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Genetic characterization of IWD and IWF strains of white leghorn through microsatellite markers

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Abstract
Genetic characterization of IWD and IWF strains of White Leghorn birds was undertaken using 5 microsatellite markers in 120 birds. A total of 33 alleles were obtained across all loci. The loci ADL102 and MCW104 showed a maximum of 4 alleles, where as the loci MCW 04, MCW 05 and MCW 29 each showed 3 alleles in the population. The expected heterozygosity estimates ranged from 0.39 (ADL102) to 0.61 (MCW004). The PIC values ranged from 0.40 for MCW29 to 0.62 for MCW004 among the populations. The Loci MCW29 in IWF demonstrated non-significant deviation for hardy Weinberg equilibrium while all other loci had significant deviation at P≤0.00.

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

Introduction
Microsatellite markers have been successfully used in many studies of genetic diversity in chickens. The microsatellite loci represent an independent evolutionary history of a population if they fulfill the conditions like Mendelian inheritance; reasonable PIC values; presence of different chromosomes/linkage groups and independent assortment (Rajkumar *et al*., 2008) [17]. Further microsatellites are best suited to assess the genetic variation available in the populations. The determination of heterozygosity and genetic distance based on microsatellite analysis is regarded as the most convenient tool and many microsatellite loci are available in chicken. The relative ease of scoring, ability to exhibit high level of polymorphism and higher heterozygosities, its application as genetic appraisal tool is quite significant. The erosion of animal genetic resources has accelerated in recent years as a consequence of development of intensive livestock production systems. Genetic variation is the base for any future breeding strategy and therefore genetic diversity within a species needs to be conserved. Molecular genetics is now opening the black box by elucidating the effect of single genes on the phenotypic expression of the trait. The study aimed to decipher the genetic structure of IWD and IWF strains in terms of genetic diversity by using microsatellite markers.

Materials and Methods
The present investigation was carried on 60 birds of each strain of White Leghorns maintained at AICRP on poultry, Rajendranagar, Hyderabad.
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 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 (Sambrook and Russell, 2001) 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% agarose gel. The concentration of the DNA was estimated by using the formula developed by Sambrook and Russell (2001).
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 5 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 resolved 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) 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 genetic diversity in terms of Allele frequency (Af), Polymorphism Information Content (PIC) and Heterozygosity of White Leghorn strains IWF and IWD were presented

Number of alleles

The number of alleles amplified at different loci in 2 population/strains is detailed in table 2. It was found that the loci ADL102, MCW104 showed maximum of 4 alleles, and the loci MCW04, MCW05 and MCW 029 each showed only 3 alleles across the population, The number of alleles within the populations ranged from 3 to 4 in both the strains. The mean number of alleles (Na) was 3.1 in IWF and 3.3 in IWD.

The allele size (bp) varied form 90bp for ADL102 to 275bp for MCW005 locus. The overall mean effective number of alleles (Ne) was ranged from 1.72 at MCW104 2.14 for ADL 102. The allele frequency ranged from as low as 0.008 in MCW 104 to as high as 0.75 for locus in ADL102. The allele frequency distribution in the present study was observed to be discrete and ranged between 0.001 to 0.867 in IWF and 0.017 to 0.85 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*., 2010) [24, 14, 13, 16, 2]. 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. Similar observations were made by Romanov and Weigend (2001) [18] for alleles at MCW004, MCW005 and MCW0014 loci, some of which were used in the present study also.

Heterozygosity

The heterozygosity is the state of an individual with different alleles of a gene at a particular locus. The number of birds, number of loci, mean number of alleles per locus, expected heterozygosity (H_e) and observed heterozygosity (H_o) estimates across the two populations are presented in Table. 2. The number of loci studied was 10 in two populations and the number of birds sampled in two populations was 60 each. The mean number of alleles amplified per locus was 4 in ADL102 and MCW104. The expected heterozygosity estimates ranged from 0.39 (ADL102) to 0.61 (MCW004) with an overall mean of 0.48±0.027. The observed heterozygosity estimates were highest in MCW 04 (0.48) and lowest in MCW104 (0.01) among the populations. These findings are in agreement with series of authors Vanhala *et al*., (1998) [24], Rajkumar *et al*., (2008) [17], Singh *et al*., (2009) [22] and Chatterjee *et al*., (2010) [2]. However few reports showed expected heterozygosity of 0.37 to 0.67 in Brazilian chicken (Rosario *et al.,* 2009), 0.31 to 0.42 in White Leghorns (Mahadeokumar *et al*., 2006) [9], 0.48 (Pipalia *et al.*, 2008) [13], 0.32 to 0.54 in 3 chicken lines (Davilia *et al.*, 2009) [5], 0.34 to 0.70 (Rhousdy *et al.,* 2013) [20] which was lower than the findings of Chattopadhyay *et al*., (2009) [3].

