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Genomic Heterogeneity of Chicken Populations in India

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ABSTRACT : A comprehensive genome profiling study was undertaken based on automated genotyping and analysis of 20 microsatellite markers that involved 155 birds representing eight different populations. The distribution of microsatellite markers in each of these breeds helped us to decipher genetic heterogeneity, population genetic structure and evolutionary relationships of the present day chicken populations in India. All the microsatellite loci utilized for the analysis were polymorphic and reasonably informative. A total of 285 alleles were documented at 20 loci with a mean of 14.25 alleles/locus. A total of 103 alleles were found to be population/strain specific of which, only 30 per cent had a frequency of more than 10. The mean PIC values ranged from 0.39 for the locus ADL158 to 0.71 for loci MCW005 or ADL267 across the genomes and 0.55 in Dahlem Red to 0.71 in *Desi* (non-descript), among the populations. The overall mean expected and observed heterozygosity estimates for our populations were 0.68 and 0.64, respectively. The overall mean inbreeding coefficients (F_{IS}) varied between -0.05 (Babcock) and 0.16 (Rhode Island Red). The pairwise F_{ST} estimates ranged from 0.06 between Aseel and *Desi* (non-descript) to 0.14 between Dahlem Red and Babcock. The Nei's genetic distance varied from 0.30 (WLH-IWD and WLH-IWF) to 0.80 (Dahlem Red and Babcock. Phylogenetic analysis grouped all the populations into two main clusters, representing i) the pure breeds, Dahlem Red and Rhode Island Red, and ii) the remaining six populations/strains. All the chicken populations studied were in the state of mild to moderate inbreeding except for commercial birds. A planned breeding is advised for purebreds to revive their genetic potential. High genetic diversity exists in *Desi* (non-descript), local birds, which can be exploited to genetically improve the birds suitable for backyard poultry. (**Key Words :** Chicken, Genetic Diversity, Heterogeneity, Phylogeny and Microsatellites)

INTRODUCTION

Poultry farming is an important livestock industry sector in India contributing to animal protein needs of the population through meat and eggs. Though the production levels in terms of eggs and meat are high in the country, the per capita availability is 45 and 1,900 grams, respectively, which are far below the recommended levels of the Indian Council of Medical Research (ICMR). Commercial poultry breeding, starting as early as the middle of the 20th century, remained largely confined to a few commercial breeds/lines and thus narrowed down genetic diversity. Widespread selective breeding has greatly reduced the number of breeds available for commercial poultry production. At present, only three groups of primary breeders dominate the international layer chicken market and four major breeders are in the broiler market (Flock and Preisinger, 2002). As a result of worldwide promotion and acceptance of a few highly specialized lines/strains, survival and existence of local important breeds seems to be at risk.

Though considerable improvement has been achieved with traditional breeding methods, some of the issues related to low heritable characters, traits that are difficult to measure and those expressing late in life, negatively correlated and sex linked traits etc., need to be addressed. Marker assisted breeding based on modern molecular approaches might help resolve these problems and improving the traditional breeding practices in augmenting production and productivity. Microsatellites and SNPs are the recent markers that are used widely in gene marker studies, as they are abundant, co-dominant, highly polymorphic and dispersed throughout the genome (Cheng et al., 1995; Crooijmans et al., 1996a; Crooijmans et al., 1996b; Vanhala et al., 1998; Romanov and Weigend, 2001; Oh et al., 2006; Qu et al., 2006; Lee et al., 2007; Shahabazi et al., 2007; Kaya and Yildiz, 2008). Microsatellites have been already validated as reliable markers in tracking chicken lineages and assessment of gene pool diversity (Vanhala et al., 1998; Romanov and Weigend, 2001). Assessing the genetic variation and genetic diversity among

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different chicken breeds using modern molecular tools is very essential for conservation and genetic improvement programmes (Osman et al., 2006). The utility of microsatellites in paternity, kinship and breed assignment analysis is well documented (Yoon et al., 2005; Fan et al., 2006). The information on chicken genome sequence and more than 2.8 million single nucleotide polymorphisms (SNPs) greatly enhanced the ability to understand chicken biology, especially with respect to identification of quantitative trait loci (QTL) and genes that control simple and complex traits (Muir et al., 2007).

This study comprised the first systematic effort to analyze genetic relatedness and heterogeneity of a selective population of farmed chicken. This population represented all the available chicken varieties such as Dahlem Red and Rhode Island Red (pure breeds), White Leghorns (synthetic layers), Babcock (a commercial layer) and Vencobb (a commercial broiler), Aseel (native breed) and *Desi* (non-descript). This study clearly revealed the extent of genetic diversity and inbreeding levels among these chickens and implications thereof for a planned breeding strategy in India.

