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Study of genetic polymorphism of various chicken breeds using microsatellite markers

B. Deshmukh*, D. Kumar, N. Kashyap[1] and D. Sharma[1]

College of Veterinary and Animal Sciences,
G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, India.

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ABSTRACT

A total number of 76 randomly selected birds i.e. 20 of Hill fowl (HF), 14 of Rhode Island Red (RIR), 14 of Kadaknath (KN), 14 of White LeghWDDorn (WLH) and 14 of White Cornish (WC) were genotyped using 25 microsatellite markers in the present study. Out of 25 microsatellite loci, 17 (~70%) were found to be polymorphic among these breeds. Across the breeds, total number of alleles ranged from 2 to 3 at polymorphic locus and average number of alleles per locus was 2.41. The allele size ranged between 98 bp and 340 bp. Among all the polymorphic microsatellite loci, observed heterozygosity were 0.441, 0.415, 0.287, 0.296 and 0.376 and expected heterozygosity were 0.435, 0.443, 0.387, 0.384 and 0.442 in HF, RIR, KN, WLH and WC, respectively. Polymorphic Information Content (PIC) estimates, across all the polymorphic loci were 0.346, 0.342, 0.305, 0.297 and 0.330 in HF, RIR, KN, WLH and WC, respectively.

Key words: Chicken, Genetic polymorphism, Hill fowl, Microsatellite.

INTRODUCTION

The poultry farming occupies an important position in Indian agricultural economy because of its immense potential to bring about rapid economic growth. The successive commercialization in poultry industry has now rigorously replaced the number of native breeds and varieties. Indian native breeds have potent, vast and versatile source of adaptive genetic variation and unique genes or gene combinations for tropical adaptability and disease resistance that can be utilized for improvement of high yielding exotic germplasm. One of such native Indian variety of chicken found in Kumaon region of Uttarakhand state which is known as Hill Fowl It has recently been identified and named as "Uttara fowl".

Characterization and estimation of genetic polymorphism using molecular tools within breed and from other breeds, is a prerequisite for developing strategies for conservation and utilization of genetic resources. Advances in molecular techniques led to assess the genetic variability at the DNA level with greater coverage of genome. Microsatellite markers are extensively being used for genetic characterization in chicken (Hillel *et al.* 2003; Haunshi and Sharma, 2006; Arya *et al.* 2011; Alipanah *et al.* 2011) since they are abundant, highly polymorphic and show co-dominant inheritance (Karaca *et al.* 1999). The observed genetic diversities at microsatellite loci may arise from the consequence of mutation, recombination and genetic drift and accumulate generation after generation in the population because they are neutral. Thus microsatellites exhibit a high degree of polymorphism among breeds and even individuals. Of all the recently developed markers, microsatellite markers are considered as the marker of choice for characterization of breeds for diversity assessment (FAO, 2007). Present study aimed to estimate the genetic polymorphism within as well as between the Hill fowl of Uttarakhand and four established chicken breeds.

MATERIALS AND METHODS

Present study was conducted on 76 birds of five chicken breeds namely Hill fowl (20), Rhode Island Red (14), Kadaknath (14), White Leghorn (14) and White Cornish (14) maintained at Instructional Poultry Farm (IPF), G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Each group represented a specific type likewise Local hill Fowl (HF)- native chicken, Rhode Island Red (RIR)- dual type, Kadaknath (KN)- Indian native chicken breed, White Leghorn (WLH)- egg type and White Cornish (WC)- meat type. About 0.5 ml of venous blood was collected from the jugular vein into 1.5 ml eppendorf tubes containing EDTA. Genomic DNA was isolated from the blood samples by Phenol: Chloroform extraction method as described by

*Corresponding author's e-mail: bharti.vet@gmail.com.
[1]Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar-243 122 , Bareilly (UP), India.

Kagami *et al.* (1990). A total of 25 informative microsatellite markers (Table 1) selected from the database were (www.thearkdb.org) used for the present study.

