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Genetic variation and genetic diversity in chicken populations using microsatellite assay

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Microsatellites are one of the recent markers widely used in gene marker studies, as they are abundant, co-dominant, highly polymorphic and dispersed throughout the genome. Microsatellites are identified as reliable markers in chicken (Romanov and Weigend 2001).

Birds (212) representing 8 populations were utilized in the present study. Blood samples were collected from the White Leghorn layer parent strains (WLH-IWD and WLH-IWF) from AICRP on Poultry Breeding, Rajendranagar, the Babcock and the Vencobb commercial birds maintained at the Department of Poultry Science, College of Veterinary Science, Hyderabad, the Aseel from the backyards of farmers of West Godavari and Srikakukam districts and non-descript (desi) birds from adjoining areas of Rajendranagar. Three di-nucleotide microsatellite markers chosen randomly from the list recommended by the FAO (Cheng *et al.* 1995) and mapped either in Compton or East Lansing reference populations were genotyped. Blood samples (0.5–2.0 ml/bird) were collected into the vacutainers containing EDTA (5.4 mg) from the wing vein. High molecular weight genomic DNA was isolated as per standard protocol of phenol-chloroform-isoamyl alcohol extraction (Sambrook and Russell 2001). The PCR assay was carried out with a 10 ml reaction mixture containing 5 picomoles of each primer, 1 μl of 1× PCR buffer (10 mM Tris-HCl (pH 8.8), 50 mM KCl), 1.5 mM $MgCl_2$, 200 μm nucleotide mix (dNTPs), 1U *Taq* polymerase and 10–20 ng of template DNA and the volume was made up by adding sterile distilled water. The (PCR) reactions performed with initial denaturation at 94°C for 5 min, cyclic denaturation at 94°C for 30 sec, primer annealing at 52°C for 45 sec and primer extension at 72°C for 1 min. This was carried out for 35 cycles followed by final extension at 72°C for 10 min. The 2 primers (ADL136 and ADL158) with similar annealing temperature and reasonable difference in product size were multiplexed and amplified in a single reaction. The PCR products were genotyped using ABI Prism 3100 capillary sequencer. One μl amplicons was mixed with the 11 μl of formamide loading dye, a denaturing agent and 0.3 ml of ROX 500 a standard internal lane marker. The mixture was denatured for 3 min at 95°C and immediately snap cooled on ice before loading into the capillary sequencing gel. The resolution patterns of the alleles were studied using the Genescan (version 3.1) software and the size estimations were done by Genotyper 3.1 software. Allele scoring was done in base pairs. The allele data were subjected to the Excel Microsatellite Tool Kit and GenAlex 6 (Peakall and Smouse 2005) for estimating various parameters. Allele frequency (Af), mean number of alleles (N_a), effective number of alleles (N_e), percentage of polymorphic loci, heterozygosity (H), polymorphism information content (PIC), genetic distance, genetic identity and departure from H-W equilibrium were studied. Fixation indices by *FSTAT* (Goudet 1995) and phylogenetic relationships by MEGA 3.1 ver. (Kumar *et al.* 2004) were estimated. The genotyper plots showing various alleles amplified at different loci are presented in Fig. 1. All the 3 microsatellite loci utilized in the present study were found to be polymorphic with a reasonable informativeness.

Alleles and allele frequency: The overall mean number of alleles obtained per locus was 16.66. A total of 50 alleles consisting of 21 at ADL136, 11 at ADL158 and 18 alleles at the locus ADL176 were amplified. Olowofeso *et al.* (2005a) observed 116 alleles in Haimen chicken populations for 15 loci with a range of 2 to 16 alleles per locus. The product sizes (bp) for different loci were 115 to 152 bp (ADL136), 185 to 217 bp (ADL158) and 175 to 194 bp (ADL176). The overall mean number of alleles (N_a) amplified was 6.46±0.57 and it varied from 4.33±0.59 in WLH-IWD to 9.00±1.06 in Aseel (Table 1). Similarly the overall mean effective number