MATERIALS AND METHODS

Experimental populations

A total of 155 birds representing eight populations were utilized in the present study. Blood samples were collected from the Dahlem Red and Rhode Island Red (purebreds), WLH-IWD and the WLH-IWF (White Leghorn D and F strains) layer parent strains from AICRP on Poultry Breeding, Rajendranagar, the Babcock, a commercial layer and the Vencobb, a commercial broiler maintained at the Department of Poultry Science, College of Veterinary Science, Hyderabad, the Aseel from West Godavari and Srikakulam districts of Andhra Pradesh and *Desi* (non-descript) birds from adjoining areas of Rajendranagar. The birds were selected randomly. The eight populations comprised two pure breeds (Dahlem Red and Rhode Island Red), two synthetic layer strains (WLH-IWD and WLH-IWF), two commercial strains (Babcock and Vencobb) and two native wild chicken (Aseel and *Desi* (non-descript)). Twenty birds from each population were genotyped, except *Desi* (non - descript) in which only 15 birds were used.

Dahlem Red is adual purpose breed with reddish brown plumage and single comb. Rhode Island Red is also a dual purpose medium heavy bird with single comb and reddish brown plumage, mainly used for egg production rather than for meat. White Leghorn is an excellent egg layer with lighter body, single comb and long wattles. The two commercial strains, Babcock and Vencobb were under strong selection for egg and meat respectively. Aseel is a game bird with compact body, long shanks and pea comb known for its martial qualities. Desi (non-descript) birds have a lean body, multicoloured plumage and single comb without any specific breed characteristics.

Microsatellites

Twenty mono/di/tri-nucleotide microsatellite marker loci mapped either in Compton or East Lansing reference populations were utilized in the present study. The markers were chosen randomly from the list recommended by the FAO (Cheng et al., 1995; Crooijmans et al., 1996a). The primer sequences and the characteristic features of these primers are presented in Table 1. High molecular weight genomic DNA was isolated from blood samples as per the standard protocol (Phenol-chloroform-isoamyl alcohol extraction). Each PCR assay was carried out with a 10 μl reaction mixture containing 5 picomoles of each primer, 1 μl of 1X PCR buffer (10 mM Tris- HCl (pH 8.8), 50 mM KCl), 1.5 mM $MgCl_2$, 200 μm nucleotide mix (dNTPs), 1 U Taq polymerase and 10-20 ng of template DNA and the volume was made up by adding sterile distilled water. The PCR reactions were performed using a Gene Amp thermal cycler 9700 (Perkin-Elmer-Cetus, USA). Initial denaturation was done at 94°C for 5 minutes. Cyclic denaturation at 94°C for 30 seconds, primer annealing at 46-55°C (specific for each primer) for 45 seconds and extension at 72°C for 1 minute were carried out for 35 cycles followed by final extension at 72°C for 10 minutes.

Multiplex PCR was carried out with either two or four primers in a single reaction giving due importance to the base pair difference and the flouroscent dye tagged to the primer. The set of loci used for multiplexing were ADL 136 and ADL 158; ADL 102 and ADL 267; MCW 048 and MCW 001; MCW 073 and MCW 014; MCW 041 and MCW 004; MCW 051, MCW 016, MCW 049 and MCW 005; MCW 043, ADL 171, ADL 172 and ADL 210. ADL 176 and MCW 075 were amplified independently.

The PCR amplicons were genotyped using an ABI Prism 3100 capillary sequencer machine (PE Applied Biosystems, ABI Prism). One μl of the amplicons was mixed with the 11 μl of 6X formamide loading dye, a denaturing agent (Sigma) and 0.3 μl 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. ROX 500, a standard size marker was used to determine the size of the fragments.

Statistical analysis

The genotyping data were subjected to the Excel

Table 1. Descriptive values for microsatellite loci utilized in eight chicken populations of India