PCR was done by mixing 17.3 µl Nuclease free water, 2.5 µl (10X) buffer, 1.5 µl (25 mM) $MgCl_2$, 1 µl (10 pM/ µl) of each forward primer and reverse primer, 0.5 µl (10 mM) dNTP mix, 0.2 µl Taq DNA polymerase (5U/µl) added with 1 µl (30-50 ng/ml) genomic DNA. PCR was done in thermal cycler (PTC-200 DNA Engine® thermal cycler, Bio-Rad, USA) with the following cycle: 3 minutes of initial denaturation (94°C) followed by 35 cycles of 30 seconds denaturation (94°C), 45 seconds annealing (55-60°C), 1 minute and 30 seconds elongation (72°C). Final elongation for 5 minutes at 72°C was given to ensure completion of amplification. Horizontal submarine agarose gel electrophoresis (1.2% agarose gel) was performed to check the amplified PCR products. Final resolution and documentation of microsatellite alleles was done on 3.4% metaphore agarose gel.

The microsatellite genotyping data obtained for hill fowl and other four chicken groups were used. Numbers of alleles were counted manually whereas allele size was estimated using AlphaDigiDoc™ 1000 software.

Observed heterozygosity (H_o) at a locus was measured by equation $H_o = H / T$, where H is the number of heterozygote individuals at a locus and T is the total number of individuals genotyped at that locus. Expected heterozygosity (H_e) at a locus was estimated using an unbiased estimator

$$HEi = 2N/(2N-1)\{1 - \sum P_j^2\}$$

where P_j is the frequency of j^{th} allele at i^{th} locus with l alleles in a population and N is the number of individuals genotyped at i^{th} locus. The PIC was calculated using equation

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2$$

where n is the number of alleles, P_i and P_j are frequencies of i^{th} and j^{th} alleles respectively, at a locus in the population (Botstein *et al*. 1980).

RESULTS AND DISCUSSION

Microsatellite polymorphism: The microsatellite markers selected for the present study covered approximately 20% of the genome. In previous studies, coverage of 12 to 21% of genome was done (Vanhala *et al*. 1998; Haunshi and Sharma, 2006), while Kaiser *et al*. (2000) covered about 57 % of the genome for estimating the genetic similarity between different chicken populations.

For each locus, number of alleles (n_a), allelic size range, observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphism information content (PIC) across all the breeds have been presented in Tables 2 and 3. Out of the 25 microsatellite loci, 17 loci (~70%) were found to be polymorphic between the breeds. Hillel *et al.* (2003) and Kumar (2009) also found that all the microsatellite loci are not polymorphic across all the populations. Total number of alleles amplified across all the microsatellite loci were 48 in HF, 45 in RIR, 44 in KN, 44 in WLH and 43 in WC, whereas total 49 alleles were amplified across all the breeds. The average number of allele per locus was almost similar among all the breeds and ranged from 1.72 in WC to 1.92 in HF. The allele size ranged between 98 bp and 340 bp. Total numbers of alleles ranged from 2 to 3 at all polymorphic loci with average number of alleles per locus being 2.41 (Table 2). Arya *et al.* (2011) using same microsatellite loci reported similar number of alleles in WLH population. Likewise, Kaiser *et al.* (2000) reported the average number of alleles per locus as 2.8 and 2.9 in two chicken populations. However, several workers have reported higher number of alleles (Kumar, 2009; Clementino *et al*. 2010; Phangchopi, 2010; Alipanah *et al*. 2011). This vast variation in number of alleles at different loci might be due to difference in choice of microsatellite markers and the population under study.

Expected heterozygosity varied with the marker as well as between the breeds. The estimates of H_o, H_e and PIC were in HF (0.441, 0.435, 0.346), RIR (0.415, 0.443, 0.342), KN (0.287, 0.387, 0.305), WLH (0.296, 0.384, 0.297) and WC (0.376, 0.442, 0.330), respectively. Over all the loci, average heterozygosity ranged from 0.384 (WLH) to 0.443 (RIR). Low heterozygosity revealed low polymorphism in the breed at microsatellite loci in the present study (Table 3). Majority of reports revealed average heterozygosity estimates of less than 0.50 in different chicken populations have been reported by several workers (Tadano *et al*. 2007; Miao *et al.* 2009; Chatterjee *et al.* 2010), however, some reports also showed high mean heterozygosity of more than 0.6 (Pandey *et al*. 2003; Shahbazi, 2007; Clementino *et al*. 2010).

