MICROSATELLITE ANALYSIS OF INDIGENOUS DUCKS OF ASSAM

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ABSTRACT

Indigenous duck breed of Assam are popular with considerable production potential with minimal input and mostly reared under semi intensive system of management. These ducks are maintained in all agro climatic zones of Assam and different from other indigenous duck genetic resources available in the country. But the genetic structure of these duck varieties was not fully studied; hence the genetic characterization of Assam ducks was assessed with 23 FAO recommended duck specific microsatellite markers using advanced automated genotyping technique. The analysis revealed that totally 91alleles were observed with the number ranging from 1 (CAUD025) to 7 (CAUD004 and APH009) and an overall mean of 3.957 ± 0.32 across the loci. The mean observed and expected heterozygosities were 0.4444 and 0.5113. All the microsatellite loci were found to be highly polymorphic except CAUD025. In Assam ducks, PIC value ranged from 0.14 (APH001) to 0.71 (CAUD004) with a mean value of 0. 4813. Nearly 14 out of 23 loci had PIC values of more than 0.5 indicating that these markers can be effectively used for genetic diversity analysis. The Chi-square test revealed that among the 23 microsatellite studied, only 12 were in Hardy-Weinberg equilibrium proportions and the rest departed from equilibrium. Selection and non-random mating could be the main reasons for this disequilibrium. The markers used in the study were found to be highly informative, explores high genetic variation in the population which could be exploited for their improvement.

Key Words: Assam Ducks, Microsatellite markers, Molecular characterization

INTRODUCTION

Indigenous ducks are evolved specifically to thrive well in the breeding tract with better adaptability and good productivity. Besides chicken production, duck farming especially small scale duck production makes a significant contribution to household economics and food security. Ducks are best maintained on free-range system because they are good foragers and it is mostly coincides with paddy cultivation. Ducks are best suited to integrate with paddy and fish farming. The leading states of India in duck population are West Bengal, Assam,

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Kerala, Andhra Pradesh, Tamil Nadu, Bihar and Orissa. The common Indian breeds/ genetic groups of ducks are Indian Runner, Nageswari, Sythetmete, Assam, Arni etc. Besides, non-descript ducks are also available in large numbers in many states of the country, contributing significantly to the total duck population. These indigenous ducks have innate potential to produce eggs and meat at considerable quantity with lesser input which are good dietary source of proteins.

In order to conserve available duck genetic resource, study on genetic diversity within and between duck populations using microsatellite markers would provide information for taking priority decisions towards preservation. According to FAO (2004) recommendations, using neutral, highly polymorphic microsatellite markers are currently the method of choice for genetic characterization and to provide information for establishing preservation priorities for livestock breeds. Microsatellite markers are widely used in genotype identification, pedigree analysis and estimation of genetic diversity and genetic distance (Romanov and Weigend, 2001; Chen *et al*., 2003; Yan *et al*., 2005). Molecular characterization and genetic diversity analysis by employing molecular tools is a pre-requisite in developing strategies for conservation and utilization of duck genetic resources for future generations. Therefore, the present study was undertaken to characterize the indigenous duck of Assam using the microsatellites.

MATERIALS AND METHODS

The indigenous duck of Assam are being reared popularly by all the duck farmers in Assam. They are the medium to large sized birds reared mainly for egg production. These ducks have different phenotypic characters as that of other indigenous ducks available in other parts of the country.

A total of 50 blood samples were collected at random from these birds in several areas of the main breeding tract were subjected to microsatellite analysis. Genomic DNA was extracted using standard Phenol-Chloroform extraction procedure (Sambrook *et al*., 1989) with slight modifications by using DNAzol reagent, instead of SDS and proteinase K.

PCR amplification and microsatellite analysis

A total of 23 microsatellite primer sets specific for ducks were used in the study as recommended by the Food and Agriculture Organization of United Nations, Rome, Italy (http://www.fao.org/dad-is). Only forward primers of each pair were labelled with one of the five fluorophore i.e. FAM, HEX, TET, FAT and PET and the reverse primers were kept unlabelled. These markers were amplified in the target DNA samples using thermal cycler (MJ Peltier). PCR reaction mixture (15µl) containing 1 µl of template DNA; 0.5 µl each of forward and reverse primers; 7.5 µl of PCR master mix and 5.5 µl of Millipore water was prepared. Amplification was carried out with initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation (95°C for 30 seconds), annealing (49.9°C to 60.0°C for 30 seconds for various primers) and extension (72°C for 5 minutes).

Amplified PCR products were checked on two per cent agarose gel and the bands developed were viewed by UV illumination (Bio-Rad, USA). The samples, which showed amplification were used for further study. The typing of microsatellite markers was made by capillary electrophoresis using automatic sequencer at Eurofins Genomics India Pvt. Ltd. Microsatellite fragment sizing was performed by the Gene MapperTm version 4.0 (Applied Bio-systems, Germany). The size standard (Gene Scan -500 ROX) peaks were used. Allele calling was performed with the software and were checked manually to avoid any false calling of alleles.

Number of alleles, effective number of alleles, Microsatellite allele frequencies, expected heterozygosity (He) and F-statistics were calculated by using POPGENE version 1.31 (Yeh *et al*., 1999).

RESULTS AND DISCUSSION

The allele number, polymorphism information content, observed and expected heterozygosity, Hardy Weinberg Equilibrium for different microsatellite loci for Assam ducks are presented in the Table.