Locus	Primer sequence (5'…3')	Bases	Repeat unit	Genebank accession No	A.T (°C)	Dye
ADL136	F TGT CAA GCC CAT CGT ATC AC	20	$(TG)_{10}$	G01561	52	FAM
	R CCA CCT CCT TCT CCT GTT CA	20	TC $(TG)_{10}$			
ADL158	F TGG CAT GGT TGA GGA ATA CA	20	$(CA)_{12}$	G01582	52	FAM
	R TAG GTG CTG CAC TGG AAA TC	20				
ADL176	F TTG TGG ATT CTG GTG GTA GC	20	$(GT)_{12}$	G01598	52	FAM
	R TTC TCC CGT AAC ACT CGT CA	20				
ADL102	F TTC CAC CTT TCT TTT TTA TT	20	$(GT)_{18}$	G01547	47	FAM
	R GCT CCA CTC CCT TCT AAC CC	20				
ADL171	F ACA GGA TTC TTG AGA TTT TT	20	$(TG)_{18}$	G01593	46	HEX
	R GGT CTT AGC AGT GTT TGT TT	20				
ADL172	F CCC TAC AAC AAA GAG CAG TG	20	$(CA)_{18}$	G01594	49	HEX
	R CTA TGG AAT AAA ATG GAA AT	20				
ADL210	F ACA GGA GGA TAG TCA CAC AT	20	$(AC)_{15}$	G01630	46	HEX
	R GCC AAA AAG ATG AAT GAG TA	20				
ADL267	F AAA CCT CGA TCA GGA AGC AT	20	$(CA)_{12}$	G01687	50	HEX
	R GTT ATT CAA AGC CCC ACC AC	20				
MCW043	F TGA CTA CTT TGA TAC GCA TGG AGA	24	$(T)_{21}$	L40073	55	FAM
	R CAC CAA GTA GAC GAA AAC ACA TTT	24				
MCW005	F ACC TCC TGC TGG CAA ATA AAT TGC	24	$(TG)_{14}$	L40048	55	HEX
	R TCA CTT TAG CTC CAT CAG GAT TCA	24				
MCW049	F AGC GGC GTT GAG TGA GAG GAG CGA	24	$(GCA)_{11}$	L40077	55	HEX
	R TCC CCA ACC CGC GGA GAG CGC TAT	24				
MCW016	F ATG GCG CAG AAG GCA AAG CGA TAT	24	$(TG)_{16}$	L40054	50	FAM
	R TGG CTT CTG AAG CAG TTG CTA TGG	24				
MCW051	F GGA ACA AGC TCT TTC TTC TTC CCG	24	$(T)_{25}$	L40079	50	FAM
	R TCA TGG AGG TGC TGG TAC AAA GAC	24				
MCW004	F GGA TTA CAG CAC CTG AAG CCA CTA	24	$(TG)_{28}$	L40047	55	HEX
	R AAA CCA GCC ATG GGT GCA GAT TGG	24				
MCW041	F CCC AAT GTG CTT GAA TAA CTT GGG	24	$(T)_{23}$	L40071	55	FAM
	R CCA GAT TCT CAA TAA CAA TGG CAG	24				
MCW075	F CGT CAA GCC AGA TGC TGA TGA GTG	24	$(T)_{19}$	L43641	55	FAM
	R ATT CCA ACC AGA AGT TTG ACT CGC	24				
MCW001	F ACT GTC ACA GTG GGG TCA TGG ACA	24	$(TG)_{9}$	L40044	50	HEX
	R ACA CGT CCT GTG TCA CAT GCC TGT	24				
MCW048	F CGT ATA GGA GGG TTT CTG CAG GGA	24	$(TG)_{17}$	L40076	55	FAM
	R AAG GAG GAA CGC ACC GCA CCT TCT	24				
MCW014	F AAA ATA TTG GCT CTA GGA ACT GTC	24	$(TG)_{8}$	L40053	55	HEX
	R ACC GGA AAT GAA GGT AAG ACT AGC	24				
MCW073	F TAT TTC ACC CAC GGG GAC GAA TAC	24	$(GAG)_{10}$	L43639	55	FAM
	R AGG GTG CTG AGA GCT GCC AAT GTC	24				

Microsatellite Tool Kit (Park, 2001) and GenAlex 6 (Peakall and Smouse, 2005) for estimating various parameters such as allele frequency, mean number of alleles (N_a), effective number of alleles (N_e), percentage of polymorphic loci, observed heterozygosity, Nei (1987) expected heterozygosity, Botstein et al. (1980) polymorphism information content (PIC), Nei (1972) genetic distance and genetic identity and Wright (1978) fixation indices by FSTAT (Goudet, 1995). Nei (1972) genetic distances were used to construct phylogenetic trees by neighbor-joining (NJ) cluster analysis with the appropriate option of computer software package MEGA 3.1 (Kumar et al., 2004).

RESULTS

Microsatellite polymorphisms in chicken

Microsatellite inferred diversity : Polymorphism is the occurrence of two or more alleles at a locus. All the microsatellite loci utilized in the present study were found to be polymorphic and reasonably informative according to Botstein et al. (1980) criteria. All 20 chromosomal loci studied were successfully amplified through genomes of all the populations studied without exception, wherein all the loci revealed varying number of alleles in all the populations (Figure 1). A total of 285 alleles were obtained among all the populations with a mean of 14.25 alleles per

