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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Study on Polymorphism of Isfahan Native Chickens Population Using Microsatellite Markers

M.T. Beigi Nasiri, F. Shokri, S. Esmaeil Khanian and S. Tavakoli
Department of Animal Production, Ramin Agricultural and Natural Resources University, Mollasani, Ahwaz, Iran

Abstract: Polymorphism of Isfahan native chickens were evaluated using ten microsatellite markers. Nine microsatellite loci were found to be polymorphic, but one of them was monomorph (MCW0216). All microsatellite loci deviate from the Hardy-Weinberg equilibrium. Heterozygosity and the Polymorphism Information Content (PIC) were calculated to determine the genetic variation. Of the nine polymorphism loci, actual and effective number of alleles (n_a, n_e) per locus ranged from 2 to 5 and 2 to 3.9014, respectively. PIC has values between 0.3750 to 0.6972 per locus (except monomorphic locus) and average of polymorphism information content based on 10 microsatellite was estimated as 0.4897 for this population. The average of heterozygosity exclusive of monomorphic locus ranged from 0.5 to 0.7437 per locus and by attention of monomorphic locus was 0.5613 per population. In general, it can be concluded that Isfahan native chickens population has approximately low genetic diversity. Therefore designing breeding project needs attention to conserving the genetic diversity, so the genetic resources will be conserved as world's national investments.

Key words: Micro satellite, native chicken, polymorphism, heterozygosity

Introduction

Animal genetic resources are a resource for future food production and environmental and socio-economic stability. Major changes in production systems, caused by the world's growing population and demand for animal products, are to be expected (Blackburn *et al*., 1998). Accurate determination of the genetic variations within animal species is a fundamental step towards conservation of the animal genetic resources (Msoffe *et al*., 2005). Recent advances in molecular technology have provided new opportunities to assess genetic variability at the DNA level (Hillel *et al*., 2003). Among the DNA methods, micro satellite DNA marker has been the most widely used, due to its easy use by simple PCR, followed by a denaturing gel electrophoresis for allele size determination and to the high degree of information provided by its large number of alleles per locus (Vignal *et al*., 2002). In this study, we describe micro satellite polymorphisms of Isfahan native chickens population. From the genotyped data, the measure of genetic variability (polymorphism) was estimated.

Materials and Methods

Sample size and sample collection: A total of 150 chicken bloods were collected from the Isfahan native chickens population.

DNA isolation, PCR protocol and electrophoresis: DNA was extracted using an optimized salting out extraction method (Miller, 1988) that guarantees long term stability of DNA samples. DNA as quantified spectrophotometrically and the concentration was adjusted to 50 ng μL^{-1}. Ten micro satellite markers were chosen from the public FAO's guidelines (6) (http://dad.fao.org./ refer / library / guidline / marker . pdf, 2004) (MCW248, MCW295, MCW14, ADL268, ADL278, LEI166) and three markers selected from the recent studies (Hillel *et al*., 2003; Crooijmans *et al*., 1997) (LEI94, ADL112, MCW216, MCW34). Micro satellite markers used in this study and their chromosomal positions are listed in Table 1. PCR reaction mixture with the final volume of 15 µL included 50ng template DNA, 1×PCR buffer, 200 µm dNTPs, 0.25 µm of each forward (F) and reverse (R) of the primers, 1U μL^{-1} Taq DNA polymerase and $mgcl_2$ (4.5-6.5 µm). Sterilized distilled water was variable and it was estimated based on decrease of PCR mixture volume. An initial denaturation step at 94°C, was followed by 25 to 35 cycles at 94°C for 30s, appropriate annealing temperature (55-65°C) for 45s, extention at 72°C for 1': 30" and finally a 2 min extention at 72°C. LEI0166 amplified with touch down PCR. In this program the annealing temperature of the reaction is decreased 1°C every one cycle from 65°C to a touch down at 60°C, at which temperature 17 cycles are carried out. Minature tubes containing amplified PCR-Products were carefully opened and equal volume of loading dye added to each tube. Each DNA sample was run through all the 10 primer pairs. 10 µL of the sample were allowed to run on % 8 denatured polyacrylamide gel and were stained by silver staining method. The stained gels were scanned and genotype data were determined Popgene (Yeh *et al*., 1999) and PIC value (Ott *et al*., 1988-2001) softwares.