The overall mean observed heterozygosity (Table 2) was 0.26 but across the loci it ranged from 0.00 (MCW104) to 0.48 (MCW004). The observations are similar to the findings with 0.28 to 0.45 (Maretto *et al*., 2013) [10], 0.00 to 0.123 (Zhou and Lamount 1999) [25], 0.003 to 0.735 (Sgdavilia 2009), 0.25 (Pratap *et al.,* 2013) [15], 0.20 to 0.79 (Rhousdy *et al.,* 2013) [20]. A range of values 0.152 to 0.25 (Kumar *et al.,* 2006), 0.64 (Rajkumar *et al*., 2008, Singh *et al*., 2009 [22] and Chatterjee *et al*., 2010) [2], 0.46 to 0.59 (Suh *et al.,* 2014) [23], 0.08 to 0.27 (Saini *et al.,* 2007) [21], 0.65 (Pandey *et al.,* 2002) [11] for heterozygosity were reported in past.The locus MCW104 was completely homozygous in both strains.

Polymorphism Information Content (PIC)

The mean PIC value observed was 0.50 and it ranged from 0.24 to 0.64. Except MCW029, MCW104 and ADL102 remaining all loci showed PIC of 0.5 indicating high degree of polymorphism. Earlier reports of (Pandey *et al*., 2002) [11] with 0.64 in Aseel, 0.62 in Ankaleswar (Pandey *et al*., 2005) [12], 0.55 in IWD and 0.51 in IWF (Rajkumar *et al*., 2007) [16] and 0.59 for multiple Indian native chicken (Ahlawat *et al*., 2007) [1], 0.364 for MCW007 to 0.723 for ADL136 (Chatterjee *et al*., 2010) [2] are in accordance with present findings with respect to PIC. However lower values for PIC were reported by Chen *et al*., (2004) [4] in Chinese chicken (0.31 to 0.52), and Mahadeokumar *et al*., (2006) [9] in White Leghorns (0.27 to 0.49), Kaya *et al.,* (2008) [7], Denizli and Gerze chickens 0.599 and 0.426.

Hardy Weinberg Equilibrium

The Hardy-Weinberg equilibrium status of the population was tested for all the loci in all the populations studied and presented in Table.3. All the loci deviated significantly from equilibrium frequency in populations studied except MCW029 in IWF and showed non significance difference. The results were in accordance with findings of Emara *et al*. (2002) [6], Pandey *et al*. (2005) [12], Rajkumar *et al*. (2008) [17] and Singh *et al*. (2009) [22] also observed deviations from equilibrium frequency in most of the loci examined.

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	ADL102	45	2.0	0.2	7
2	MCW005	55	1.5	0.1	5
3	MCW004	58	1.5	0.1	5
4	MCW104	56	1.5	0.1	5
5	MCW029	58	1.5	0.1	5

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

Pop	Locus	No. of samples (N)	Product size (bp)	No. of alleles (Na)	No. of effective alleles (Ne)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Polymorphic Information content (PIC)
IWF	ADL102	60	90-120	4	2.14	0.05	0.51	0.54
	MCW005	60	258-275	3	2.20	0.23	0.52	0.55
	MCW004	60	208-220	3	2.81	0.48	0.61	0.64
	MCW104	60	198-210	2	1.72	0.00	0.39	0.42
	MCW29	60	209-224	3	1.74	0.38	0.41	0.42
IWD	ADL102	60	90-120	4	1.36	0.03	0.24	0.27
	MCW005	60	258-275	3	2.26	0.23	0.52	0.56
	MCW004	60	208-220	3	2.34	0.37	0.51	0.57
	MCW104	60	190-210	4	1.64	0.00	0.37	0.39
	MCW29	60	209-224	3	1.57	0.26	0.41	0.36