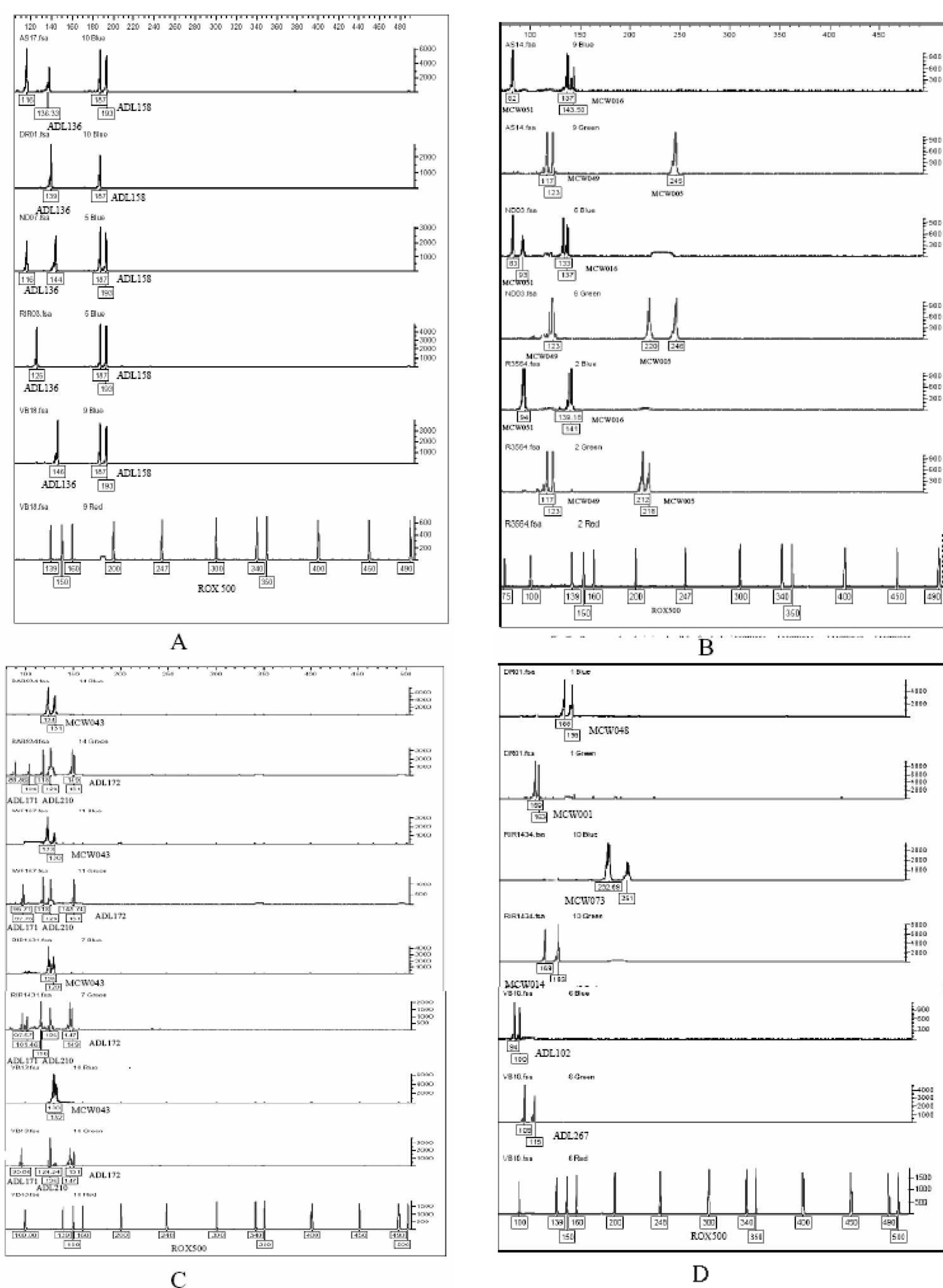

Figure 1. Microsatellite profiles of chicken populations studied for 20 genomic loci. Genotyper plots depicting the allele sizes across the genome for different loci (A) ADL136 and ADL158; (B) MCW051 and MCW016, MCW049 and MCW005; (C) MCW043, ADL171, ADL172 and ADL210; (D) MCW001, MCW073, ADL102 and ADL267.

locus. The highest (26) total number of alleles was amplified at locus MCW005 and the lowest (7) at locus MCW001 (Table 2). The product size (bp) varied form 76 bp for MCW051 to 256 bp for MCW005 locus. The mean number of alleles ranged from 4.70±0.40 in WLH-IWD to 6.75±0.44 in *Desi* (non-descript), whereas the mean effective number of alleles per population/strain varied between 2.69 for Dahlem Red and 4.15 for non-descript chickens (Table 3). The allele frequency ranged from as low as 2.50 to 2.78 percent in almost all the populations/strains and loci (except MCW073) to as high as 85.00 per cent in Babcock for allele size of 187 bp at locus ADL158.

Population/strain specific alleles : We observed a total of 103 population specific alleles out of 285 alleles amplified. Aseel and *Desi* (non-descript) populations showed the highest number of specific alleles across all the loci. However the frequency of most of these population/strain specific alleles was low and only 31 out of 103 (30%) alleles had a frequency of more than 10 per cent. It is assumed that alleles with frequency levels above 10 per

Table 2. Description of the twenty microsatellite markers used in eight chicken populations from India

Locus	bp	N	Na	Ne	He	PIC	F_{IS}
ADL136	115-152	21	6.13	3.43	0.69	0.62	0.38
ADL158	185-217	10	3.50	1.96	0.47	0.39	-0.28
ADL176	175-194	18	8.63	4.25	0.76	0.71	0.18
ADL102	87-110	16	5.13	2.97	0.63	0.56	0.25
ADL171	88-112	15	5.50	3.88	0.66	0.59	-0.01
ADL172	131-159	11	4.75	2.89	0.64	0.56	0.25
ADL210	115-132	15	6.13	3.97	0.77	0.71	-0.10
ADL267	97-130	24	7.00	4.41	0.76	0.70	-0.02
MCW043	115-133	13	5.75	4.11	0.75	0.69	0.06
MCW005	210-256	26	8.63	4.13	0.76	0.71	0.09
MCW049	111-129	10	4.75	2.82	0.64	0.57	-0.17
MCW016	125-145	11	5.38	3.27	0.68	0.61	-0.29
MCW051	76-99	13	6.25	3.75	0.73	0.67	0.14
MCW004	171-209	12	4.88	3.30	0.69	0.63	-0.06
MCW041	140-162	15	5.38	3.03	0.65	0.59	0.08
MCW075	167-177	9	4.13	2.79	0.63	0.55	0.21
MCW001	155-167	7	3.63	2.77	0.63	0.54	-0.05
MCW048	164-198	9	4.25	3.31	0.70	0.62	-0.03
MCW014	168-185	11	3.88	2.74	0.63	0.56	0.12
MCW073	230-254	19	5.63	4.30	0.76	0.70	0.32
Mean	76-256	14.25	5.46	3.36	0.68	0.61	0.06

bp: allele size range, N: number of alleles, Na: mean number of alleles, Ne: effective number of alleles.
He: expected heterozygosity, PIC: polymorphism information content, F_{IS}: Wright's fixation index.

cent may be more suitable to tag to a particular population /strain.

