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Analysis of Genetic Structure and Diversity of Chai Chicken Breed Using Microsatellite Markers

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Abstract: Genetic diversity of 10 microsatellite locis were analyzed in Chai chicken breeds. Allele frequency, effective number of alleles, heterozygosity, Polymorphism Information Content (PIC), F-statistics, migration rate were calculated. The results showed that the average observed Heterozygosity (Ho) and PIC amongst populations ranged from 0.5353-0.5614 and 0.5108-0.5199, respectively. The genetic diversity was very rich and was of high selective potency. To analyze population structure, pairwise Fst coefficients explained only 8.4% variability from the breed differences, the remaining variability stems from individual differences. Gene flow of microsatellite loci was 2.7794 indicate no significant genetic differentiation among populations.

Key words: Chai chichen, microsatellite, genetic diversity, genetic differentiation, PIC, China

INTRODUCTION

Chai chichen is associated with fitness, flavor and survival which is indigenous to the mountainous areas of the Taihang and guaranteed the sustainability of many Hebei families for centuries. Since the middle of the 20th century these populations have been gradually substituted by commercial breeds marked by a massive importation and use of exotic breeds. Nowadays, remaining Chai chicken being raised only by conservation farms and the demand of consumers has been focused on flavor (Xi, 2005).

The germplasm resources and characterize the genetic diversity of the Chai chicken in different conservation farms are unknown and the genetic differentiation among farms has never been evaluated. The knowledge of genetic relationships is indispensable for choosing productive individuals and establishing selection programs.

Microsatellite markers are very useful to analyze the genetic diversity and genetic variability within and differences between populations (Hull *et al*., 2008; Yang *et al*., 2008; Olivieria *et al*., 2008; Zhang *et al*., 2008; Pandey *et al*., 2005; Alvarez *et al*., 2008) because of their high variability, high mutation rate, large number, distribution through out the genome, co-dominant inheritance and neutrality (Boyce *et al*., 1996; Gour *et al*., 2006). The objectives of this study were to study the estimates of genetic variability and population structure 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 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}

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

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

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Table 2: The name, sequence, annealing temperature and position of primers

Locus	Forward sequence	Reverse sequence	Annealing temperature (°C)
ADL268	CTCCACCCCTCTCAGAACTA	CAACTTCCCATCTACCTACT	49.0
ADL278	CCAGCAGTCTACCTTCCTAT	TGTCATCCAAGAACAGTGTG	50.0
MCW67	GAGATGTAGTGCCACATTCCGAC	GCACTACTGTGTGCTGCAGTTT	55.0
MCW248	TTGCATTAACTGGGCACTTTC	GTTGTTCAAAAGAAGATGCATG	55.0
MCW183	ATCCCAGTGTCGAGTATCCGA	TGAGATTTACTGGAGCCTGCC	55.0
MCW330	TGGACCTCATCAGTCTGACAG	AATGTTCTCATAGAGTTCCTGC	63.0
MCW134	GGAGACTTCATTGTGTAGCAC	ACCAAAAGACTGGAGGTCAAC	58.0
MCW120	CTATGTAAAGCTTGAATCTTCA	ATTCCTGGGTGCTAATTTACC	57.0
MCW150	TCCTGACTGAAATGGTACAGC	CATGAAAACCTTTGCCCTCAG	61.0
LEI0066	GATCAGATGCATCCAAAGTTC	GAAGCAGGAAAATAGAAAAGGC	56.0

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). The PCR primers are shown in Table 2. 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 markers) and visualized by silver staining. The images data was analyzed using the Kodak Digital Science ID Image Analysis Soft ware.

Statistical analyses: Data analysis allele frequency, the observed and expected mean heterozygosity (Nei, 1973). Heterozygosity for each group, Mean Number of Alleles perlocus (MNA) and the exact test for Hardy-Weinberg equilibrium, F-statistics (Fit, Fis and Fst) for each locus, pair-wise Fst (Weir and Cockerham, 1984) between populations and the estimate of average inbreeding coefficient (Fis) were performed using the Genepop computer package (ver. 3.3). The number of migrants per generation (Nm = (1-Fst)/4Fst) (Wright, 1969) an indirect estimate of gene flow was calculated. Polymorphism Information Content (PIC) for each breed was according to Botstein *et al*. (1980).

