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Analysis of Genetic Diversity in Qingyuan Partridge Chickens Based on Microsatellite Markers

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Abstract: In the present study, we report a genetic diversity study of Qingyuan partridge chicken by use of 30 microsatellite markers. Qingyuan partridge chicken is a light-body type breed with good meat quality, which is famous for its 3 yellow, 2 thin and 1 partridge morphology features in China. Microsatellite genotypes were derived and allelic and genotypic frequencied, hererozygosities and gene diversity were estimated. A total of 154 alleles were distinguished. All the microsatellites were polymorphic, with mean allelic number of 5.1, ranging 2-9 per locus. The expected hererozygosity in the population ranged between 0.500 and 0.839, with mean of 0.685, 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. Our data suggest that the Qingyuan partridge chicken population has experienced a genetic bottleneck in the recent past.

Key words: Genetic diversity, microsatellites, genetic bottleneck, Qingyuan partridge chicken

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.

Qingyuan partridge chicken or Qingyuan chicken is an important indigenous breed among them. It mainly distributes in Qingyuan city, Guangdong province, P.R. China and got its name by the fact that there are lots of sesame-like pocks interspersing the back feathers of hens. Qingyuan chicken is a light-body type breed with good meat quality, which is famous for its 3 yellow, 2 thin and 1 partridge morphology features, i.e., yellow beak, shanks and skin; thin head and bone; partridge feather. The numbers of Qingyuan partridge 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 Qingyuan partridge 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

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genetic relationships among highly inbred chicken lines (Zhou *et al*., 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 Qingyuan partridge chickens. The population structure, genetic variability and genetic bottlenecks in Qingyuan partridge chickens were evaluated. The present study, gives an account of the existing within-breed genetic variability in Qingyuan partridge chickens and the genetated 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 Qingyuan partridge chickens.

MATERIALS AND METHODS

Experimental population: A total 60 individuals from Qingyuan partridge chicken breed were genotyped. These individuals were randomly selected from a centre of poultry resource in Qingyuan city, Guangdong 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 *et al*., 2001).

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 μL PCR volume included 50 ng of genomic DNA template, 1.0 μM of each primer, 200 μM 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.

The PCR products were subjected to 8% polyacrylamide gel in 1 × TBE buffer and electrophoresed

Table 1: The information of the 30 microsatellite markers

Markers	Chr.	Total no. alleles	Effective no. alleles	Range allele sizes (bp)	Ho	He	F_{IS}	PIC	Temperature (°C)
ADL136	9	9	6.200	117-163	1.000	0.839	-0.184**	0.818	53
ADL166	5	8	4.383	114-151	1.000	0.772	-0.284**	0.741	55
ADL185	2	9	4.891	113-155	1.000	0.796	-0.245**	0.767	60
ADL195	1	4	2.921	95-124	0.893	0.658	-0.350**	0.560	52
ADL210	11	5	2.325	108-121	1.000	0.570	-0.751**	0.476	55
ADL212	2	8	3.363	83-110	1.000	0.703	-0.416**	0.655	52
ADL225	13	7	5.725	118-158	0.915	0.825	-0.100*	0.802	59
ADL123	11	2	2.000	86-98	1.000	0.500	-1.000**	0.375	53
ADL201	Z	2	2.000	118-128	1.000	0.500	-1.000**	0.375	53
ADL176	2	6	4.976	172-202	1.000	0.799	-1.000**	0.770	52
LEI0166	3	4	2.439	254-290	1.000	0.590	-0.691**	0.504	58
LEI0066	14	6	3.647	298-360	1.000	0.726	-0.370**	0.683	56
LEI0094	4	7	4.388	158-223	1.000	0.772	-0.287**	0.738	63
MCW0295	4	3	2.649	80-96	1.000	0.622	-0.601**	0.551	62
MCW0014	6	4	2.138	181-204	1.000	0.532	-0.877**	0.423	63
MCW0067	10	4	3.372	164-193	1.000	0.704	-0.414**	0.649	65
MCW0081	5	4	3.279	96-126	0.983	0.695	-0.407**	0.643	63
MCW0183	7	4	2.563	287-351	0.683	0.610	-0.112	0.558	64
MCW0294	Z	3	2.388	311-359	1.000	0.582	-0.716**	0.492	63
MCW0330	17	4	3.060	257-289	0.729	0.673	-0.074	0.617	63
MCW0085	4	6	2.632	275-312	1.000	0.620	-0.608**	0.546	57
MCW0264	2	4	3.905	208-263	1.000	0.744	-0.337**	0.696	56
MCW0134	9	4	2.601	275-311	1.000	0.616	-0.619**	0.538	58
MCW0104	13	8	3.104	182-245	0.847	0.678	-0.242**	0.619	64
MCW0145	1	2	2.000	186-195	1.000	0.500	-1.000**	0.375	61
MCW0150	3	4	3.920	217-267	1.000	0.745	-0.334**	0.697	61
MCW0032	5	7	5.335	269-354	1.000	0.813	-0.223**	0.789	56
MCW0004	3	5	4.181	167-207	1.000	0.761	-0.307**	0.718	64
MCW0120	7	7	5.266	261-312	1.000	0.810	-0.226**	0.783	57
MCW0147	8	4	2.556	83-104	0.964	0.609	-0.578**	0.539	54
Mean		5.1	3.474		0.967	0.685	-0.418**	0.618	