Heterozygosity

The overall mean polymorphism information content values ranged from 0.39 for ADL158 to 0.71 for MCW005 and ADL267 across the loci (Table 2) and 0.55 in Dahlem Red to 0.71 in *Desi* (non-descript) among the populations (Table 3). The highest and lowest PIC values were observed for 0.84 in *Desi* (non-descript) for MCW043 and 0.25 in Babcock for ADL158. All twenty loci utilized in the present investigation were found to be polymorphic in all the populations studied.

Heterozygosity is the state of an individual with different alleles of a gene at a particular locus and it is the measure of genetic diversity in the populations. The overall mean expected and observed heterozygosity estimates were 0.68±0.004 and 0.64±0.005 (Table 3) in our populations. The mean expected heterozygosity across the populations ranged from 0.63 (Dahlem Red) to 0.77 (*Desi* (non-descript)). The expected heterozygosity was highest in *Desi* (non-descript) birds for locus MCW043 (0.89) and least in Babcock for ADL158 (0.27). Those birds with highest mean number of alleles amplified per locus had the highest expected as well as observed heterozygosity.

The mean observed heterozygosity levels among the populations ranged from 0.55 in Dahlem Red to 0.73 in *Desi* (non-descript). The loci ADL136 and MCW075 were completely homozygous in Dahlem Red and WLH-IWD birds, and thus accorded zero heterozygosity. Six loci were

Table 3. Genetic diversity within chicken populations in India

Population	No. of birds	No. of loci	Mean No. of alleles/locus (N_a)	Effective No. of alleles/locus (N_e)	No. of population /strain specific alleles	Expected heterozygosity (H_e)	Observed Heterozygosity (H_o)	PIC	F_{IS}
Dahlem Red	20	20	4.80±0.37	2.69±0.15	0.35±0.15	0.63±0.004	0.55±0.006	0.55	0.13
Rhode Island Red	20	20	5.60±0.46	3.78±0.26	0.40±0.13	0.72±0.006	0.61±0.006	0.66	0.16
WLH-IWD	20	20	4.70±0.40	3.00±0.23	0.50±0.15	0.65±0.005	0.61±0.006	0.58	0.07
WLH-IWF	20	20	5.05±0.45	3.03±0.27	0.60±0.31	0.64±0.008	0.63±0.006	0.56	0.01
Babcock	20	20	4.85±0.45	3.17±0.28	0.50±0.15	0.66±0.007	0.70±0.005	0.59	-0.05
Vencobb	20	20	5.60±0.37	3.52±0.28	0.55±0.17	0.70±0.006	0.68±0.005	0.64	0.04
Aseel	20	20	6.40±0.61	3.63±0.35	1.10±0.24	0.69±0.007	0.63±0.006	0.63	0.08
Desi (non-descript)	15	20	6.75±0.44	4.15±0.24	1.20±0.19	0.77±0.004	0.73±0.007	0.71	0.06
Overall	19.38	20	5.46±0.37	3.36±0.21	0.65±0.20	0.68±0.004	0.64±0.005	0.61	0.06

Table 4. Nei's genetic distance (below the diagonal) and pair-wise F_{ST} estimates (above the diagonal) matrix between the populations

	Dahlem Red	Rhode Island Red	WLH- IWD	WLH-IWF	Babcock	Vencobb	Aseel	*Desi* (non-descript)
Dahlem Red	**	0.07	0.13	0.13	0.14	0.10	0.11	0.10
Rhode Island Red	0.36	**	0.09	0.09	0.11	0.07	0.09	0.07
WLH-IWD	0.71	0.49	**	0.07	0.09	0.10	0.11	0.10
WLH-IWF	0.68	0.48	0.30	**	0.10	0.11	0.12	0.10
Babcock	0.80	0.65	0.43	0.44	**	0.09	0.11	0.09
Vencobb	0.59	0.41	0.53	0.58	0.49	**	0.09	0.08
Aseel	0.64	0.55	0.62	0.67	0.67	0.52	**	0.06
Desi (non-descript)	0.58	0.53	0.66	0.59	0.55	0.53	0.36	**

completely heterozygous with all heterozygotic alleles in the six populations, namely, ADL136 and ADL158 in WLH-IWD; ADL267 in WLH-IWF/Babcock/ Aseel/ *Desi* (non-descript); MCW043 in Rhode Island Red; MCW049 in Babcock; MCW016 in Vencobb/Aseel/*Desi* (non-descript).

