Research Journal of Poultry Sciences 6 (1): 13-17, 2013
ISSN: 1993-5285

Genetic Structure and the Conservation of Genetic Resources of Chai Chicken

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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

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

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

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) 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 5min 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 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

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 (MNA) 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 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 II). 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 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

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	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	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	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	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	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	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	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	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	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	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	3.2604	0.5614	0.4040	0.5199

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

(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 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 deficit of heterozygotes were detected.

The estimation of Fst provided a significant value (average Fst = 0.0840). It is generally accepted that Fst values under 0.05 indicate negligible genetic differentiation while those >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 >1 and <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 conspecific bird populations are little differentiated and it is difficult to find local populations or subspecies with Fst >0.05. Therefore, the Fst 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

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

Modal	Test	I	II	III	Total
IAM	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
	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
TPM	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
	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
SMM	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
	Standardized test				
	P	0.019375	0.009703	0.013705	0.01253
	Wilcoxon test				
	P (one tail for H deficiency)	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

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.

ACKNOWLEDGEMENT

This research was supported by the China Agriculture Research System (CARS-42-Z06), 333 training projects of Jiangsu (BRA2010162) and agricultural science and technology research project of Yangzhou (YZ2010050).

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