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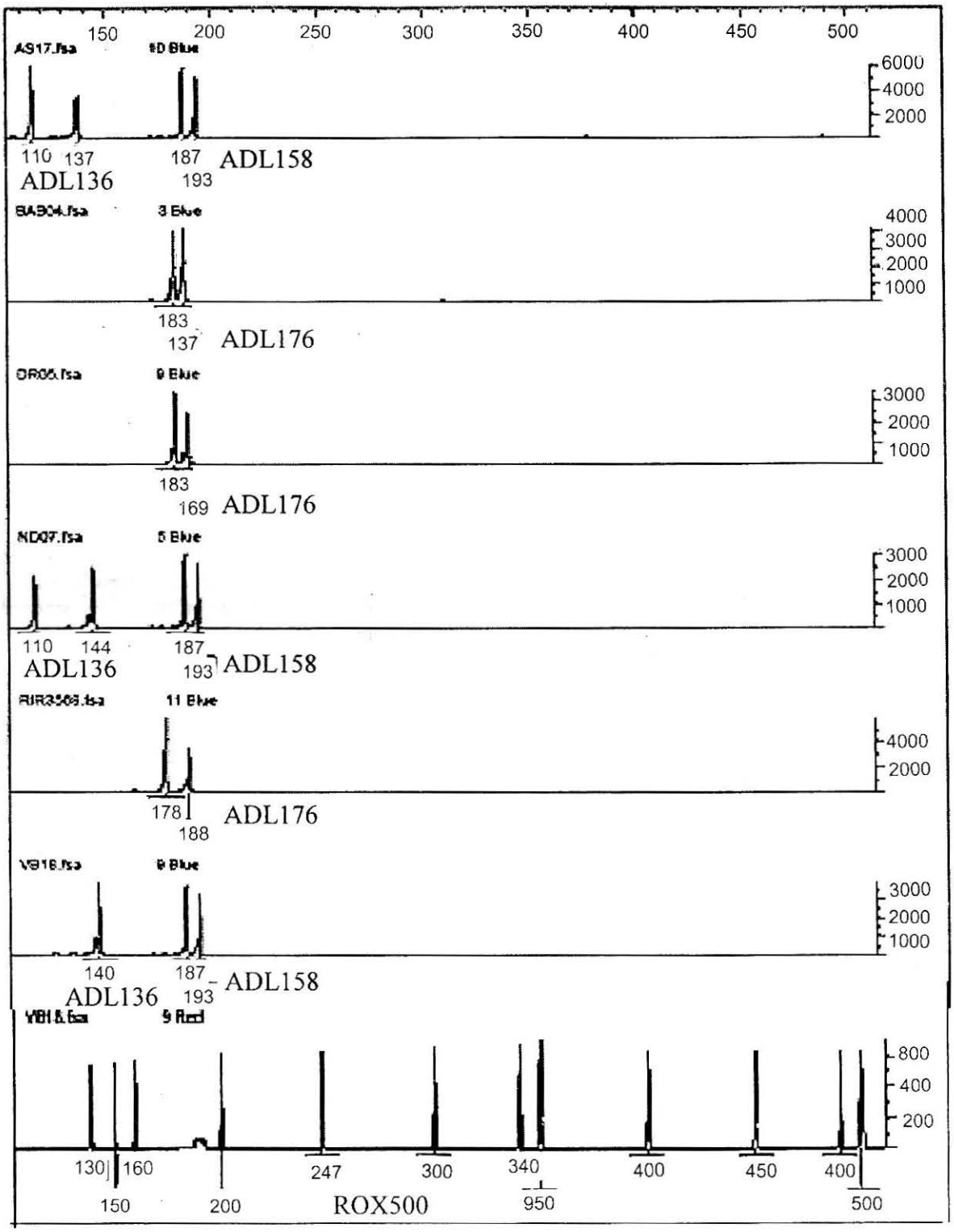

Fig. 1. Genotyper plots depicting the alleles for loci ADL 136 and ADL 158 and ADL 176.