Average PIC value ranged from 0.297 in WLH to 0.346 in HF in present study which was low (Table 3). Arya *et al.* (2011) reported similar low level of PIC in white leghorn. Tadano *et al*. (2007) also reported low mean polymorphic information content ranging from 0.250 to 0.478; however, some reports revealed higher PIC estimates in chicken breeds (Cheng *et al*. 2003; Qu *et al*. 2006).

These results suggest that genetic polymorphism within group of all the chicken breeds including Hill Fowl, are somewhat similar in magnitude which might be due to small population size and closed flock. Furthermore, polymorphism between all the breeds was more or less equal.

TABLE 1: Details of microsatellite markers

Markers	Forward & Reverse Primer Sequence	Repeat Motifs	T_a (°C)	Map Location
ADL0019	F- TGCTGCCTAGACCAGTTCAA R-TCTGCTGGGATTATGTGTCA	$(AC)_{10}$	57.8	1
ADL0112	F- GGCTTAAGCTGACCCATTAT R- ATCTCAAATGTAATGCGTGC	$(TG)_9$	55	10
ADL0150	F- ATGCCAAGCATTACAGAAGC R- CCTGCAGCACCTTTATCTCT	$(CA)_{11}$	56.7	1
ADL0176	F- TTGTGGATTCTGGTGGTAGC R- TTCTCCCGTAACACTCGTCA	$(GT)_7$	57.8	2
ADL0257	F- ATCTTGAAACCTCACAAAGC R- TCTTCCAACCTATTTTTAGT	$(TGT)_9$	55	2
ADL0268	F-CTCCACCCCTCAGAACTA R-CAACTTCCCATCTACCTACT	$(TG)_{13}$	55	1
ADL0273	F- GCCATACATGACAATAGAGG R- TGGTAGATGCTGAGAGGTGT	$(TG)_{11}$	55	Z
LEI0094	F-GATCTCACCAGTATGAGCTGC R-TCTCACACTGTAACACAGTGC	$(CA)_{16}$	56.7	4
LEI0139	F- ACATTTGAGATGAAGCTTGCC R- GGTATCTAGTGCATATGATGC	$(CA)_{19}$	56.7	1
LEI0174	F-CCATTACCTGTAGCACTGGGCC R- TTAAAGGGCATTCCCGCATG	$(TG)_{24}$	55	1
LEI0234	F-ATGCATCAGATTGGTAATCAA R-CGTGGCTGTGAACAATATG	$(TTTC)_6$	55	2
MCW0007	F- GCAGAAGTGTTCTCTGTTCAT R- ACCCAAACTGGAAGGGTCTCA	$(AT)_{11}$	56.7	1
MCW0014	F-AATATTGGCTCTAGGAACTGTC R-GGAAATGAAGGTAAGACTAGC	$(TG)_8$	57.8	6
MCW0037	F-CGGTGCCATCAATTACCTATTA R-AGCTCACATGACACTGCGAAA	$(TG)_7$	57.8	3
MCW0067	F- GCACTACTGTGTGCTGCAGTTT R-GATGTAGTTGCCACATTCCGAC	$(TG)_{11}$	57.8	10
MCW0069	F-CACTCGAGAAAACTTCCTGCG R- GCTTCAGCAAGCATGGGAGGA	$(TG)_{11}$	55	26
MCW0081	F- GTTGCTGAGAGCCTGGTGCAG R- CCTGTATGTGGAATTACTTCTC	$(GT)_{12}$	55	5
MCW0098	F- GCACTACTGTGTGCTGCAGTTT R- GATGTAGTTGCCACATTCCGAC	$(TG)_{11}$	55	4
MCW0103	F-AACTGCGTTGAGAGTGAATGC R-TTTCCTAACTGGATGCTTCTG	$(CA)_8$	55	3
MCW0154	F- GATCTGTTTTATCACACACAC R- CCATTTCCTTTGTTATCAGGC	$(CA)_{20}$	56.7	Z
MCW0183	F-ATCCCAGTGTCGAGTATCCGA R-TGAGATTTACTGGAGCCTGCC	$(TG)_{11}$	55	7
MCW0214	F- CAACAGTAACCATACATCTGC R- TACCTGGATTCTTTCATCAGG	$(CA)_9$	55	5
MCW216E	F-GGGTTTTACAGGATGGGACG R-AGTTTCACTCCCAGGGCTCG	$(CA)_4$	57.8	13
MCW0256	F- GATGGGGCACTGTGGGTCC R- TGGTTTCCATCAAGCAGTTCC	$(TG)_9$	57.8	19
MCW0330	F- TGGACCTCATCAGTCTGACAG R- AATGTTCTCATAGAGTTCCTGC	$(AC)_4$	55	17

