# Genetic Structure and the Conservation of Genetic Resources of Chai Chicken

[1]Zhen-Hua Zhao, [2]Jian-Zhong Xi, [2]Qing Jia, [2]Shou-Feng Li and [1]Hua-Yun Huang
[1]Chinese Academy of Agricultural Sciences, Poultry Institute,
225003 Yangzhou, Jiangsu Province, P.R. China
[2]College of Animal Science and Technology, Hebei Agriculture University,
071001 Baoding, Hebei Province, P.R. China

**Abstract:** With growing concern about maintenance of genetic variation and conservation of gene resources, the question arises on how to preserve the gene pool of a species. In this study, genetic diversity of 10 microsatellite locis was analyzed in Chai chicken breed. Allele frequency, effective number of alleles, heterozygosity, Polymorphism Information Content (PIC), F-statistics, migration rate and genetic bottleneck hypothesis were calculated. Number of alleles observed across the microsatellite loci varied from 3-8 with an overall mean of 5.0. Average observed Heterozygosity (Ho) and PIC amongst populations ranged from 0.5353-0.5614 and 0.5108-0.5199, respectively. To analyze population structure, pair wise Fst coefficients explained only 8.4% variability from the breed differences, the rest variability stems from individual differences. Gene flow of microsatellite loci was 2.7794, the result showed no significant genetic differentiation among populations. Bottleneck analysis indicated heterozygosis was obvious and group did not in the balance between mutation and excursion for its bottleneck. Researchers suggest that the moderate level of genetic diversity retained in Chai populations has the potential to supply sufficient genetic diversity for the species conservation through the recruitment of Chai resources in origin region and individuals exchanging among conservation farms.

**Key words:** Chai-chicken, microsatellite loci, genetic structure, bottleneck, conservation

## INTRODUCTION

Hebei Chai chicken is indigenous to the mountainous areas of the Taihang and guaranteed the sustainability of many Hebei families for centuries. It is valuable genetic resources of human being because of some good qualities including good endurance, strong antidisease, flavor and getting on well with others easily (Xi, 2005). However, the breed was faced with danger to extinct in its native breeding region because this population has been gradually substituted by commercial breeds marked by a massive importation and used of exotic breeds since the middle of the 20th century. Now-a-days, remaining Chai chicken being raised only by conservation farms, it will be tragic commentary if this unique breed is lost because there is a lack of concern for the conservation and improvement of this breed under field conditions. These adaptation and unique characteristics might have been diluted due to intermixing, sub-structuring and/or consequent genetic drift in the population over time.

The germplasm resources and characterize the genetic diversity of the Chai chicken in different conservation farms are unknown and the genetic differentiation among farms have never been evaluated. The knowledge of genetic relationships is indispensable for choosing productive individuals and establishing selection programs. Microsatellite loci are best suited for using to analyze the degree and pattern of genetic variability within and differences between populations and widely used for breed characterization and relationship among indigenous breeds (Hull *et al.*, 2008; Yang *et al.*, 2008). The objectives of this study were to study the estimates of genetic variability, population structure to evaluate the genetic bottleneck hypothesis in this breed and to provide basic molecular data for the research and scientific basis for the conservation and utilization of Chai chicken.

## MATERIALS AND METHODS

**Samples:** A total of 115 individual blood samples of 3 groups (i.e., Ninjing, Zanhuang and Yixian) were collected from the preservation farms (Table 1). The samples are unrelated which have no common grandparents within

**Corresponding Author:** Jia Qing, College of Animal Science and Technology, Hebei Agriculture University, 071001 Baoding, Hebei Province, P.R. China

two or three generations. Blood samples were collected into vacuum tubes with 1:1 decomposing solution (containing 10 mmol L$^{-1}$ Tris-HCl, 100 mmol L$^{-1}$ EDTA and 2% SDS) as anticoagulant. All blood samples were stored at -80°C before analyses.

