the chickens in this study were from the same population. Scoring bias may be possible for a few loci but not for all loci. All the loci were found to be neutral in Ewens–Watterson neutrality test, so selection is not the cause of heterozygote deficiency. Thus the most plausible explanation for heterozygote deficiency is inbreeding in this population as indicated by the high value of $F_{IS}$ (0.184). The deviations are understandable, as in artificial breeding the original structure is disturbed and random mating cannot be expected. Although in practice, hens were inseminated with semen from cocks that were not siblings, the general practice of breeding in chickens was to allow a cock for 10-50 hens. After a long time, this practice may lead to small effective population size or mating between individuals with common ancestor, thus, inbreeding could not be avoided in breeding.

In brief, the present study reveals that Yangzhou Chicken has substantial genetic variation and polymorphism across the loci, which is beneficial to be selected into commercial strains. There is fairly high degree of inbreeding but the speculated bottleneck was found to be absent. This is the high time but still safe enough to strengthen the conservation programme. For improvement of this breed having unique attributes like moderate egg weight, suitable egg color, adaptability and fitness with high meat quality, efforts should be firstly made to strengthen the conservation programme, including constructing core population and preservation area. Breeding strategies should therefore be designed to amplify the population size and simultaneously avoid inbreeding. The information generated in this study will greatly aid in the establishment of effective breeding for Yangzhou Chicken and may further be utilized for studying differentiation and relationships among different chicken breeds.

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