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Micro-satellite based diversity estimation of Local hill fowl (Uttara fowl): A unique poultry strain of Uttarakhand

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There are 17 defined breeds of domestic fowl (*Gallus domesticus*) in India (Acharya and Bhat 1984). Apart from these defined breeds there are several populations available in Nainital and Pithoragarh districts of Uttarakhand which possess totally different phenotypes from the reported breeds. These populations evolved in various environments represent unique sets of genetic diversity. The Himalayan region is full of diverse genetic resources with respect to animals and birds. In few pockets of Nainital and Pithoragarh districts of Uttarakhand. Uttara fowl is generally reared under backyard system. Two types of populations of Uttara fowl are found in the state. One is Shank feathered variety which is found in Pithoragarh district and another variety having crown like structure on their head is found in Nainital district of the state. Uttara fowls are hardy, good foragers and resistant to many diseases as well as the harsh climate of the habitat. The Uttara fowl is of local importance in the region due to as its gives the nutritional as well as economic security to the rearing families. But there is no information available at molecular level in the literature about this important germplasm. Breed characterization (Phenotypic and molecular) is a primary step in designing appropriate management and conservation programmes of livestock in developing countries. Characterization of genetic diversity by employing molecular tools is a prerequisite in developing strategies for conservation and utilization of poultry genetic resources. Therefore, this study was planned to evaluate the genetic diversity of the Uttara fowl. As only single study has been cited in the intro, a bit more citations of the studies in intro is needed describing the impact of work done in other fowl breeds and such comparative information is lacking in the Local hill fowl.

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Present study was carried out by randomly selected 50 Uttara fowl maintained at Instructional Poultry Farm, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). 1 ml of blood was collected from wing vein using 0.5M EDTA as an anticoagulant. DNA was isolated using standard protocol (Sambrook *et al.* 1989). PCR reactions were carried out in a volume of 25 µl containing 50–100 ng genomic DNA, 1.5 mM $MgCl_2$, 20 pM of each primer, 0.5mM dNTP, one Unit of *Taq DNA* polymerase. Denaturation, annealing and extension steps for 30 cycles were carried out using thermocycler. Amplified products were electrophoresed at 4ºC on 8% non-denaturing polyacrylamide gel containing acrylamide and bis-acralymide in the ratio of 29:1. The gel was run at 250 V for 4 h in 1× TBE and stained with 0.1% silver nitrate following the standard protocol. The gel was visualized and documented under white light of gel documentation system. Amplified PCR products were used for genotyping of Uttara fowl using ABI Avant 3100 Automated DNA sequencer and Gene mapper software version 3.0.

The statistical analysis was carried out using POPGENE software (Yeh *et al.* 1999). The observed number of alleles and effective number of alleles under each locus in the population, the observed heterozygosity and expected heterozygosity on the basis of allele frequency were calculated. Shannon's information Index was used to find out the number of alleles for a specific locus.

A total of 25 micro-satellite loci were used for this study. The 25 primers recommended by FAO and specific for *Gallus gallus* were used. The loci were amplified using these primers and tagged with HEX and FAM dyes. Tests for pair-wise linkage (genotypic) disequilibrium among the micro-satellite loci were done using FSTAT version 2.9.3 an update version 1.2 (Goudet 1995) for 25 micro-satellite loci whose genotypes were determined directly.

Various measures of genetic variation including F_{IS} are presented in the Table 1. All the 25 micro-satellite loci were polymorphic and a total of 158 alleles were observed. Sufficient allelic diversity was found in Uttara fowl with

Table 1. Heterozygosity and polymorphism for co-dominant microsatellite data for Uttara fowl

Loci	No. of alleles	Size range	Na	Ne	I	Ho	He	Chi-Sq.	Prob.	Level of significance	Fis
HUJ 002	5	120–134	5.000	3.524	1.369	0.675	0.716	18.079	0.054	NS	0.058
HUJ003	8	151–177	8.000	4.549	1.712	0.667	0.780	47.930	0.011	*	0.145
LEI—64	8	287–309	8.000	5.885	1.872	0.646	0.830	62.547	0.000	***	0.222
LEI—74	5	277–313	5.000	2.462	1.153	0.417	0.594	26.787	0.003	**	0.298
LEI—82	7	243–283	7.000	2.319	1.166	0.617	0.569	46.219	0.001	**	0.098
LEI—90	5	202–214	5.000	3.447	1.377	0.729	0.710	41.184	0.000	***	–0.085
LEI—98	5	154–166	5.000	3.156	1.284	0.681	0.683	14.739	0.142	NS	–0.027
LEI—120	6	272–296	6.000	2.175	1.085	0.438	0.540	51.097	0.000	***	0.003
LEI—122	10	267–301	10.000	3.630	1.614	0.617	0.725	91.912	0.000	***	0.190
LEI—147	9	253–289	9.000	4.148	1.706	0.646	0.759	91.290	0.000	***	0.148
LEI—155	3	93–101	3.000	1.802	0.768	0.292	0.445	11.175	0.011	*	0.149
LEI—166	5	250–260	5.000	3.542	1.432	0.417	0.718	49.288	0.000	***	0.345
LEI—174	6	224–252	6.000	4.380	1.580	0.667	0.772	59.744	0.000	***	0.419
LEI—180	5	185–201	5.000	3.830	1.457	0.667	0.739	29.617	0.001	***	0.136
MCW-84	3	91–95	3.000	1.976	0.753	0.125	0.494	73.571	0.000	***	0.747
MCW-213	10	277–315	10.000	3.547	1.568	0.542	0.718	137.825	0.000	***	0.246
MCW-217	7	143–167	7.000	3.938	1.479	0.563	0.746	46.937	0.001	***	0.246
MCW-228	11	216–250	11.000	6.188	2.042	0.638	0.838	100.227	0.000	***	0.239
MCW-250	5	224–238	5.000	1.706	0.849	0.438	0.414	55.037	0.000	***	–0.057
MCW-261	5	238–252	5.000	3.086	1.238	0.500	0.676	28.402	0.002	**	0.260
MCW-262	5	63–71	5.000	2.281	1.071	0.650	0.562	7.721	0.656	NS	–0.157
MCW-266	6	157–167	6.000	4.581	1.583	0.646	0.782	38.794	0.001	***	0.174
MCW–305	7	252–264	7.000	3.835	1.546	0.638	0.739	44.979	0.002	**	0.137
MCW-317	6	225–245	6.000	1.835	0.987	0.425	0.455	40.163	0.000	***	0.066
MCW-328	6	249–261	6.000	3.415	1.406	0.575	0.707	57.258	0.000	***	0.187
Mean	158		6.32	3.409		0.539	0.668				0.168