Fixation indices (F- statistic)

Fixation indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. In purebred, Dahlem Red and Rhode Island Red there was considerable homozygosity/inbreeding. All the alleles at loci ADL136 in Dahlem Red and MCW075 in WLH-IWD were fixed, as the coefficient of inbreeding was 1.00. All the 20 loci except MCW073 showed negative inbreeding coefficients (F_{IS} values) in one or more populations indicating the presence of more heterozygotes. The F_{IS} values ranged from -0.29 for MCW016 to 0.38 for ADL136 (Table 2). The locus MCW073 had inbreeding effects with positive inbreeding coefficients (F_{IS}) in all the populations investigated. Babcock, a commercial layer, had negative F_{IS} value (-0.05) indicating high genetic variation and heterogeneity. WLH-IWF was stable with low F_{IS} value (0.01) and less homozygotes. The remaining four populations, WLH-IWD, Vencobb, Aseel and *Desi* (non-descript) had positive F_{IS} ranging from 0.04 to 0.08 (Table 3). The F_{ST} values varied from 0.06 between Aseel and non-descript to 0.14 between Dahlem Red and Babcock (Table 4).

Genetic distance and phylogenetic analysis

The Nei's genetic distance matrix (Nei, 1972) is presented in the Table 4. The genetic distance was least between WLH-IWD and WLH-IWF (0.30) and highest between Dahlem Red and Babcock (0.80). 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 0.75. Dahlem Red and Babcock were further apart with a lowest identity index value of 0.45.

The dendrogram (NJ method) showing the phylogenetic relationship among the eight chicken populations studied is presented in Figure 2. The eight populations were grouped in two main clusters, one cluster representing Dahlem Red and Rhode Island Red pure breeds and the other representing the remaining six populations/strains (two commercial, two synthetic strains and two native wild chickens). The second group was divided into three sub clusters i.e., Aseel and *Desi* (non-descript); Babcock and Vencobb and WLH-IWD and WLH-IWF. The individual genetic distances between the birds were estimated and trees were built using the NJ method in MEGA 3.1. The circular tree representing all the birds is presented in Figure 2. The individual birds clustered according to their lineage.

DISCUSSION

This study was the first attempt to analyze comparative population structure of important commercial breeds of chicken in India along with some native stock of birds. We used genome-derived microsatellite markers for deducing genomic diversity and evolutionary history of chickens. The microsatellite loci represent an independent evolutionary history of a population if they fulfill the conditions like Mendelian inheritance with reasonable PIC values, presence on different chromosomes/linkage groups and independent assortment. The loci genotyped in the present study satisfied all the conditions and were suitable for biodiversity and population genetic studies. All the loci were found to be polymorphic in all the populations with moderate to high informativeness. We used a fairly stable, reproducible and high-resolution method based on a universally accepted set of polymorphic loci.

The present study revealed much greater microsatellite variation in the chicken compared to earlier reports (Crooijmans et al., 1996a; Crooijmans et al., 1996b, Kaya and Yıldız 2008). The present findings were comparable to the microsatellite variations in Indian native chicken breeds observed by Pirany et al. (2007). The number of alleles and their size range obtained in the present study were higher than those reported by other authors (Cheng et al., 1995; Crooijmans et al., 1996a; Crooijmans et al., 1996b; Vanhala et al., 1998; Romanov and Weigend, 2001; Pandey et al.,

2002; 2003; 2005; Shahbazi et al., 2007; Kaya and Yıldız, 2008). Osman et al. (2006) reported three to 18 alleles per locus in Japanese and foreign chicken populations. The allele numbers in ADL158 (10 alleles) and ADL172 (11 alleles) observed in Korean native and foreign chicken were similar to the alleles in the present study for the two loci (Kong et al., 2006). The number of alleles observed for MCW005 (27) and MCW016 (11) loci (Pirany et al., 2007) were in agreement with the allele number in the present investigation whereas higher number of alleles (20) were recorded for ADL158. Higher average number of alleles (18.74 per locus) was observed by Qu et al. (2008) in Chinese indigenous chicken breeds than in the present study. The differences in allele number and allele size may be because of genotyping of unrelated local populations and commercial strains, which harbored a high degree of genetic variation. The average number of alleles obtained for various populations in the present study were consistent with the recommended level of at least four 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). However, the unusual number of alleles observed at loci ADL176, ADL267, MCW005 and MCW073 need to be confirmed by sequencing the loci.

The effective number of alleles (N_e) is the non-linear function of the expected heterozygosity in the population. It gives an idea about "how wider is the allele frequency distribution in the populations?" The lower effective number of alleles than the observed number of alleles across the loci in the present investigation indicated that the allele frequencies were widely distributed since the alleles with frequencies away from the average (0.5) contribute very little to the effective number of alleles. The mean effective number of alleles reported in the literature were 3.09 in Aseel, 3.39 in Miri, 3.15 in Nicobari (Pandey et al., 2002) 2.91 in Ankaleswar, (Pandey et al., 2005) and 4.05± 0.03 in Haimen chicken breeds (Olowofeso et al., 2005a). These findings are in accordance with our findings which fall within the present range.