Table 1. Estimates of different parameters observed for 8 chicken populations studied

Population	Sample size	Expected hetero-zygosity	Observed hetero-zygosity	Mean number of alleles	Effective number of alleles	PIC	F_{IS}
Dahlem Red	30	0.55±0.010	0.42±0.010	5.00±0.55	2.20±0.22	0.48	0.24
Rhode Island Red	30	0.66±0.019	0.45±0.010	6.00±0.66	3.27±0.73	0.60	0.32
WLH-IWD	30	0.63±0.011	0.69±0.010	5.33±0.53	2.71±0.37	0.55	–0.11
WLH-IWD	30	0.61±0.016	0.92±0.005	4.33±0.59	2.82±0.78	0.51	–0.53
Babcock	30	0.68±0.027	0.54±0.010	9.00±0.55	4.43±1.75	0.65	0.22
Vencobb	25	0.66±0.019	0.45±0.012	5.33±0.31	3.30±1.00	0.61	0.33
Aseel	22	0.67±0.034	0.35±0.013	9.00±1.06	4.31±1.66	0.63	0.48
non-descript	15	0.78±0.008	0.68±0.020	7.67±0.30	4.11±0.47	0.72	0.14
Overall	212	0.65±0.018	0.56±0.010	6.46±0.57	3.39±0.87	0.59	0.13

of alleles (N_e) was 3.39±0.87 and ranged from 2.20±0.22 in Dahlem Red to 43±1.75 in Babcock. The number of alleles and their size range obtained in the present study were higher than those reported by other authors (Vanhala *et al.* 1998, Romanov and Weigend 2001 and Pandey *et al.* 2005). The differences in allele number and allele size may be attributable to the more sensitive method of genotyping and study of the unrelated local populations and commercial strains, which harbored high degree of genetic variation. The average number of alleles obtained for various populations were consistent with recommended level of at least 4 alleles per locus for a microsatellite to be used in the estimation of genetic diversity and genetic distances as suggested by Wimmers *et al.* (2000). The large allele size distribution and the number per locus displayed in the present study will be of immense help in the use of these markers for further studies involving different chicken populations (Olowofeso *et al.* 2005a).

The allele frequency ranged from 1.67% in IWF at locus ADL176 (allele size 178 bp) to 79.55% in Babcock at locus ADL158 (allele size 187 bp). The allele frequency distribution in the present study was discrete, which was also reported by Romanov and Weigend (2001) and Olowofeso *et al.* (2005a), in spite of its normal distribution as per step-wise mutation model (SMM).

Alleles (2) were population/strain specific, but only 14.28% (3 alleles) had the frequency of more than 10%, which may be more reliable and can be used as markers to identify the population/strain. A total of 27, 15 and 12 unique alleles observed by Romanov and Weigend (2001) in Jungle fowl, selected lines and German native breeds, respectively, substantiated the more number of specific alleles observed. The locus ADL136 recorded highest number of specific alleles (4) in Aseel. The number of breed/line specific alleles of 4 in Aseel by Pandey *et al.* (2002) was similar to that observed in the present investigation. The overall mean PIC values ranged from 0.41 for ADL158 to 0.73 for ADL176 across the loci and 0.48 (Dahlem Red) to 0.72 (non-descript) among the populations. The PIC values for the 2 loci were above 0.50 except ADL158 indicating the loci were moderate to highly informative. The mean PIC values of 0.64 in Haimen chicken (Olowofeso *et al.* 2005a), 0.64 in Aseel (Pandey *et al.* 2002) and 0.62 in Ankaleswar (Pandey *et al.* 2005) were within the range of PIC values obtained in the present study.