**DNA extraction and PCR amplification:** Genomic DNA was isolated from blood using a modified Phenol/Chloroform Extraction Method. Blood was digested in 300 µL lysis buffer (10 mmol L$^{-1}$ Tris-HCl, 1 mmol L$^{-1}$ EDTA, 100 mmol L$^{-1}$ NaCl, pH 8.0) with 8 µL proteinase K (10 mg mL$^{-1}$) for 12 h at 55°C. The extraction was repeated three times. After precipitation by adding two volumes of ice-cold ethanol, DNA was isolated by centrifugation and then stored at -20°C for future use. DNA pellets were re-suspended in 30 µL TE buffer and the total genomi DNA was quantified using agarose gel electrophoresis. The DNA concentration was calculated according to the standards.

Ten microsatellite markers were investigated including ADL268, ADL278, MCW67, MCW248, MCW183, MCW330, MCW134, MCW120, MCW150 and LEI0066 (from GenBank). All primers were synthesized by the Shanghai Bioasia Bio-Tech. Co., Ltd.

PCR reactions were carried out in a 20 µL volume comprising 20-50 ng of genomic DNA, 2 pmol of each primer, 0.2 µL of 5 U µL$^{-1}$ Taq polymerase, 0.8 µL of 10 mmol dNTPs, 2 µL of 10×buffer and 2 µL of 25 mmol MgCl. PCR amplifications were performedas follows: an initial denaturation step at 95°C for 5 min followed by 30 cycles of 1 min at 94°C, 1 min at annealing temperature (from 49-62°C) and 1 min at 72°C and a final extension step at 72°C for 10 min.

The amplification products were separated by electrophoresis on 8% non-denaturing polyacrylamide gels along with DNA marker (pBR322 DNA/Msp I markers) and visualized by silver staining. The images data was analyzed using the Kodak Digital Science ID image analysis software.

**Statistical analyses:** Data analysis of allele frequency, the observed and expected mean heterozygosity for each group (Nei, 1973) Mean Number of Alleles per locus (MNA) and the exact test for Hardy-Weinberg equilibrium were performed using the POPGENE computer package. F-statistics (Fit, Fis and Fst) for each locus, pair-wise Fst (Weir and Cockerham, 1984) between populations, the estimate of average inbreeding coefficient (Fis) the number of migrants per generation (Nm = (1-Fst)/4Fst) (Wright, 1969) an indirect estimate of gene flow were done using FSTAT. Polymorphism Information Content (PIC) for each breed was determined according to Botstein *et al.* (1980). Finally the bottleneck hypothesis was investigated using bottleneck (Cornuet and Luikart, 1996). All the three models of mutation were used to calculate H$_{eq}$, the strict one stepwise Mutation Model (Ota and Kimura, 1973) the Infinite Allele Model (Kimura and Crow, 1964) and Two-Phase Model (Di Rienzo *et al.*, 1994).

## RESULTS AND DISCUSSION

**Genetic diversity:** Various measures of genetic diversity are presented in Table 2. The F-statistics estimates of population structure are presented in Table 3. The total number of alleles for the 10 microsatellite locis in three groups was 151 and the Mean Number of alleles (MNE) per locus was 5.03. MCW330 showed the highest number of alleles per locus (9) while MCW248 the lowest (3). Group specific alleles were 22 (14.6%) they were detected in each group and possessed rather low frequencies (>10%). The values of Ho ranged from 0.3578-0.6883 with the mean of 0.5488. The values of He ranged from 0.2300-0.5750 with the mean of 0.4064. The observed

Table 1: The names of herds and the amount of samples

| Farms | Places | Number | | |
|---|---|---|---|---|
| | | Females | Males | Total |
| I | Ninjing | 36 | 9 | 45 |
| II | Zhanhuang | 28 | 7 | 35 |
| III | Yixian | 28 | 7 | 35 |