T_a, annealing temperature

TABLE 2: Number of alleles and allelic size range in Hill fowl and different chicken breeds at various microsatellite loci

Locus	Hill Fowl		Rhode Island Red		Kadaknath		White Leghorn		White Cornish		Overall population	
	No of alleles	Size (bp)	No of alleles	Size (bp)	No of alleles	Size (bp)	No of alleles	Size (bp)	No of alleles	Size (bp)	No of alleles	Size (bp)
ADL0019	3	98- 124	3	98- 124	2	98- 110	2	98- 110	2	98- 110	3	98-124
ADL0112	1	136	1	136	1	136	1	136	1	136	1	136
ADL0150	1	166	1	166	1	166	1	166	1	166	1	166
ADL0176	3	194-216	3	194-216	3	194-216	3	194-216	2	194-204	3	194-216
ADL0257	3	171-213	2	195-213	2	195-213	3	171-213	2	195-213	3	171-213
ADL0268	2	102-118	2	102-118	2	102-118	2	102-118	2	102-118	2	102-118
ADL0273	1	156	1	156	1	156	1	156	1	156	1	156
LEI0094	3	210-254	3	210-254	3	210-254	2	210-234	3	210-254	3	210-254
LEI0139	1	318	1	318	1	318	1	318	1	318	1	318
LEI0174	2	186-254	2	186-254	3	186-254	3	186-254	2	186-216	3	186-254
LEI0234	3	240-332	3	240-332	1	240	2	240-332	2	240-332	3	240-332
MCW0007	2	316-340	2	316-340	2	316-340	2	316-340	2	316-340	2	316-340
MCW0014	2	200-220	2	200-220	2	200-220	2	200-220	2	200-220	2	200-220
MCW0037	1	168	1	168	1	168	1	168	1	168	1	168
MCW0067	2	188-216	2	188-216	2	188-216	2	188-216	2	188-216	2	188-216
MCW0069	2	174-198	2	174-198	2	174-198	2	174-198	2	174-198	2	174-198
MCW0081	3	118-140	2	118-140	2	118-140	1	140	3	118-140	3	118-140
MCW0098	1	284	1	284	1	284	1	284	1	284	1	284
MCW0103	1	324	1	324	1	324	1	324	1	324	1	324
MCW0154	2	176-192	2	176-192	2	176-192	2	176-192	2	176-192	2	176-192
MCW0183	2	306-336	1	306	2	306-336	2	306-336	1	306	2	306-336
MCW0214	2	286-308	2	286-308	2	286-308	2	286-308	2	286-308	2	286-308
MCW216E	1	150	1	150	1	150	1	150	1	150	1	150
MCW0256	2	186-206	2	186-206	2	186-206	2	186-206	2	186-206	2	186-206
MCW0330	2	306-322	2	306-322	2	306-322	2	306-322	2	306-322	2	306-322
Across all loci	48	98- 340	45	98- 340	44	98- 340	44	98- 340	43	98- 340	49	98- 340

bp, base pairs

TABLE 3: Observed heterozygosity, Expected heterozygosity and Polymorphism information content estimates at different microsatellite loci in Hill fowl and other chicken breeds