The allele frequency distribution in the present study was observed to be discrete, which was also reported by many authors (Freimer and Slatkin, 1996; Vanhala et al., 1998; Romanov and Weigend, 2001; Olowofeso et al., 2005a). The allele frequency of microsatellites should distribute normally as per SMM model (Ohta and Kimura, 1973). The single base pair differences observed for some of the di/tri-nucleotide repeat alleles might be attributable to point mutations (deletions/insertions) in the flanking region. Similar observations were made by Romanov and Weigend (2001) for alleles at the loci ADL158, MCW004, MCW005

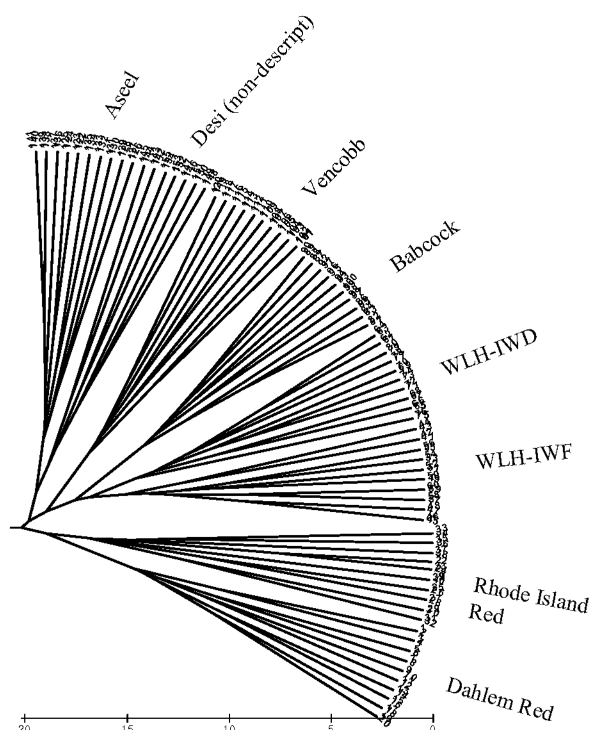

Figure 2. Neighbor-joining dendogram among eight chicken populations in India. Based on Nei (1972) genetic distances from Table 4.

and MCW0014 that were used in the present study also.

About 45 per cent of the total unique alleles identified were observed in Aseel and *Desi* (non-descript) populations. Zhou and Lamont (1999) reported 19 line specific alleles for UCD-001, a jungle fowl line, that was similar to the present finding of 22 and 24 specific alleles in Aseel and *Desi* (non-descript) populations, respectively. A total of 27, 15 and 12 unique alleles was observed by Romanov and Weigend (2001) in jungle fowl, some selected lines and German native breeds, respectively. Also, the PIC values observed in our study were broadly congruent to those observed by various investigators: 0.64 in Hiamen chicken (Olowofeso et al., 2005a), 0.64 in Aseel (Pandey et al., 2002) and 0.62 in Ankaleswar (Pandey et al., 2005) etc. Kong et al. (2006) reported lower PIC (0.55) values in Korean native and foreign chicken populations.

Our population study revealed a high degree of polymorphism and genetic variation with an overall expected unbiased heterozygosity (Nei, 1987) of 0.68± 0.004, which was in accordance with expected heterozygosity (0.67±0.04) observed in Turkish native chicken (Kaya and Yildizi, 2008). Shabhazi et al. (2007) reported a range of 0.62 to 0.79 expected heterozygosity in Iranian native chicken which was similar to the present estimate (0.63 to 0.77) in Indian chicken populations. The high heterozygosity estimates in our populations were due to existence of a large number of heterozygous alleles. The average observed heterozygosity estimates obtained in the present study were in accordance with the estimated ranges of 0.49 to 0.77 in chicken populations from Bolivia, India, Nigeria and Tanzania (Wimmers et al., 2000); 0.65-0.70 in Hiamen chicken populations (Olowofeso et al., 2005a); 0.45 to 0.75 in European chicken populations (Hillel et al., 2003); 0.54 to 0.68 in Korean native and foreign chicken (Kong et al., 2006) and the estimates of 0.68 in Ankaleswar poultry (Pandey et al., 2005); 0.65 in Aseel, 0.68 in Miri and 0. 64 in Nicobari (Pandey et al., 2002) and 0.61 in three Indian breeds (Pandey et al., 2003).

The highest observed (0.73) and expected (0.77) heterozygosities obtained in the *Desi* (non-descript) populations in the present study is consistent with the fact that these birds were maintained in Rajendranagar villages as backyard poultry where no selection was practiced. The number of alleles was also higher in this population. Aseel, a game bird population that recorded the observed and expected heterozygosities of 0.63 and 0.69, respectively, was also maintained in village flocks especially in tribal areas. The high heterozygosity may be attributable to low selection pressure where no organized breeding was followed. In Rhode Island Red, though selection was practiced for several years, the genetic variation still existed in high degree. Despite extensive use of this breed in commercial breeding the diversity still present indicating good genetic management of the population. This may be the reason that the breed is extensively used in developing new breeds of either type, i.e., layers and broilers. The two White Leghorn strains (WLH-IWD and WLH-IWF), a commercial layer (Babcock) and the pure breed Dahlem Red, which were under a selection programme for high egg production, exhibited less variation compared to the others.

