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Microsatellite DNA Typing for Assessment of Genetic Variability in Guangxi Three-yellow Chickens

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Abstract: In the present study, researchers report a genetic diversity study of Guangxi Three-yellow chicken by use of 30 microsatellite markers. Guangxi Three-yellow chicken is a light-body type breed with good meat quality which is famous for its three yellow morphology features in China. Microsatellite genotypes were derived and allelic and genotypic frequencied, hererozygosities and gene diversity were estimated. A total of 212 alleles were distinguished. All the microsatellites were polymorphic with mean allelic number of 7.067 ranging 2-11 per locus. The expected hererozygosity in the population ranged between 0.500 and 0.847 with mean of 0.734 indicating considerable genetic variation in this population. The F_{IS} values indicated low levels of inbreeding in the population. Genetic bottleneck hypotheses were also explored. The data suggest that the Guangxi Three-yellow chicken population has not experienced a genetic bottleneck in the recent past.

Key words: Genetic diversity, microsatellites, genetic bottleneck, Guangxi Three-yellow chicken, population

INTRODUCTION

With its long history of animal husbandry and diversified geographical conditions, China has a wide variety of indigenous poultry resources. There are 108 native chicken breeds recorded in China (Chen *et al*., 2004). Conservation of genetically unique breeds/ populations is of top priority to prevent loss of genetic diversity within each domestic species. Nevertheless, conservation measures are however, expensive to implement and as a result not all breeds or populations will be included. Unique and genetically diverse populations should therefore be identified in order to cover the widest range of genetic variability.

Guangxi Three-yellow chicken chicken is an important indigenous breed among them. It mainly distributes in Guangxi province, P.R. China. Guangxi Three-yellow chicken is a light-body type breed with good meat quality which is famous for its three yellow features i.e., yellow beak, yellow shanks and yellow skin. The numbers of Guangxi Three-yellow chickens have been drastically reduced due to the introduction of modern commercial chicken breeds and the limited resources available for conservation measures. In these circumstances, it is necessary to design more efficient conversation strategies for Guangxi Three-yellow chickens. Genetic variation is the basic material for animal breeding but the genetic resources required for the future are difficult to predict. Though decisions on conservation have to rely upon a range of information including the degree of endangerment, adaptation to a specific environment, possession of traits of current or future economic importance, possession unique traits of scientific interest and the cultural or historical value of the breed, molecular markers may serve as an important initial guide to evaluate breeds as genetic resources (Barker, 1999; Ruane, 1999; Weigend and Romanov, 2001).

Within the framework of breed conservation, genetic characterization is important in guarding breed integrity and is a prerequisite for managing genetic resources. Among the currently used molecular marker systems for genetic characterization, microsatellites are widely adopted to quantify genetic variation within and among breeds because of their extremely informative polymorphic nature, their abundance in the genome and the ease of amplification and typing by PCR (Rosenberg *et al*., 2002).

Studies on chicken biodiversity based on microsatellite marker included estimation of genetic diversity in commercial broiler and layer lines (Crooijmans *et al*., 1996), assessment of conversation efficiency of Dagu chicken and Beijing Fatty chicken (Qu *et al*., 2004) and analysis of genetic relationships among highly inbred chicken lines (Zhou and Lamond,

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1999) among African, Asian and South American local chickens (Wimmers *et al*., 2000) between various populations of domestic and jungle fowl (Romanov and Weigend, 2001) in 52 chicken populations (Hillel *et al*., 2003) and in Chinese native chicken populations (Du *et al*., 2004; Qu *et al*., 2006).

In this study, 30 microsatellite markers were used to investigate genetic diversity in Guangxi Three-yellow chickens. The population structure, genetic variability and genetic bottlenecks in Guangxi Three-yellow chickens were evaluated. The present study gives an account of the existing within-breed genetic variability in Guangxi Three-yellow chickens and the generated data can be used to determine genetic relationships with other indigenous as well as exotic chicken breeds. The results may also contribute to a more efficient conversation effort on Guangxi Three-yellow chickens.