Locus	Hill Fowl			Rhode Island Red			Kadaknath			White Leghorn			White Cornish		
	Ho	He	PIC	Ho	He	PIC	Ho	He	PIC	Ho	He	PIC	Ho	He	PIC
ADL0019	0.800	0.512	0.397	0.857	0.638	0.541	0.929	0.516	0.374	1.000	0.519	0.375	1.000	0.519	0.375
ADL0176	0.450	0.550	0.477	0.571	0.561	0.453	0.357	0.542	0.442	0.539	0.446	0.389	0.083	0.489	0.359
ADL0257	0.211	0.198	0.181	0.714	0.519	0.375	0.071	0.198	0.173	0.231	0.539	0.418	0.167	0.391	0.305
ADL0268	1.000	0.514	0.375	0.846	0.508	0.369	1.000	0.524	0.375	1.000	0.522	0.375	0.833	0.522	0.375
LEI0094	0.842	0.585	0.474	0.846	0.563	0.456	0.615	0.625	0.532	0.500	0.389	0.305	0.727	0.558	0.432
LEI0174	0.600	0.492	0.365	0.571	0.519	0.375	0.357	0.675	0.577	0.143	0.603	0.517	0.364	0.520	0.373
LEI0234	0.471	0.587	0.492	0.000	0.615	0.502	0.000	0.000	0.000	0.000	0.233	0.195	0.200	0.200	0.164
MCW0007	0.647	0.451	0.342	0.546	0.416	0.318	0.222	0.366	0.286	0.154	0.517	0.374	0.833	0.507	0.368
MCW0014	0.188	0.175	0.156	0.250	0.431	0.328	0.083	0.228	0.195	0.000	0.000	0.000	0.000	0.485	0.356
MCW0067	0.333	0.413	0.321	0.500	0.389	0.305	0.250	0.431	0.328	0.500	0.389	0.305	0.143	0.254	0.215
MCW0069	0.000	0.457	0.346	0.154	0.492	0.361	0.000	0.349	0.280	0.000	0.476	0.354	0.571	0.508	0.370
MCW0081	0.579	0.653	0.564	0.143	0.138	0.124	0.100	0.100	0.091	0.000	0.000	0.000	0.778	0.628	0.505
MCW0154	0.100	0.467	0.352	0.000	0.508	0.370	0.000	0.349	0.280	0.000	0.138	0.124	0.000	0.508	0.370
MCW0183	0.200	0.287	0.239	0.000	0.000	0.000	0.000	0.533	0.375	0.000	0.303	0.239	0.000	0.000	0.000
MCW0214	0.105	0.102	0.095	0.636	0.507	0.367	0.500	0.389	0.305	0.462	0.517	0.374	0.182	0.485	0.356
MCW0256	0.625	0.484	0.359	0.182	0.312	0.253	0.083	0.228	0.195	0.500	0.522	0.375	0.091	0.507	0.367
MCW0330	0.353	0.471	0.352	0.231	0.409	0.316	0.308	0.517	0.374	0.000	0.423	0.325	0.417	0.431	0.328
Across all loci	0.441	0.435	0.346	0.415	0.443	0.342	0.287	0.387	0.305	0.296	0.384	0.297	0.376	0.442	0.330

H_o, Observed Heterozygosity; H_e, Expected Heterozygosity; PIC, Polymorphism Information Content

All the microsatellite loci showed low polymorphism within and between different chicken breeds, though they were informative enough.