Heterozygosity and F statistic: The expected heterozygosity estimates ranged from 0.55 (Dahlem Red) to 0.78 (non-descript) with an overall mean of 0.65. The observed heterozygosity estimate was the highest in WLH-IWD (0.92) and lowest in Aseel (0.35) among the populations studied. The mean estimates of expected heterozygosities of 0.6 in Rhode Island Red (Vanhala *et al.* 1999) and 0.45 in broiler lines (Emara *et al.* 2002) were lower than the mean heterozygosity estimates obtained.

The mean values of F_{IS}, F_{ST} and F_{IT} overall the loci were 0.13±0.08, 0.16±0.03 and 0.27±0.07, respectively. The F_{IS} values ranged from –0.37 for locus ADL158 to 0.40 for ADL136, F_{ST} values varied from 0.13 for ADL158 to 0.17 for ADL136 and F_{IT} values varied from–0.20 for ADL158 to 0.50 for ADL136. The F_{IS} values varied from–0.53 (WLH-IWD) to 0.48 (Aseel) with an overall estimate of 0.13 among the chicken populations studied (Table 1). The pair-wise F_{ST} estimates between the populations varied from 0.02 between WLH-IWD and WLH-IWF to 0.14 between Dahlem Red and non-descript. The high estimates for Dahlem Red (0.24) and Rhode Island Red (0.32) purebreds revealed that there was a considerable inbreeding/homozygosity in the populations at the 3 loci studied. This was justified by the fact that the 2 breeds were under selection for many generations. Emara *et al.* (2002) in broiler lines and Pandey *et al.* (2005) in Ankaleswar poultry population reported 19 and 24% variation within populations, respectively, which was lower than the present estimates. Vanhala *et al.* (1998) observed 13 to 48% variation within 8 chicken lines. The 2

Table 2. Genetic distance (below the diagonal) and genetic identity (above the diagonal) matrix between eight chicken populations

	Dahlem Red	Rhode Island Red	WLH-IWF	WLH-IWD	Babcock	Vencobb	Aseel	Non-descript
Dahlem Red	**	0.75	0.60	0.66	0.60	0.58	0.58	0.40
Rhode Island Red	0.29	**	0.65	0.68	0.66	0.72	0.64	0.66
WLH-IWD	0.52	0.43	**	0.94	0.68	0.52	0.48	0.43
WLH-IWF	0.42	0.38	0.06	**	0.65	0.51	0.47	0.42
Babcock	0.51	0.41	0.38	0.43	**	0.69	0.76	0.55
Vencobb	0.54	0.32	0.66	0.67	0.38	**	0.60	0.49
Aseel	0.54	0.45	0.73	0.75	0.28	0.51	**	0.67
Non-descript	0.91	0.42	0.85	0.86	0.60	0.71	0.41	**

strains of White Leghorn-WLH-IWD and WLH-IWF showed no inbreeding with negative F_{IS} values, which were under proper breeding plan for many generations. Aseel, a local breed had highest (0.48) F_{IS} estimate, which might be because of the fact that the blood samples were collected from a flock that has been maintained as a closed flock with limited number of cocks and hens, leading to increased homozygosity.

About 16% differentiation between the sub-populations (F_{ST}) observed in the chicken populations was within the range of moderate genetic differentiation on a scale defined by Wright (1978). Moderate to high genetic differentiation of 20 to 60% between the populations (F_{ST}) and 15 to 66 % among the total population (F_{IT}) in 8 chicken lines from Europe were higher than those in the present study (Vanhala *et al.* 1998). Pandey *et al.* (2005) observed 21% variation among the Ankaleswar poultry populations from India.