MATERIALS AND METHODS

Experimental population: A total 60 individuals from Guangxi Three-yellow chicken breed were genotyped. These individuals were randomly selected from a centre of poultry resource in Yulin city, Guangxi Province.

DNA isolation: Per individual, 0.4 mL whole blood was collected from the ulnar vein with heparin as anticoagulant. Then, 4 mL of DNA lysate solution [2 M urea, 100 mM Tris-HCl (pH 8.0), 1% SDS, 100 mM EDTA] was added and the mixture was stored at 4°C. DNA was isolated by using a phenol/chloroform based method (Sambrook and Russell, 2001). DNA was quantified by spectrophotometer and the concentration was adjusted to 50 ng uL^{-1}.

Genotyping: The DNA polymorphism was assessed at 30 microsatellite loci (Table 1). These markers are randomly distributed across the chicken genome and most of these markers are part of the set of 30 microsatellites recommended by FAO (2004).

The 25 uL PCR volume included 50 ng of genomic DNA template, 1.0 uM of each primer, 200 uM of each dNTP, 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase. The amplification protocol comprised of an initial denaturation and enzyme activation phase at 95°C (15 min) followed by 35 cycles of denaturation at 95°C (1 min), primer annealing at temperature varying between 52 and 65°C (1 min) and extension at 72°C (1 min) and a final extension at 72°C for 10 min. The obtained fragments were detected on 2.0% agarose gel.

Table 1: The information of the 30 microsatellite markers

Markers	Chr.	Total No. of alleles	Effective No. of alleles	Range allele sizes (bp)	Ho	He	F_{IS}	PIC	Temperature (°C)
ADL123	11	5.000	2.866	108-142	0.932	0.651	-0.427**	0.583	53
ADL136	9	9.000	5.768	131-181	0.949	0.829	-0.137**	0.807	53
ADL166	5	10.000	6.376	130-174	1.000	0.847	-0.172**	0.830	55
ADL176	2	7.000	3.819	186-215	1.000	0.738	-0.344**	0.699	52
ADL185	2	7.000	3.495	126-170	1.000	0.712	-0.398**	0.700	60
ADL195	1	9.000	4.954	114-173	0.714	0.801	0.110*	0.776	52
ADL201	Z	3.000	2.047	137-151	0.864	0.512	-0.690**	0.404	53
ADL210	11	8.000	3.056	116-144	0.983	0.670	-0.460**	0.614	55
ADL211	9	6.000	4.020	102-133	1.000	0.756	-0.315**	0.719	53
ADL212	2	6.000	2.991	98-119	1.000	0.670	-0.485**	0.610	52
ADL225	13	9.000	5.211	141-191	0.983	0.808	-0.210**	0.780	59
LEI0094	4	11.000	4.654	171-236	1.000	0.782	-0.271**	0.753	63
LEI0066	14	6.000	3.685	298-360	1.000	0.726	-0.370**	0.683	56
MCW0067	10	6.000	4.055	180-224	1.000	0.759	-0.311**	0.723	65
MCW0081	5	7.000	3.639	115-154	0.983	0.725	-0.349**	0.675	63
MCW0085	4	7.000	4.266	270-325	1.000	0.767	-0.296**	0.730	57
MCW0014	6	7.000	5.961	181-232	1.000	0.831	-0.195**	0.809	63
MCW0183	7	7.000	3.284	291-350	0.627	0.700	0.103	0.663	64
MCW0264	2	8.000	5.478	224-267	1.000	0.815	-0.219**	0.791	56
MCW0294	Z	4.000	2.396	311-359	1.000	0.581	-0.716**	0.493	63
MCW0330	17	4.000	3.225	252-288	0.464	0.686	0.343**	0.630	63
MCW0120	7	8.000	5.492	262-314	1.000	0.816	-0.217**	0.791	57
MCW0134	9	7.000	5.038	273-341	0.982	0.801	-0.218**	0.771	58
MCW0150	3	4.000	3.936	217-267	1.000	0.745	-0.334**	0.697	61
MCW0147	8	7.000	3.070	153-238	0.627	0.678	0.074	0.622	54
MCW0145	1	2.000	2.000	186-195	1.000	0.500	-1.000**	0.375	61
MCW0004	3	8.000	3.725	185-221	0.776	0.730	-0.059	0.705	64
MCW0032	5	9.000	5.254	238-346	0.983	0.811	-0.205**	0.785	56
MCW0104	13	11.000	2.556	187-243	0.474	0.602	0.235**	0.576	64
MCW0295	4	10.000	4.365	95-127	0.949	0.769	-0.227**	0.738	62
Mean	-	7.067	4.023	-	0.910	0.734	-0.243**	0.683	-