RESULTS AND DISCUSSION

Genetic diversity: Various measures of genetic diversity are shown in Table 3. The F-statistics estimates of population structure are shown in Table 4. 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 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 and 0.4654 Farm. Obviously, the mentioned data showed a relatively higher genetic diversity in Chai chicken as shown in Fig. 1.

Inter-population genetic variability: The overall means for the Wright's 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 to a larger extent to a 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).

Table 3: 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	5.0000	3.9028	0.6589	0.5740	0.5946	5.0000	4.0198	0.6750	0.5800	0.6005	5.0000	4.0828	0.6883	0.5750	0.6052
ADL278	4.0000	2.1670	0.6689	0.4380	0.6126	4.0000	2.1598	0.6694	0.4328	0.6187	4.0000	2.1616	0.6758	0.4300	0.6389
MCW67	4.0000	3.0198	0.5461	0.3520	0.4421	4.0000	3.2206	0.5472	0.3607	0.4442	4.0000	3.1375	0.5461	0.3508	0.4481
MCW248	3.0000	2.8598	0.3578	0.2300	0.2938	3.0000	2.5429	0.4200	0.2750	0.3018	3.0000	2.6682	0.4578	0.2360	0.3135
MCW183	5.0000	3.2206	0.4194	0.2900	0.3732	5.0000	3.4258	0.4731	0.2970	0.3764	5.0000	3.0568	0.4986	0.2950	0.3784
MCW330	8.0000	4.3375	0.6370	0.5130	0.5870	8.0000	4.3003	0.6390	0.5150	0.5828	9.0000	4.3010	0.6450	0.5150	0.5840
MCW134	5.0000	3.7782	0.4325	0.3360	0.4950	5.0000	3.7258	0.4383	0.3321	0.4946	5.0000	3.7847	0.4490	0.3360	0.4964
MCW120	7.0000	4.1010	0.4930	0.4050	0.6760	7.0000	4.0985	0.4960	0.4050	0.6775	7.0000	4.1157	0.4978	0.4040	0.6843
MCW150	4.0000	2.6985	0.5203	0.4300	0.5800	4.0000	2.6027	0.5260	0.4860	0.5812	4.0000	2.6522	0.5286	0.4360	0.5845
LEI0066	5.0000	2.6752	0.6190	0.4270	0.4540	5.0000	2.6506	0.6130	0.4750	0.4548	5.0000	2.6437	0.6270	0.4260	0.4654
Average	5.0000	3.2760	0.5353	0.3995	0.5108	5.0000	3.2747	0.5497	0.4158	0.5132	5.1000	3.2604	0.5614	0.4040	0.5199

Table 4: The F-statistics and migration 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

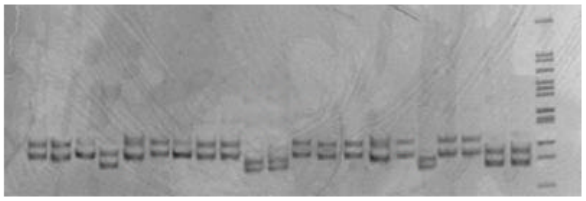

Fig. 1: The results of PCR

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 by Wei *et al*. (2008) in a study on Xuefeng black bone chicken (0.6285 and 0.5496, respectively) with 23 locus and Bai *et al*. (2007) studied on Bian chicken breed (0.6671 and 0.7457, respectively) and Qian *et al*. (2006) studied on Wuding chicken by 25 microsatellite loci (0.6957 and 0.6382, respectively) and found also higher richness estimates than Chahua breed (0.3514 and 0.3143, respectively) studying by Ye *et al*. (2006) with 7 microsatellite loci.

Overall heterozygosity estimates were comparable with what found in 50 European chicken breeds (lines) by 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 breeds have higher 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 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 Mgration rate (Nm) found in Chai chicken was 2.7794. When Nm value was over 1, It shows no significant genetic differentiation and under 1 show genetic differentiation because of genetic drift, while those over 4 show a great deal of gene exchange (Allendorf, 1983). 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 could not be found to justify not 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 by Barrowclough (1983) many conspecific bird populations are little differentiated and it is difficult to find local populations or subspecies with Fst values greater than 0.05. Therefore, the Fst values are in the ranks of usual observations in birds.

CONCLUSION

In this study, 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, helps to better understand the genetic diversity and population structure. 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.

RECOMMENDATIONS

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 and the recruitment of Chai resources in origin region. To date, the exhaustions of natural resources in different mountains are severe and production are highly needed to restore and complement Chai populations.

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