Table 1: Characterization of micro satellite markers used

Name	Chr	Primer sequence (5' -> 3') Forward Reverse	Genbank (Accession Number)	Observed alleles size range (bp)
ADL0268	1	CTCCACCCCTCTCAGAACTACAACTTCCCATCTACCTACT	G01688	95 -122
LEI0166	3	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	X85531	254-310
MCW0295	4	ATCACTACAGAACACCCTCTCTATGTATGCACGCAGATATCC	G32051	92-105
MCW0014	6	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	None	159-182
ADL0278	8	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	G01698	147-170
MCW0248	1	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	G32016	223-283
MCW0034	2	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG	None	205-291
LEI0094	4	GATCTCACCAGTATGAGCTGC TCTCACACTGTAACACAGTGC	X83246	247-342
MCW0216	13	GGGTTTTACAGGATGGGACG AGTTTCACCTCCCAGGGCTCG	AF030586	144
ADL0112	10	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC	G01725	131-144

Table 2: Observed and expected heterozygosities, number of alleles per locus and Polymorphic Information Content (PIC)

Locus	Observed Allele	Effective Allele	PIC-value	Exp-Het*	Ave-Het	Shannon index
MCW0014	4	3.1365	0.6181	0.6835	0.6812	1.2109
LEI0094	5	3.9014	0.6972	0.7462	0.7437	1.4058
MCW0295	4	2.6902	0.5538	0.6304	0.6283	1.1008
MCW0248	4	2.7843	0.5777	0.643	0.608	1.1408
MCW0034	4	2.0816	0.4041	0.5213	0.5196	0.7912
MCW0216	1	1.0000	0.0000	0.0000	0.0000	0.0000
ADL0268	5	2.9449	0.5986	0.6632	0.661	1.2189
ADL0112	4	3.4682	0.6564	0.714	0.7117	1.2954
ADL0278	3	2.1126	0.4161	0.5284	0.5266	0.805
LEI0166	2	2	0.375	0.5017	0.5	0.6931
Mean	3.6	2.6125	0.4897	0.5632	0.5613	0.9662

*Expected homozygosity were computed using Levene (1949)

Data analysis: Popgene software was used to estimate the observed and expected heterozygosity (Yeh *et al.*, 1999).

Expected theoretical heterozygosity from the Hardy-Weinberg assumptions was calculated using the formula (Hedrick, 1999).

$$H_i = 1-\Sigma^{n}_{i=1}P_i^2$$

Where: P_i as ith allele frequency.

Effective number of alleles (n_e) was calculated using the formula (Hedrick, 1999).

$$n_e = \frac{1}{\sum_{i=1}^{n} P_i^2}$$

Where: P_i as ith allele frequency

So Polymorphism Information Content (PIC) was estimated using allele frequencies in each polymorphic micro satellite locus, using the formula (Olowofeso *et al.*, 2005).

$$PIC = 1-\Sigma^{n}_{i=1} P_i^2-2 \Sigma^{n}_{i=1} \Sigma_{i=1}^{n} P_i^2 P_j^2$$

Where:P_i and p_j are frequencies of corresponding alleles.

Results and Discussion

The Hardy-Weinberg Equilibrium (HWE) test showed that all loci deviated from HWE. There are several explantations for the deviations. In this population selection and migration were the important reasons for this deviation. It is possible that such deviation may result the presence of null alleles and wrong genotyping (Vanhala *et al.*, 1998). But in this study, no homozygous null individuals were found.

Among 10 micro satellite loci, only MCW0216 was monomorph and the other loci demonstrates their utility as informative molecular markers for native chickens. Number of alleles, PIC values, shanon index and average of heterozygosity for each locus were depicted in Table 2. It was found that a comparing heterozygosity with PIC, all PIC values were less than their related heterozygosity. The relation between PIC and heterozygosity is not direct but they are closely related. The obtained results from Table 2 indicated that usually, loci with more alleles contain higher rate of heterozygosity and PIC values, but it isn't absolute. Because of affecting frequency of alleles. For in.stant in this study ADL0268 had 5 alleles, while it has the average heterozygosity and PIC values lower than ADL0112. Since the most polymorphic was LEI0094 with 5 alleles, average heterozygosity of LEI0094 was the highest (0.7437). At the other extreme, LEI0166 was the least polymorphic with 2 alleles and average heterozygosity 0.5. The mean Shannon information index in this work with attention of MCW0216 was 0.9662.

Average heterozygosity for this population exclusive of MCW0216 and with attention of monomorph locus were

0.5613±0.2142, 0.6237±0.0885, respectively. So gene diversity in this population is low. The level of genetic diversity in this study have been similar or different to other values reported for different chicken populations using micro satellite markers (Olowofeso *et al*., 2005; Vanhala *et al*., 1998). The variation in results could be adduced to differences in location, sample sizes, experimental chicken and sources of the micro satellite markers used. The low genetic diversity in this population could be attributed to its improvement breeding history for some traits. There for designing breeding project needs attention for conserving of genetic diversity, until the genetic resources will be conserved as world's national investments.

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