Genetic distance and phylogeny: The lower genetic distance (0.06) between WLH-IWF and WLH-IWD in the present study indicated that the populations were completely isolated but had been separated recently over a short period of time. The genetic distance was highest between Dahlem Red and non-descript (0.91) on expected lines (Table 2). Pandey *et al.* (2002) observed that the genetic distance between Nicobari and Aseel and Mini and Aseel breeds varied from 0.59 to 0.94. Emara *et al.* (2002) reported the genetic distance estimates for broiler lines ranging from 0.22 to 0.41, which were within the range of genetic distances of 0.06 to 0.91, obtained in the present investigation. The Nei's genetic similarity or identity matrix is just reciprocal of the genetic distance matrix, the higher the genetic distance, the lower the genetic identity and *vice versa*. The WLH-IWD and WLH-IWF were closer with maximum genetic identity index of 0.94. Dahlem Red and non-descript were more wide apart with least identity index value of 0.45. The 8 populations were grouped in to 2 main clusters, one cluster representing Aseel and non-descript and the other representing the remaining 6 populations/strains (2 commercial, 2 synthetic strains and 2 purebreeds). Aseel and non-descript which share a common habitat and natural conditions clustered separately from others populations. The remaining 6 populations were grouped into 3 sub clusters, i.e, Dahlem Red and Rhode Island Red, WLH-IWD and WLH-IWF and Babcock and Vencobb. The clustering of Dahlem Red and Rhode Island Red birds were as expected, since Dahlem Red was developed from Rhode Island Red and other German native birds over a period of time. The WLH-IWD and WLH-IWF the 2 synthetic strains that shared the common ancestry were developed from White Leghorn breed hence clustered together with high degree of relationship. However, the commercial layer breed of Babcock clustered with a broiler breed Vencobb, which may be due to an ancestral relationship during the breed formation stage. The existence of broiler inheritance in layer stocks during the breed formation stage was reported by Wandelt and Wolters (1996). Since both are commercial breeds, which harbor high genetic variation and polymorphism, might be another reason.

Hardy-Weinberg Equilibrium: The populations were tested for departure from Hardy-Weinberg equilibrium frequencies for all the 3 loci. The allele frequencies at ADL136 locus departed significantly from equilibrium frequency in all the 8 populations. ADL158 and ADL176 departed in 4 (Rhode Island Red, WLH-IWD, WLH-IWF and non-descript) and 6 (Rhode Island Red, WLH-IWD, WLH-IWF, Babcock, Vencobb and Aseel) populations, respectively. The White Leghorn strains, WLH-IWD and WLH-IWF populations departed in all the 3 loci studied. The possible explanations for the significant deviations of observed frequencies from expected frequencies were genetic selection, presence of null alleles, small sample size and genotyping errors (Vanhala *et al.* 1998 and Emara *et al.* 2002). In pure breeds and commercial breeds, which were under selection for several generations the departure from equilibrium, frequency may be due to selection pressure. Some of the loci may be linked to economically important genes that were under intense selection pressure leading to fixation of the alleles and change in the gene frequencies, thus deviation from the H-W equilibrium frequency. In addition, presence of null alleles, which could not amplify in PCR leading to overestimation of particular homozygote or heterozygote and deviation in the frequencies causing the departure from the equilibrium.

SUMMARY

Birds (212) representing 8 populations—2 layer strains of White Leghorn (WLH-IWD and WLH-IWF), 2 dual-purpose breeds (Rhode Island Red and Dahlem Red), a commercial layer (Babcock), a commercial broiler (Vencobb), a Indian breed (Aseel) and non-descript (*desi*) chicken native—were studied for assessing the polymorhism, genetic variation, genetic diversity and genetic relationships between the populations. The overall mean number of alleles (N_a) and effective number of alleles (N_e) were 6.46±0.57 and 3.39±0.87, respectively. The overall mean PIC values across the loci ranged from 0.41 for ADL158 to 0.73 for ADL176 and across the populations from 0.48 (Dahlem Red) to 0.72 (non-descript). The mean expected and observed heterozygosity levels were 0.65±0.02 and 0.56±0.01, respectively. Phylogenetic analysis grouped the populations into 2 main clusters, one representing Aseel and non-descript, other representing the remaining 6 populations/strains. All the 3 loci deviated significantly from equilibrium frequencies in most of the populations.

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