*p<0.05; **p<0.01

at 200 V for 2 h. The DNA bands on the gel were viewed by silver staining. Allele-size scoring was performed with RFLPscan 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 = The number of alleles
p_i = Frequency of the allele i
p_j = Frequency of the allele j

To detect whether the Qingyuan partridge chicken population has experienced a recent reduction in the effective population size or a genetic bottleneck, 2 different approaches were followed. In the first approach, based on heterozygosity excess, 3 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. These 2 approaches were conducted using Bottleneck ver. 1.2.02 software (Cornuet and Luikart, 1996).

RESULTS AND DISCUSSION

All microsatellite loci typed were polymorphic. The number of alleles/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 154 alleles were observed in the Qingyuan chicken breed. The allele frequency data revealed a reasonable amount of polymorphism (Table 1). The number of observed alleles varied between 2 (MCW0145, ADL123 and ADL201) and nine (ADL136 and ADL185), with overall mean number of alleles per locus of 5.1. FAO has specified a minimum of 4 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 Qingyuan chicken breed. The overall effective number of alleles was less than the observed value across all the loci and ranged from 2 (MCW0145, ADL123 and ADL201) to 6.200 (ADL136), with mean of 3.747.

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 Qingyuan chicken population ranged between 0.375 and 0.818, with mean of 0.618. 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 0.967, which was higher than the expected heterozygosity (Table 1). Average expected heterozygosity (gene diversity) within the Qingyuan population ranged from 0.500 (MCW0145, ADL123 and ADL201) to 0.839 (ADL 136), with overall mean of 0.685. 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), but lower than the value in the 78 Chinese indigenous chicken breeds (Qu *et al*., 2006). Qingyuan partridge 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 -0.418. All loci showed excess of heterozygotes, with a negative F_{IS} value. On an average, there is a significant excess (41.8%) of heterozygotes in the Qingyuan partridge chicken population ($p<0.01$). All of the 30 microsatellite markers, except MCW0183 and MCW0330, contributed to this result significantly. This suggests that the Qingyuan partridge chicken breed retains considerable genetic variability and low levels of inbreeding, despite its declining population in the breeding region.

According to the results of Bottleneck ver. 1.2.02 software, ADL201 is a monomorphic locus, so, we analyzed the bottleneck based on the other 29 loci. The conclusion from the bottleneck analysis is in the recent past. The first approach based on heterozygosity excess works 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-size (equilibrium) population. All of the calculated p-values (Table 2) was

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

Test	Exc. H exp	Exc. H obs	Def. H obs	p-value
Sign test				
IAM	14.410	26	3	0.00001*
TPM	15.400	26	3	0.00003*
SMM	16.030	24	5	0.00169*
Standardized differences test (T_2 value)				
IAM	6.307			0.00000*
TPM	4.705			0.00000*
SMM	2.702			0.00344*
Wilcoxon test (probabilities-one tail for H excess)				
IAM				0.00000*
TPM				0.00007*
SMM				0.00150*

*Deviation from the mutational equilibrium p<0.05

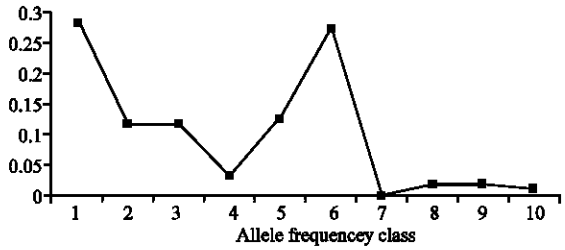

Fig. 1: Mode-shift analysis for test for genetic bottleneck in Qingyuan partridge chickens

significant (p<0.05), demonstrating that the null hypothesis of mutation-drift equilibrium is not fulfilled in this population. 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 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. The observed distribution suggests that the breed encountered a genetic bottleneck in the recent past. The concordance in the results of 2 approaches revealed the existence of a recent-past demographic reduction in the Qingyuan partridge population.

The significant level of variability in Qingyuan partridge 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. Nevertheless, according to our result, the breed encountered a genetic bottleneck in the recent past, which might be caused by its recent shrinking population size. Thus, it is now critical to initiate planned and organized breeding. To make a start, a Qingyuan breed society should be formed in advance, which should be educated and supported for comprehensive safeguarding and upgrading of the breed to make it economically sustainable.

CONCLUSION

In general, 30 microsatellite markers used in the present were polymorphic. Qingyuan partridge chicken has considerable genetic variation and low levels of inbreeding. However, the Qingyuan partridge chicken population has experienced a genetic bottleneck in the recent past, so, it is now critical to initiate planned and organized breeding of this breed.

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