The Pharma Innovation Journal 2018; 7(5): 556-558

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(5): 556-558

www.thepharmajournal.com
Received: 18-03-2018
Accepted: 19-04-2018

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Molecular characterization of IWD and IWF white leghorn chicken using microsatellite markers

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Abstract
Genetic characterization of IWD and IWF strains of White Leghorn birds was undertaken based on analysis of 10 microsatellite markers that involved 120 birds. The distribution of microsatellite markers in each of these strains helped decipher genetic heterogeneity, population genetic structure and evolutionary relationships. All the microsatellite loci utilized for the analysis were polymorphic and reasonably informative; overall 4 primers (ADL176, MCW0014, ADL0210 and ADL158) showed PIC more than 0.5 which are very informative markers. Among the 5 loci the highest (4) number of alleles were obtained for loci ADL176, ADL0210, and the lowest (2) at the locus MCW0110. However the loci MCW0014 and ADL158 revealed 3 alleles in two strains studied. The number of alleles ranged from 2 to 4 in both strains, observed heterozygosity ranged from 0.23 (ADL158) to 0.40 (ADL210) in IWF and 0.22 (ADL 158) to 0.40 (MCW014) in IWD.

Keywords: White leghorn, heterozygosity, PIC, IWD, IWF, microsatellites

Introduction
Poultry production is an important livestock sector contributing to a high proportion of animal protein for human needs through meat and eggs. Genetic variation is the base for any future breeding strategies in all farm animal species and therefore genetic diversity within a species needs to be conserved. Genetic selection plays a major role in improvement in production efficiency of layers and broilers and brought about 85 to 90 percent of the changes that occurred in broiler growth rate over 50 years (Sharma and Chatterjee, 2006) [14].
Assessing the genetic diversity among chicken breeds by using molecular tools is essential for designing future conservation and genetic improvement programmes (Osman *et al*., 2006) [6]. Among the DNA markers, microsatellites or short tandem repeats (STR's) are most reputed markers of choice as they provide a polymorphic and robust marker system since they are abundant, co-dominant, randomly available across genome, having high information content due to variable number of repeats, high mutation rate, ability to decipher moderate to high level of variability, amenability to PCR and ease of genotyping (Pandey *et al*., 2005; Kaya and Yildi, 2008) [8, 4].

Material & Methods
The present investigation was carried on two strains of White Leghorns *viz.*, IWD and IWF maintained at AICRP on poultry, Rajendranagar, Hyderabad.
The IWD and IWF were selected for egg production based on 64 week egg production using Osborne Index. The Two strains of white leghorn chicken IWD, IWF utilized in the present study were under selection for high egg production (EP40) based on Osborne index since 1971. The selection for the last 9 generations was based on EP64. The blood samples were collected from 9th generation birds.

Isolation of genomic DNA
Blood samples (0.5-2.0 ml per bird) were collected into vacuutainers (3ml) containing EDTA (5.4 mg) from the wing vein. The blood samples were mixed gently and stored at -20 ^{0}C until further processing. High molecular weight genomic DNA was isolated by standard phenol-chloroform-extraction and ethanol precipitation method and stored at -20^{0} C for further usage. The quantity of the genomic DNA was measured by nanodrop (JENWAY Genova Nano) and the quality was evaluated by electrophoresis on 0.8 per cent agarose gel.

The concentration of the DNA was estimated by using the formula developed by.
The purity of DNA was determined by the ratio of optical absorbance (A) at 260 and 280 nm of wavelength. The A260/A280 ratio ranging from 1.6 to 2.0 was considered as relatively pure DNA and only such samples were used for PCR amplification.