Table 2: Average genetic paramters of 10 microsatellite loci in Chai-chicken

| | Farm I | | | | | Farm II | | | | | Farm III | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Na | Ne | Ho | He | PIC | Na | Ne | Ho | He | PIC | Na | Ne | Ho | He | PIC |
| ADL268 | 5 | 3.9028 | 0.6589 | 0.5740 | 0.5946 | 5 | 4.0198 | 0.6750 | 0.5800 | 0.6005 | 5.0 | 4.0828 | 0.6883 | 0.5750 | 0.6052 |
| ADL278 | 4 | 2.1670 | 0.6689 | 0.4380 | 0.6126 | 4 | 2.1598 | 0.6694 | 0.4328 | 0.6187 | 4.0 | 2.1616 | 0.6758 | 0.4300 | 0.6389 |
| MCW67 | 4 | 3.0198 | 0.5461 | 0.3520 | 0.4421 | 4 | 3.2206 | 0.5472 | 0.3607 | 0.4442 | 4.0 | 3.1375 | 0.5461 | 0.3508 | 0.4481 |
| MCW248 | 3 | 2.8598 | 0.3578 | 0.2300 | 0.2938 | 3 | 2.5429 | 0.4200 | 0.2750 | 0.3018 | 3.0 | 2.6682 | 0.4578 | 0.2360 | 0.3135 |
| MCW183 | 5 | 3.2206 | 0.4194 | 0.2900 | 0.3732 | 5 | 3.4258 | 0.4731 | 0.2970 | 0.3764 | 5.0 | 3.0568 | 0.4986 | 0.2950 | 0.3784 |
| MCW330 | 8 | 4.3375 | 0.6370 | 0.5130 | 0.5870 | 8 | 4.3003 | 0.6390 | 0.5150 | 0.5828 | 9.0 | 4.3010 | 0.6450 | 0.5150 | 0.5840 |
| MCW134 | 5 | 3.7782 | 0.4325 | 0.3360 | 0.4950 | 5 | 3.7258 | 0.4383 | 0.3321 | 0.4946 | 5.0 | 3.7847 | 0.4490 | 0.3360 | 0.4964 |
| MCW120 | 7 | 4.1010 | 0.4930 | 0.4050 | 0.6760 | 7 | 4.0985 | 0.4960 | 0.4050 | 0.6775 | 7.0 | 4.1157 | 0.4978 | 0.4040 | 0.6843 |
| MCW150 | 4 | 2.6985 | 0.5203 | 0.4300 | 0.5800 | 4 | 2.6027 | 0.5260 | 0.4860 | 0.5812 | 4.0 | 2.6522 | 0.5286 | 0.4360 | 0.5845 |
| LEI0066 | 5 | 2.6752 | 0.6190 | 0.4270 | 0.4540 | 5 | 2.6506 | 0.6130 | 0.4750 | 0.4548 | 5.0 | 2.6437 | 0.6270 | 0.4260 | 0.4654 |
| Average | 5 | 3.2760 | 0.5353 | 0.3995 | 0.5108 | 5 | 3.2747 | 0.5497 | 0.4158 | 0.5132 | 5.1 | 3.2604 | 0.5614 | 0.4040 | 0.5199 |

number of alleles across the loci was more than the effective number of alleles as expected. The Polymorphic Information Content (PIC) showed that most of the loci were highly informative which varied from a maximum of 0.6843 in MCW150 to a minimum of 0.3135 in MCW248. PIC for the 3 groups ranged between 0.4540 (Farm I) and 0.4654 (Farm III). Obviously, the above mentioned data showed a relatively higher genetic diversity in Chai chicken.