observed number of alleles varying from 3 (LEI-155, MCW-84) to 11 (MCW-228) with overall mean number of alleles per locus as 6.32. The mean number of alleles in different populations in the present study were little lower than the earlier reports in native Indian chicken (Pandey *et al.* 2002, 2005; Pirany *et al.* 2007; Rajkumar *et al.* 2008; Chatterjee *et al.* 2010), which might be due to the fact that the three populations are under long-term selection and the two native breeds are random mated but in closed populations. The effective number of alleles ranged between 1.706 (MCW-250) and 6.188 (MCW-228). The mean effective number of alleles (3.409) was lower than mean observed numbers of alleles (6.32). The lower effective number of alleles than the observed number of alleles across the loci in the present investigation indicated that allele frequencies were widely distributed. The present findings are in accordance with the earlier reports in Indian native chickens (Pandey *et al.* 2002, 2005, Pirany *et al.* 2007, Rajkumar *et al.* 2008, Chatterjee *et al.* 2010). The use of micro-satellites with a range of polymorphism reduced the risk of overestimating genetic variability, which might occur with microsatellite exhibiting only high polymorphism. Genetic variability is also measured as the amount of actual or potential heterozygosity (Table 1). Expected heterozygosity was higher than the observed heterozygosity indicating high level of information of the chosen micro-satellite set. The observed and expected heterozygosity values ranged from 0.125 (MCW-84) to 0.729 (LEI-90), and from 0.414 (MCW-250) to 0.838 (MCW-228), respectively. Similar observations were recorded in different Indian native breeds by earlier workers (Chatterjee *et al.* 2010). The high heterozygosity estimates for most of the loci in the present study were probably 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 0.45 to 0.77 in Asian, African and European chicken populations (Hillel *et al.* 2003; Rajkumar *et al.* 2008). In assessing diversity estimates from different studies, it should be mentioned that the values are not directly comparable, as different micro-satellite set were used by different workers. These values have only suggestive indication of diversity in the population. Observed heterozygosity was slightly lower than expected heterozygosity showing a departure from Hardy-Weinberg Equilibrium (HWE) and possibility of inbreeding. Most of the loci were in HWE and significant deviation from HWE had been observed only in 5 out of 25 loci ($P<0.01$). This could be linked to very low positive F_{IS} values obtained in this breed. The values ranged from –0.027 (LEI-98) to 0.747 (MCW-84). Only 1.6% of entire set of 25 loci contributed to the overall heterozygote deficiency. The mean Fýs for 25 microsatellite loci was 0.168.

The study was planned to examine the applicability of chicken micro-satellites in assessing the genetic variation

and it can be concluded that microsatellites markers can be used to evaluate the genetic variability in Uttara fowl. The number of alleles per locus ranged from 3 to 11. The average expected heterozygosity was 0.668. This breed is of local use and subjected to traditional husbandry management. Thus strong artificial selection, intensive use of elite sires and artificial insemination is not frequent resulting in higher variability and better adaptation to natural environment. The breed is reared by local people of hilly region of Uttarakhand following their own breeding schemes and usually using their own sires. The findings of this study would be further utilized to compare the structure of repeat motifs and study the evolution of micro-satellites across hill fowl. Therefore, this would provide further insight into the evolution of micro-satellites and genetic divergence of birds of different orders in the class Aves.

SUMMARY

Nainital and Pithoragarh districts of Uttarakhand in Himalayan region have 2 types of poultry populations. Uttara fowl is reared under backyard system. But no information is available in the literature of Uttara fowl. The aim of the study was to analyze the genetic diversity in Local hill fowl of Uttarakhand (Uttara Fowl) using panel of micro-satellite markers recommended by FAO. The 50 blood samples were collected from randomly selected Uttara fowl. A total of 25 micro-satellite loci were used for this study. All the analyzed 25 loci were polymorphic and a total of 158 alleles were observed in the present study of Uttara Fowl. The observed and expected heterozygosity ranged from 0.292 (LEI-155) to 0.729 (LEI-90) and from 0.414 (MCW–250) to 0.838 (MCW-228) in Uttara fowl, respectively. Wright's fixation index (Fis) values among loci ranged from –0.085 (for LEI-90) to 0.747(MCW-84). The mean Fis for 25 microsatellite loci was estimated 0.168. Deviation from Hardy-Weinberg equilibrium was observed in Uttara fowl in the commercial cross. The overall population heterozygote deficiency was 0.168. The existence of sufficient genetic diversity within Local hill fowls, estimated through molecular markers analysis would further aid in a conservation scheme, enabling the planning of new strategies for the improvement of *in situ* conservation schemes.

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