PCR amplification of different allelic segments
The different allelic segments pertaining to 10 primers were amplified in a thermal cycler with initial denaturation at 95 ^{0}C for 5 minutes followed by 34 cycles of 94 ^{0}C for 1 minute for cyclic denaturation, 55 ^{0}C for 30 sec for primer annealing, 72 ^{0}C for 30 sec for primer extension and final extension at 72 ^{0}C for 5 min.
PCR amplification was carried out in a 200 µl tube with 10 µl reaction mixture containing 2.5 µl of each primer (5 pM), 1µl of 10X PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 µl dNTP's (200 µM), 0.1 µl Taq polymerase (1U) and 2 µl of template DNA and the volume was made up to 10 µl by adding the sterile distilled water.

Resolution of alleles and allele scoring
The PCR amplified products were electrophoresed on 0.8% non-denaturing polyacrylamide gel containing acrylamide and bis-acrylamide in the ratio of 29:1. The gel was run at 160V for 6 hrs in 1X TBE and genotyped by silver staining method following the standard protocol (Bhattacharya *et al*., 2007) [1] and the gel was visualized and genotyped under gel documentation system (Syngene). The genotype of every allele was determined manually from the gel. Genotyping involved the recording of the homozygous or heterozygous state of the alleles as well as the size of the respective alleles. Allele size was estimated by comparison with a standard ladder DNA marker.

Results & Discussion
Microsatellites are the markers of choice due to their polymorphism as well as higher reliability. The present investigation was carried out with the objectives of studying microsatellite diversity in terms of Allele frequency (Af), Polymorphism Information Content (PIC) and Heterozygosity of White Leghorn strains IWF and IWD.

Allele frequency (Af)
The frequency of various alleles at different loci in different populations/strains and in individual birds is detailed in Table.2. All the five loci utilized in the present investigation were found to be polymorphic in all the populations studied. Overall 4 primers (ADL176, MCW0014, ADL0210 and ADL158) showed PIC more than 0.5 which are very informative markers. Among the 5 loci the highest (4) number of alleles were obtained for loci ADL176, ADL0210, and the lowest (2) at the locus MCW0110. However the loci MCW0014 and ADL158 revealed 3 alleles in the two strains studied. The number of alleles ranged from 2 to 4 in both the strains. The amplification of alleles in ADL176 and MCW0014 are shown in fig 1 and 2.
The allele frequency distribution in the present study was observed to be discrete and ranged between 0.017 to 0.867 in IWF and 0.050 to 0.692 in IWD as reported by many authors (Vanhala *et al*., 1998; Pirany *et al.*, 2007; Pipalia *et al.*, 2008; Rajkumar *et al*., 2007 and Chatterjee *et al*., 2010a) [15, 10, 9, 2, 12]. The single base pair differences observed for some of the di/tri nucleotide repeat alleles might be due to the point mutations in the flanking region. These findings are in accordance with the earlier reports of Romanov and Weigend (2001) [13] with respect to ADL158 and MCW0014 loci which were used in the present study.

Polymorphism Information Content (PIC)
All the microsatellite loci utilized in the present study were found to be polymorphic with a reasonable informativeness.
The mean PIC is an ideal index to measure the polymorphism of alleles. The PIC value of more than 0.5 indicates high polymorphism, 0.25 to 0.50 a moderate and less than 0.25, a low polymorphism. The PIC values for all the loci were above 0.50 except for MCW110. Mean PIC estimates of remaining 4 loci ranged from 0.61 (ADL210) to 0.50 (MCW014) and is in agreement with the published mean PIC values of 0.64 in Aseel (Pandey *et al*., 2002) [7], 0.62 in Ankaleswar (Pandey *et al*., 2005) [8], 0.55 in IWD and 0.51 in IWF (Rajkumar *et al*., 2007) [12] and 0.59 for multiple Indian native chicken 0.364 for MCW007 to 0.723 for ADL136 (Chatterjee *et al*., 2010a) [2]. Mean PIC values lower than those observed in the present study were reported by Chen *et al*., (2004) [3] in Chinese chicken (0.31 to 0.52), and Mahadeokumar *et al*., (2006) [5] in White Leghorns (0.27 to 0.49), Kaya *et al.*, (2008) [4] in Denizli and Gerze chickens 0.599 and 0.426.