**Genetic variability:** The overall means of the F-statistics for population subdivision were significantly different from zero for all loci. The relatedness among the individuals in the given sample was also significantly

Table 3: The F-statistics and Mgration rate at 10 microsatellite loci

| Locus | Fis | Fit | Fst | Nm |
|---|---|---|---|---|
| ADL268 | -0.4046 | -0.1982 | 0.1960 | 1.4513 |
| ADL278 | -0.3341 | -0.2470 | 0.0691 | 3.5767 |
| MCW67 | 0.3170 | 0.4520 | 0.0111 | 1.0130 |
| MCW248 | -0.4030 | -0.2810 | 0.0303 | 0.6160 |
| MCW183 | 0.2140 | 0.4120 | 0.0445 | 0.7410 |
| MCW330 | -0.3090 | -0.2440 | 0.1480 | 4.7250 |
| MCW134 | 0.1500 | 0.1950 | 0.0870 | 4.5560 |
| MCW120 | -0.2950 | -0.2290 | 0.1520 | 4.6850 |
| MCW150 | -0.1970 | -0.1260 | 0.0500 | 3.9990 |
| LEI0066 | 0.0500 | 0.1390 | 0.0520 | 2.4310 |
| Average | -0.1212 | -0.0127 | 0.0840 | 2.7794 |

different from zero. The Fst an estimator of genetic differentiation among these samples was rang from 0.0111-0.1960 with the mean of 0.0840. In the overall population the Fst was partly due to the genetic differentiation among breeds (8.4%) and the most of that is due to the significant homozygote excess within breeds (91.6%). The highest Nm was observed in MCW330 loci (4.7250). The smaller Nm was obtained in MCW248 loci (0.6160). In mutation-drift-equilibrium, heterozygosity excess/deficiency under different mutation models generated by the Bottleneck showed that there were significant deficiency of heterozygosity (Table 4). Standardized test in all modal and Wilcoxon test in SMM Modal revealed that heterozygosis was obvious and group did not in the balance between mutation and excursion for its bottleneck.

The research in this study showed considerable genetic diversity. Compared with native chicken breeds, average heterozygosity and polymorphic information content was slightly lower than what observed in Xuefeng black bone chicken (0.6285 and 0.5496, respectively) with 23 locus (Wei *et al.*, 2008) Bian chicken breed (0.6671 and 0.7457, respectively) (Bai *et al.*, 2007) and Wuding chicken by 25 microsatellite loci (0.6957 and 0.6382, respectively) (Qian *et al.*, 2006). It also higher

Table 4: Mutation-drift-equilibrium, heterozygosity excess/deficiency under different mutation models in Chai chicken population

| Modals | Test | I | II | III | Total |
|---|---|---|---|---|---|
| | Sign test | - | - | - | - |
| | Hee | 6.8 | 6.8 | 7.1 | 6.88 |
| | Hd | 2 | 2 | 2 | 2 |
| | He | 8 | 8 | 8 | 8 |
| | P | 0.0760 | 0.0766 | 0.0906 | 0.0793 |
| IAM | Standardized test | - | - | - | - |
| | P | 0.004712 | 0.004278 | 0.033418 | 0.003286 |
| | Wilcoxon test | - | - | - | - |
| | P (one tail for Hdeficiency) | 0.6474 | 0.6690 | 0.6581 | 0.6546 |
| | P (one tail for H excess) | 0.26544 | 0.3416 | 0.3131 | 0.3142 |
| | P (two tails for H excess and deficiency) | 0.4309 | 0.6832 | 0.4573 | 0.4683 |
| | Sign test | - | - | - | - |
| | Hee | 6.93 | 7.23 | 7.38 | 7.30 |
| | Hd | 2 | 4 | 2 | 3 |
| | He | 8 | 6 | 8 | 7 |
| | P | 0.1332 | 0.3280 | 0.1628 | 0.1585 |
| TPM | Standardized test | - | - | - | - |
| | P | 0.007843 | 0.008866 | 0.055862 | 0.004712 |
| | Wilcoxon test | - | - | - | - |
| | P (one tail for Hdeficiency) | 0.1896 | 0.2114 | 0.1844 | 0.1954 |
| | P (one tail for H excess) | 0.9189 | 0.7972 | 0.8289 | 0.8631 |
| | P (two tails for H excess and deficiency) | 0.3792 | 0.4228 | 0.3841 | 0.4009 |
| | Sign test | - | - | - | - |
| | Hee | 7.23 | 7.03 | 7.53 | 7.38 |
| | Hd | 3 | 4 | 5 | 4 |
| | He | 7 | 6 | 5 | 6 |
| | P | 0.1528 | 0.3146 | 0.1729 | 0.1707 |
| SMM | Standardized test | - | - | - | - |
| | P | 0.019375 | 0.009703 | 0.013705 | 0.01253 |
| | Wilcoxon test | - | - | - | - |
| | P (one tail for Hdeficiency) | 0.0003 | 0.0060 | 0.0025 | 0.0105 |
| | P (one tail for H excess) | 0.8999 | 0.9946 | 0.8166 | 0.8904 |
| | P (two tails for H excess and deficiency) | 0.0005 | 0.0119 | 0.0004 | 0.0210 |