*p<0.05; **p<0.01

The PCR products were subjected to 8% polyacrylamide gel in 1×TBE buffer and electrophoresed at 200 voltages for 2 h. The DNA bands on the gel were viewed by silver staining. Allele sizes were estimated using a 10-bp ladder (Invitrogen Life Technologies, Carlsbad, USA). Allele-size scoring was performed with RFLP scan software package (Scanalytics, Division of CSP, Billerica, USA).

Statistical analysis: Genotypes were assigned for each individual based on allele size data. Allele frequencies and expected Heterozygosity (He) (Nei, 1987) for each locus were estimated with Microsatellite-Toolkit for Excel (Park, 2001). Genetic differentiation within breed was examined by F_{IS} for each locus as implemented in FSTAT program (Version 2.9.3, Goudet, 2002). Significance of the F_{IS} was determined from permutation tests with the sequential Benferroni procedure applied over all loci. Polymorphism Information Content (PIC) for each locus was obtained according Botstein *et al*. (1980):

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

Where:

n = Number of alleles
p_i = Frequency of the allele i
p_j = Frequency of the allele j

To detect whether the Guangxi Three-yellow chicken population has experienced a recent reduction in the effective population size or a genetic bottleneck, two different approaches were followed. In the first approach based on heterozygosity excess, three different tests namely a sign test, a standardized differences test and a Wilcoxon sign-rank test were employed under different models of microsatellite evolution like the Infinite Allele (IAM), Stepwise Mutation (SMM) and Twophased (TPM) Models of mutation. The second approach was the graphical representation of the mode-shift indicator proposed by Cornuet and Luikart (1996). These two approaches were conducted using Bottleneck v1.2.02 software (http://www.ensam.inra.fr/URLB; Cornuet and Luikart, 1996).

RESULTS AND DISCUSSION

All microsatellite loci typed were polymorphic. The number of alleles per locus, effective number of alleles, expected Heterozygosity (He) and Polymorphism Information Content (PIC) were shown in Table 1. Across the 30 microsatellites studied, a total of 212 alleles were observed in the Guangxi Three-yellow chicken breed. The allele frequency data revealed a reasonable amount of polymorphism (Table 1). The number of observed alleles varied between two (MCW0145) and eleven (LEI0094 and MCW0104) with overall mean number of alleles per locus of 7.067. FAO has specified a minimum of four distinct alleles per locus for proficient judgment of genetic differences between breeds. By this criterion, 30 microsatellites employed in this study showed ample polymorphism for evaluating genetic variation within Guangxi Three-yellow chicken breed. The overall effective number of alleles was less than the observed value across all the loci and ranged from 2 (MCW0145) to 6.376 (ADL166) with mean of 4.023.

Genetic markers showing PIC values higher than 0.5 are normally considered as informative in population genetic analyses (Botstein *et al*., 1980). In this study, PIC values in the Guangxi Three-yellow chicken population ranged between 0.375 and 0.830 with mean of 0.683. Reasonably high PIC values observed for most of the markers are indicative of the usefulness of microsatellites for biodiversity evaluation in this breed.

Mean observed heterozygosity, averaged over the 30 loci was 0.910 which was higher than the expected heterozygosity (Table 1). Average expected heterozygosity (gene diversity) within the Guangxi Three-yellow population ranged from 0.500 (MCW0145)-0.839 (ADL166) with overall mean of 0.734. This value exceeded the value reported in the 52 European chicken breeds (Hillel *et al*., 2003), higher than the values estimated for commercial breeds (Crooijmans *et al*., 1996) and also higher than the value in the 78 Chinese indigenous chicken breeds (Qu *et al*., 2006). Guangxi Three-yellow chickens thus seem to harbour a good amount of genetic variation.