Population specific alleles: At LEI0094 locus, allele 254 bp was rare allele and present in nil to very low frequencies (0.00 to 0.08) except in KN (0.27). At LEI0234 locus predominance of allele 240 bp was found in KN, WLH and WC (0.88 to 1.00), whereas, predominance of allele 304 bp in HF (0.56) and of allele 240 bp in RIR (0.57) was seen. At

TABLE 4: Allelic frequencies at different microsatellite loci in Hill fowl and other chicken breeds

Locus	Allele size (bp)	HF	RIR	KN	WLH	WC
ADL0019	98	0.38	0.32	0.46	0.50	0.50
	110	0.60	0.50	0.54	0.50	0.50
	124	0.03	0.18	0.00	0.00	0.00
ADL0176	194	0.15	0.36	0.61	0.12	0.38
	204	0.63	0.57	0.32	0.73	0.63
	216	0.23	0.07	0.07	0.15	0.00
ADL0257	171	0.08	0.00	0.00	0.04	0.00
	195	0.89	0.50	0.11	0.58	0.25
	212	0.03	0.50	0.89	0.38	0.75
ADL0268	102	0.50	0.58	0.50	0.50	0.50
	118	0.50	0.42	0.50	0.50	0.50
LEI0094	210	0.47	0.58	0.54	0.75	0.41
	234	0.45	0.35	0.19	0.25	0.55
	254	0.08	0.08	0.27	0.00	0.05
LEI0174	186	0.60	0.50	0.25	0.57	0.55
	216	0.40	0.50	0.32	0.21	0.45
	254	0.00	0.00	0.43	0.21	0.00
LEI0234	240	0.32	0.57	1.00	0.88	0.90
	304	0.56	0.14	0.00	0.00	0.00
	332	0.12	0.29	0.00	0.13	0.10
MCW0007	316	0.32	0.27	0.78	0.46	0.58
	340	0.68	0.73	0.22	0.54	0.42
MCW0014	200	0.09	0.29	0.13	0.00	0.36
	220	0.91	0.71	0.88	1.00	0.64
MCW0067	188	0.72	0.75	0.71	0.75	0.86
	216	0.28	0.25	0.29	0.25	0.14
MCW0069	174	0.67	0.62	0.79	0.36	0.57
	198	0.33	0.38	0.21	0.64	0.43
MCW0081	118	0.24	0.93	0.95	0.00	0.44
	130	0.29	0.00	0.00	0.00	0.11
	140	0.47	0.07	0.05	1.00	0.44
MCW0154	176	0.35	0.57	0.79	0.07	0.57
	192	0.65	0.43	0.21	0.93	0.43
MCW0183	306	0.83	1.00	0.50	0.17	1.00
	336	0.17	0.00	0.50	0.83	0.00
MCW0214	286	0.05	0.41	0.25	0.54	0.36
	308	0.95	0.59	0.75	0.46	0.64
MCW0256	186	0.63	0.18	0.88	0.50	0.59
	206	0.38	0.82	0.13	0.50	0.41
MCW0330	306	0.35	0.27	0.54	0.29	0.29
	322	0.65	0.73	0.46	0.71	0.71

HF, Hill Fowl; RIR, Rhode Island Red; KN, Kadaknath; WLH, White Leghorn; WC, White Cornish

locus MCW0081, allele 118 bp had very high frequency (0.93 to 0.95) in RIR and KN, while, allele 140 bp was the only allele present in WLH (Table 4). Though the alleles showed distinct differences in frequencies between the populations but population specific allele could not be identified for any of the chicken breed under study. However, earlier workers have reported several population specific alleles like Hillel *et al*. (2003) identified 32 population specific alleles among 52 populations and 22 loci. Likewise, Nakamura *et al*. (2006) used 25 microsatellite markers and identified five population specific alleles for Nagoya breed. Kumar, (2009) reported 48 population specific alleles, of which 21, 9, 6, 10 and 2 were specific to RJF, WLH, KN, AS and RC respectively. Hence no breed specific microsatellite marker could be identified, however some markers showed alleles present in only few populations, however in low frequency.

CONCLUSION

Selection of marker in the genetic diversity studies is critical and may influence the results therefore the microsatellite markers to be used should be highly polymorphic. The microsatellite loci explored in this study revealed low polymorphism within and between different chicken breeds. There was no breed specific microsatellite marker found, still some markers showed alleles present in only few breeds of chicken, though in low frequency. Concluding that, microsatellite markers proved to be very extensive in their capabilities to detect polymorphism and thus, deemed as the marker of choice for genetic diversity studies between the chicken breeds.

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