richness estimates than Chahua breed (0.3514 and 0.3143, respectively) studying by Wang with 7 microsatellite loci (Ye *et al.*, 2006). Overall heterozygosity estimates were comparable with what found in 50 European chicken breeds (lines) (Hillel *et al.*, 2003) while they are similar with chai chicken except that Yurlovcrower in Russia and Broiler dam line D were slightly higher (0.62). The results suggested that Chai chicken breed has high genetic diversity compared to others which showed comparable results in terms of mean number of alleles per locus. In this case their heterozygosity estimates were slightly lower, however, the highly significant deficiency of heterozygotes were detected.

The estimation of Fst provided a significant value (Average Fst value = 0.0840). It is generally accepted that Fst values under 0.05 indicate negligible genetic differentiation while those over 0.25 indicate a great deal of genetic differentiation (Weir, 1996). The average value of the Migration rate (Nm) found in Chai chicken was 2.7794. It show no significant genetic differentiation when Nm value was over 1 and under 1 show genetic differentiation because of genetic drift while those over 4 show a great deal of gene exchange (Keyghobadi *et al.*, 2005). Therefore, genetic isolation was only demonstrated among these farms. All of them were situated several hundred kilometres away from each other and belonged to different owners and previous data did not suggest genetic interchanges among farms. The value of the Fixation Index (Fst) showed that approximately 8.4% of the genetic variability came from breed differences and the rest came from individual differences. In the light of the estimated Fst and Nm values, managers were consulted in a search for possible genetic relationships among farms and similar circumstances but could not find any evidence to justify significant Fst. Breeds for both farms were selected randomly regardless of their origin when constitute conservation group so some of them has common ancestor. This could provide a reasonable explanation for low Fst and Nm values. However, the analysis of Fst values in birds has important peculiarities as demonstrated (Barrowclough, 1983) many and conspecific bird populations are little differentiated and it is difficult to find local populations or subspecies with Fst values >0.05. Therefore, the Fst values are in the ranks of usual observations in birds.

The speculation of genetic bottleneck was found in Hebai Chai chicken. However, the difference of values in different models is as per expectation. The higher heterozygosity deficiency exhibited by SMM was due to over sensitivity of this model for microsatellite mutation. These values were of similar magnitude obtained by POPGENE and FSTAT. The standardized difference tests indicated significant departure of the population from the mutation-drift-equilibrium under two-phase and single-step mutation models. However, sign tests under bottleneck hypothesis could not detect any significant departure from mutation-drift-equilibrium in the population but it also shown high probability of bottleneck effect.