The F_{IS} estimates ranged between -0.074 and -1.000, with average of -0.243. On an average, there is a significant excess (24.3%) of heterozygotes in the Guangxi Three-yellow chicken population ($p<0.01$). All of the 30 microsatellite markers except ADL195, MCW0183, MCW0147 and MCW0004 contributed to this result significantly. This suggests that the Guangxi Three-yellow chicken breed retains considerable genetic variability and low levels of inbreeding, despite its declining population in the breeding region.

The finding from the bottleneck analysis is absence of bottleneck in Guangxi Three-yellow chicken in the recent past. The first approach based on heterozygosity excess research on the principle that in a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium gene diversity (Heq) which is computed from the observed number of alleles (k) under the assumption of a constant

Table 2: Number of loci with heterozygosity excess/deficiency and probabilities obtained from three microsatellite evolution models for bottleneck test

Tests	Exc. H exp	Exc. H obs	Def. H obs	p-value
Sign test				
IAM	17.550	25	5	0.02032
TPM	17.660	24	6	0.01249
SMM	17.650	15	15	0.21179
Standardize differences test				
(T2 value)	-	-	-	-
IAM	5.422	-	-	0.00000*
TPM	2.910	-	-	0.00181*
SMM	-2.415	-	-	0.00786*
Wilcoxon test (probabilities-one tail for H excess)				
IAM	-	-	-	0.00000*
TPM	-	-	-	0.00007*
SMM	-	-	-	0.72194

Deviation from the mutational equilibrium $p<0.05$

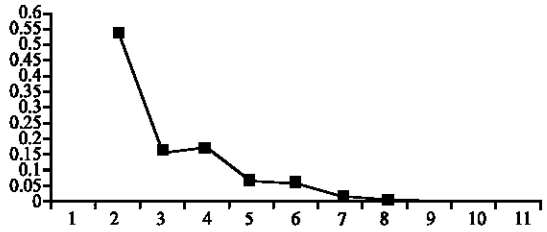

Fig. 1: Mode-shift analysis for test for genetic bottleneck in Guangxi three-yellow chicken chickens

size (equilibrium) population. None of the calculated P values (Table 2) was significant ($p>0.05$), demonstrating that the null hypothesis of mutation-drift equilibrium is fulfilled in this population. The second approach, the allele frequency spectrum visualized by the qualitative graphical method is shown in Fig. 1. The microsatellite alleles were organized into 10 frequency classes which permit checking whether the distribution followed the normal L-shaped form where alleles with low frequencies (0.01-0.1) are the most numerous.

When a population goes through a bottleneck rare alleles tend to be lost and the average number of alleles per locus or allelic diversity is reduced. Heterozygosity, however is not reduced proportionally because rare alleles contribute little to heterozygosity. The difference between allelic diversity and heterozygosity is used as the basis for statistical tests to detect presence of recent genetic bottleneck (Piry *et al*., 1999). The observed distribution suggests that the breed encountered a genetic bottleneck in the recent past. The concordance in the results of two approaches revealed the absence of a recent-past demographic reduction in the Guangxi three-yellow population.

CONCLUSION

In this study, the significant level of variability in Guangxi Three-yellow chicken breed is indicative of a valuable reservoir of genetic diversity in this breed. This fact, coupled with its evident environment adaptation and high economical value, emphasizes the importance of genetic regulation and conservation of this important indigenous breed as a valuable pure breed and its sustainable utilization in agricultural exploitation as a source for indigenous chicken improvement to achieve better production. The extension of the ongoing attempt at molecular-genetic characterization to other indigenous chicken breeds for determining genetic relationships will help in prioritizing the breeds on the basis of their genetic makeup and phylogenetic ranking for effective conservation and improvement programmes.

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