Fixation indices

The higher estimates of F_{IS} for Dahlem Red (0.13) and Rhode Island Red (0.16), the purebreds in the present study, revealed that there was a considerable inbreeding/ homozygosity in these populations. This was consistent with the fact that the two breeds were under selection for many generations. A planned strategy involving out-crossing and recurrent reciprocal selection is suggested to revive the genetic potential. The three commercial populations/strains (WLH-IWD, WLH-IWF and Vencobb), Aseel and *Desi* (non- descript) had positive F_{IS} values indicating the moderate heterozygotes in the populations. The Babcock showed more heterozygotes with negative F_{IS} values at the majority of loci, which could be due to the fact that all the commercial breeds were maintained with a proper breeding plan so as to avoid inbreeding. In the case of Aseel and *Desi* (non-descript), there was probably no selection pressure of any type, but the mild inbreeding thus observed might be due to small groups of hens maintained in backyards of farmer's houses with continuous use of one or two cocks for several years. About 15 per cent differentiation between the sub-populations (F_{ST}) observed in the chicken populations in the present study was within the range of moderate genetic differentiation on a scale defined by Wright (1978). Ji et al. (2005) observed 17 per cent variation due to breed differences in Chinese chicken populations. Qu et al. (2006) observed 6 to 20 percent variation between subpopulations of Chinese indigenous chicken. Significantly high genetic differences were documented (39 to 51 per cent) between Red Jungle Fowl and other Japanese Chicken breeds (Tadano et al., 2008).

The Dahlem Red, a dual-purpose breed used in commercial poultry breeding, was greatly differentiated (F_{ST} = 14%) from the commercial layer Babcock, while it was little differentiated from the Rhode Island Red (F_{ST} = 7%). The commercial broiler Vencobb was less differentiated from the Aseel (F_{ST} = 9%), the native game bird of Andhra Pradesh and a non-descript (F_{ST} = 8%) population. Table 4 summarises the pair-wise F_{ST} estimates between the populations. Some of the loci with moderate F_{ST} values may identify the chromosomal regions approaching fixation as a result of genetic selection (Emara et al., 2002). However, with low F_{ST} values in the present study, the populations may not be able to detect the chromosomal regions approaching fixation.

Genetic distance and phylogenetic analysis

The genetic distance between populations provides a relative estimate of the time that might have elapsed since the population existed as a unified and cohesive unit and helps in characterizing the breeds or lines in the populations. The lower genetic distance between WLH-IWF and WLH-IWD and between Aseel and *Desi* (non-descript) in the present study indicated that the populations were completely isolated but had been separated recently over a short period of time. The maximum distance was observed between Babcock, a commercial layer, and Dahlem Red (0.80), a purebred layer strain.

Pandey et al. (2002) observed that the genetic distance between Nicobari and Aseel and Miri and Aseel breeds varied from 0.59 to 0.94. Similarly, Emara et al. (2002) reported that the genetic distance estimates for broiler lines ranged from 0.22 to 0.41, which were within the range of genetic distances obtained in the present investigation. Similarly, Olowofeso et al. (2005b) reported genetic distances in Port-city chicken populations which were relatively contrasting with those observed in the present study.

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. Within the second group, the remaining six populations were grouped into three sub clusters, i.e, Aseel and *Desi* (non-descript), WLH-IWD and WLH-IWF and Babcock and Vencobb. The former two breeds were on the expected lines mainly because Aseel and *Desi* (non-descript) almost share a common habitat under natural conditions. The WLH-IWD and WLH-IWF, the two synthetic strains that shared a common ancestry, were developed from the White Leghorn breed and hence clustered together with a high degree of relationship.

Contrasting, however, was the commercial layer breed of Babcock that clustered with a broiler breed Vencobb and this may be due to an ancestral relationship at the breed formation stage. Another reason could be the New Hampshire inheritance that might have been introduced into layer strains to maintain resistance and sturdiness. The existence of broiler inheritance in layer stocks during the breed formation stage has been previously reported by Wandelt and Wolters (1996). Yet another reason could be the fact that, since both the above breeds are commercial ones, they might harbour high genetic variation and polymorphism.

Romanov and Weigend (2001) also reported a phylogenetic tree topology according to breed/strain characters in eight chicken populations representing Jungle fowls, selected lines and commercial birds which clustered separately in three groups. Similar types of phylogeny reconstructions and clustering patterns based on the breed/geographical origins were observed by many authors (Vanhala et al., 1998; Zhou and Lamont, 1999; Pandey et al., 2002; Chen et al., 2004; Vijh et al., 2004). The circular tree constructed from the pair-wise inter-individual distances showed that individual birds clustered according to their breed and their geographical origin.

CONCLUSIONS

The present study revealed the comprehensive genetic diversity, polymorphisms and evolutionary relationships in domesticated chicken populations. All the microsatellite loci typed were polymorphic with reasonable to high informativeness. Most of the alleles deviated from the equilibrium frequency. The birds were clustered true to their breed. It may be concluded that the chicken populations studied were in the state of moderate heterogeneity except for commercial birds. A planned breeding is advised for purebreds to revive their genetic potential. High genetic diversity exists in *Desi* (non-descript) birds, which can be tapped to improve the birds suitable for backyard poultry.

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