Heterozygosity
Heterozygosity is the unit of measurement for population diversity and variation. In the present study, observed heterozygosity ranged from 0.23 (ADL158) to 0.40 (ADL210) in IWF and 0.22 (ADL158) to 0.40 (MCW014) in IWD. Average observed heterozygosity estimates obtained in the present study were lower than the published reports of several Indian chicken populations 1.00 (ADL158), 0.65 (ADL210) in IWF and 0.95 (ADL158) and 0.50 (MCW014) in IWD.

Marker	Average Heterozygosity IWF	Average Heterozygosity IWD
ADL0176	0.42	0.28
MCW 0014	0.23	0.40
ADL210	0.45	0.35
ADL158	0.25	0.22
MCW110	0.33	0.33

Table 1: PCR conditions for microsatellite markers

S. No	Primer	Annealing temperature (^{0}C)	$MgCl_2$ concentration (mM)	Taq Polymerase concentration (Unit)	Primer concentration (Picomoles)
1	ADL176	55	1.5	0.1	5
2	MCW014	55	1.5	0.1	5
3	ADL210	55	1.5	0.1	5
4	ADL158	55	1.5	0.1	5
5	MCW110	55	1.5	0.1	5

Table 2: Allelic frequencies at different microsatellite loci in two strains of IWD and IWF

S. No	Locus	Allele No.	Allele size (bp)	IWF	IWD
1	MCW0110	1	110	0.633	0.667
		2	120	0.367	0.333
2	ADL176	1	168	0.150	0.092
		2	188	0.575	0.692
		3	176	0.133	0.083
		4	200	0.142	0.133
3	MCW0014	1	175	0.867	0.467
		2	184	0.117	0.267
		3	198	0.017	0.267
4	ADL158	1	188	0.175	0.142
		2	194	0.550	0.525
		3	208	0.275	0.333
5	ADL0210	1	125	0.475	0.575
		2	128	0.400	0.242
		3	130	0.075	0.050
		4	135	0.050	0.133

Table 3: Average PIC values for different microsatellite markers

Marker	PIC values
ADL0176	0.55
MCW 0014	0.50
ADL210	0.61
ADL158	0.59
MCW110	0.46

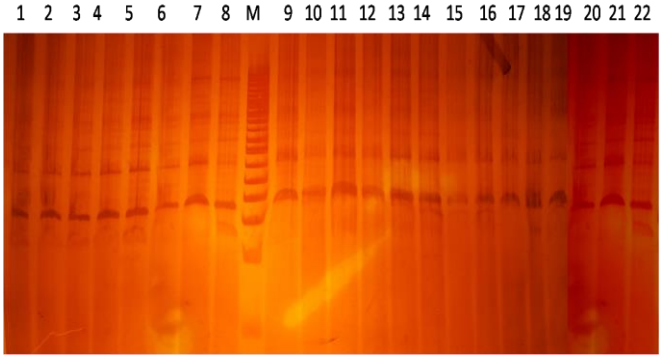

Fig 1: PAGE gel showing the allelic pattern of locus ADL176 in IWD

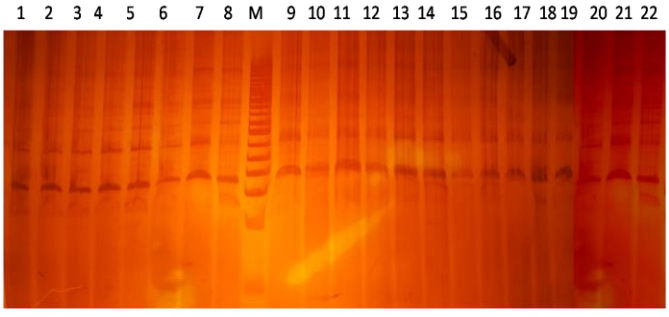

Fig 2: PAGE gel showing the allelic pattern of locus in MCW014 in IWF

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