## CONCLUSION

Chai chicken is mainly distributed in Hebei Province, located in Southeastern part of Taihang Mountains region approximately 1200 years ago and has genetically differentiated with excellent characteristics. This is the first attempt to specifically quantify the genetic diversity of the Chai chicken with microsatellite markers which helps to better understand the genetic diversity and population structure. There is worldwide recognition of the need for the conservation of livestock diversity and for characterization of breeds and populations including their genetic differentiation and relationships. Therefore, it is extremely urgent and necessary to conserve Chai chicken. Since, there are certain traits or genes unique to the mountains region they should be conserved as different units of management and conservation even though they have a weak differentiation. Populations of all three groups still possess some genetic diversity. Therefore, conservation management should now concentrate on an effective long-term protection of this remaining diversity. Two tentative and constructive plans or measures are suggestive of the following: the preservation of genetic diversity. More healthy individuals should be exchange at three farms to increase the observed heterozygosity, a key valuable index for the estimates of genetic diversity. The recruitment of Chai resources in origin region. To date, the exhaustions of natural resources in different mountains are severe and it was urgent to restore and complement Chai populations. Researchers confirm that the protection of the Chai chicken with molecular markers is highly advisable. Therefore, researchers advise further fragments to be put under protection in order to maintain the overall genetic diversity and to ensure the long-term survival of Chai chicken.

## ACKNOWLEDGEMENTS

## REFERENCES

Bai, W.L., R.H. Yin and G.Q. Yang, 2007. Analysis on population genetic diversity of Bian Chicken breed using microsatellite DNA markers. Acta Agric. Univ. Jiangxiensis, 29: 423-428.

Barrowclough, G.F., 1983. Biochemical Studies of Microevolutionary Processes. In: Perspectives in Ornithology, Brush, A.H. and G.A. Clark, Jr. (Eds.). Cambridge University Press, New York, pp: 223-261.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Cornuet, J.M. and G.L. Luikart, 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144: 2001-2014.

Di Rienzo, A., A.C. Peterson, J.C. Garza, A.M. Valdes, M. Slatkin and N.B. Freimer, 1994. Mutational process of simple-sequencerepeat loci in human populations. Proc. Nat. Acad. Sci., 91: 3166-3170.

Hillel, J., M.A.M. Groenen, M. Tixier-Boichard, A.B. Korol and L. David *et al.*, 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genet. Sel. Evol., 35: 533-557.

Hull, J.M., B.N. Strobel, C.W. Boal, A.C. Hull and C.R. Dykstra *et al.*, 2008. Comparative phylogeography and population genetics within *Buteo lineatus* reveals evidence of distinct evolutionary lineages. Mol. Phylogenet. Evol. 49: 988-996.

Keyghobadi, N., J. Roland and C. Strobeck, 2005. Genetic differentiation and gene flow among populations of the alpine butterfly, *Parnassius smintheus*, vary with landscape connectivity. Mol. Ecol., 14: 1897-1909.

Kimura, M. and J.F. Crow, 1964. The number of alleles that can be maintained in a finite population. Genetics, 49: 725-738.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA., 70: 3321-3323.

Ota, T. and M. Kimura, 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res., 22: 201-204.

Qian, L.D., T. Chen and J.L. Huo, 2006. Microsatellite DNA variation within the wuding chicken breed. J. Yunnan Agric. Univ., 21: 651-656.

Wei, L., S.G. Liu and X.W. Shi, 2008. Genetic diversity of the Xuefeng black bone chicken based on microsatellite markers. Biodiv. Sci., 16: 503-508.

Weir, B.S. and C.C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358-1370.

Weir, B.S., 1996. Genetic Data Analysis II: Methods for Discrete Population Genetic Data. 2nd Edn., Sinauer Association, Sunderland, Massachusetts, USA.

Wright, S., 1969. Evolution and the Genetics of Populations. University of Chicago Press, Chicago.

Xi, J.Z., 2005. Ivestigation of Chai chicken and its phylogeny taxonomic. Master Thesis, Hebei Agriculture University, Hebei, China.

Yang, C., X.P. Zhu and X.W. Sun, 2008. Development of microsatellite markers and their utilization in genetic diversity analysis of cultivated and wild populations of the mud carp. J. Genet. Genomics, 35: 201-206.

Ye, L.H., Y.W. Miao, J.L. Huo, H.J. Wei and J.P. Liu *et al.*, 2006. Genetic diversity of Chahua chicken based on microsatellite markers. J. Zool. (Chinese), 41: 37-42.