Table 3: Chi-square calculated values for testing the Hardy -Weinberg equilibrium at various loci in the populations

S.No.	Locus	IWF		IWD	
		Df	χ^2	Df	χ^2
1	ADL102	6	140.33^{***}	6	145.99^{***}
2	MCW5	3	75.45^{***}	3	71.83^{***}
3	MCW004	3	49.06^{***}	3	25.56^{***}
4	MCW104	1	60.00^{***}	6	180.00^{***}
5	MCW29	3	2.92^{NS}	3	11.06^{*}

*** significant at P≤ 0.001, ** significant at P≤ 0.01, * significant at *P*≤ 0.05,
^{NS} Not significant, *df* = (Number of heterozygotes - Number of homozygotes)

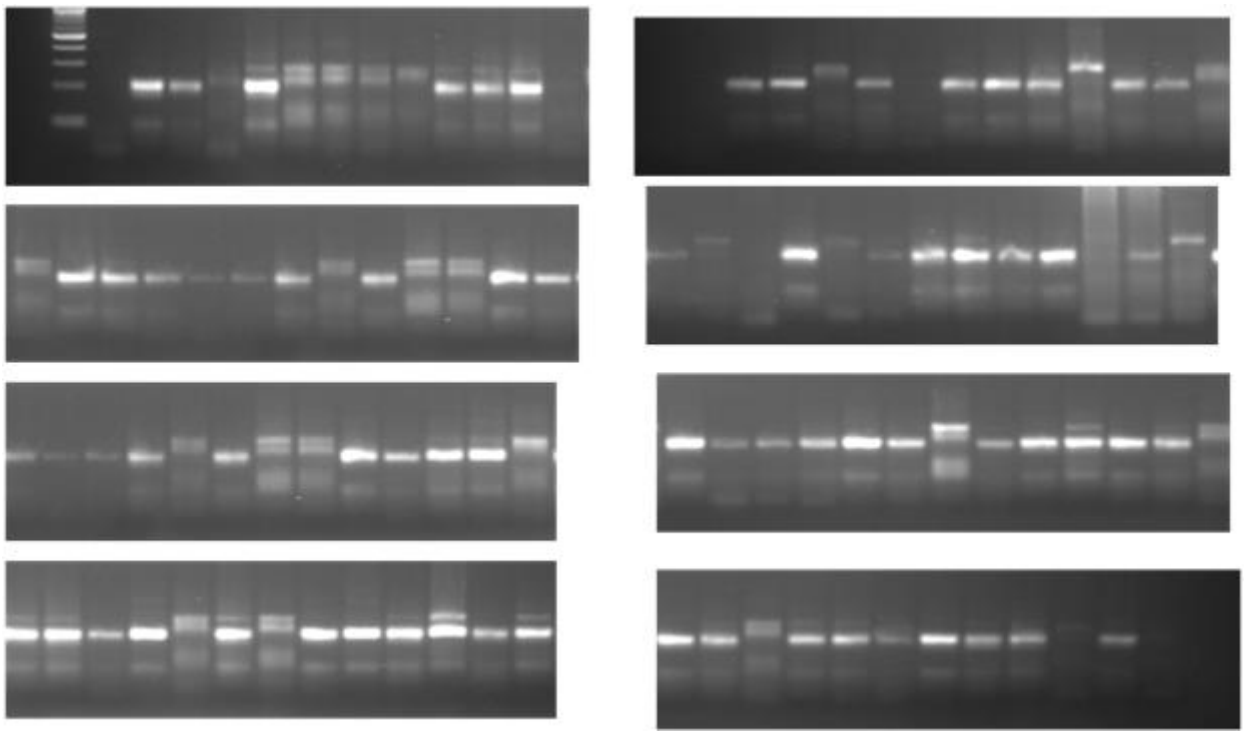

Fig 1: Agarose gel electrophoresis showing allelic pattern of ADL 102

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