Table.Microsatellite alleles, observed and expected heterozygosities, PIC, and Hardy-Weinberg equilibrium (HWE) values for *Assam* ducks

Microsatellite Marker	n_a	n_e	H_o	H_e	PIC	HWE	
						χ^2	d.f
CAUD010	4	1.1868	0.1111	0.1574	0.1560	35.03**	6
CAUD011	4	3.1765	0.1111	0.6852	0.6497	43.44**	6
CAUD013	6	3.4105	0.5556	0.7068	0.6949	66.88**	15
CAUD016	4	2.1672	0.2778	0.5386	0.5051	18.82**	6
CAUD017	2	1.8000	0.3333	0.4444	0.3457	1.42^{NS}	1
CAUD019	5	1.9343	0.5000	0.4830	0.4771	26.80**	10
CAUD022	3	2.2192	0.7778	0.5494	0.5259	6.70^{NS}	3
CAUD023	4	2.8297	0.4444	0.6466	0.6378	9.50^{NS}	6
CAUD024	4	2.4828	0.9444	0.5972	0.5939	13.52*	6
CAUD025	1	1.0000	0.0000	0.0000	0.0000	-	-
CAUD026	3	2.6667	0.8889	0.6250	0.5550	5.22^{NS}	3
CAUD027	4	2.1966	0.3889	0.5448	0.5418	5.87^{NS}	6
CAUD031	5	3.5217	0.8333	0.7160	0.6972	7.92^{NS}	10
CAUD032	5	2.0062	0.5000	0.5015	0.4566	4.67^{NS}	10
CAUD033	2	1.3144	0.0556	0.2392	0.2106	13.16**	1

CAUD035	4	2.2116	0.5000	0.5478	0.5368	8.96^{NS}	6
CAUD001	4	2.4089	0.6111	0.5849	0.5105	37.23**	6
CAUD004	7	3.4839	0.3889	0.7130	0.7109	73.43**	21
APH001	2	1.1803	0.1667	0.1528	0.1411	0.10^{NS}	1
APH007	5	2.8929	0.3889	0.6543	0.6366	14.12^{NS}	10
APH009	7	3.1610	0.2222	0.6836	0.6354	73.33**	21
APH010	2	1.9059	0.5556	0.4753	0.3624	0.36^{NS}	1
MCW328	4	2.0571	0.6667	0.5139	0.4887	4.07^{NS}	6
Mean	**3.957**	**2.3137**	**0.4444**	**0.5113**	**0.4813**	-	-
S. E.	**0.32**	**0.16**	**0.06**	**0.04**	**0.04**	-	-

n_a – observed number of alleles , n_e- Effective number of alleles, H_o- Observed heterozygosity, H_e- Expected heterozygosity, PIC- Polymorphism Information Content, χ^2- Chi- square value and d.f- Degrees of freedom .

NS- Not Significant *- Significant **- Highly significant

Microsatellite allelic diversity

In Assamducks the number of alleles ranged from 1 to 7 with mean number of alleles as 3.957 ± 0.32 across all 23 microsatellite loci.

The total number of alleles observed was 91. The lowest number of one allele was observed in CAUD025 and the highest number of seven alleles was observed in CAUD004 and APH009 locus. Higher number of total alleles than those observed in the present study was reported by earlier authors (236 alleles in 24 loci for six Chinese duck breeds by Li *et al*., 2006; 281 alleles in 20 loci among six Chinese duck populations by Wu *et al*., 2008). In contrast to this, lesser number of alleles were also reported by several authors (50 alleles in 21 primers for Moti Indian native ducks by Alyethodi and Kumar, 2010; 48 alleles in 9 loci among two Indian duck populations by Kumar *et al*., 2011).

The effective number of alleles was ranging from 1.1803 to 3.5217 with mean value of 2.3137 ± 0.16. Higher effective number of alleles than those in the present study was reported (4.80 and 3.60) by Li *et al*. (2006) and Kumar *et al*. (2011) respectively. The variations in the observed and expected number of alleles can be attributed to the genetic variability of the duck populations, number and type of microsatellite primers utilised for analysis and the difference within the duck population under study.

All the 23 microsatellite loci studied were highly polymorphic. This finding is in agreement with the reports of Li *et al*. (2006), Gaur *et al*. (2009), Su and Chen (2009), Alyethodi and Kumar (2010) and Kumar *et al*. (2011). The effective number of alleles is also an index used to reveal the genetic diversity of duck populations and the highly polymorphic loci indicated that these microsatellite loci could be used as effective markers for molecular characterization and genetic diversity analysis among different duck breeds. Lower mean effective and observed number of alleles, obtained in the present study is the indicative of lesser frequencies of alleles, which might be due to closed nature of the populations.

Polymorphism information content

In general the polymorphism information content (PIC) values are suggestive of high polymorphic nature of the microsatellite loci analysed. The higher the PIC value the more informative a marker. The PIC is a good index for genetic diversity evaluation. When PIC is more than 0.5, the locus has high diversity; when PIC is less than 0.25, the locus has low diversity; and the locus has intermediate diversity when PIC is in between 0.25 and 0.5. The mean PIC value obtained in the present study was 0.4813 ± 0.04. As observed in the present study, Alyethodi and Kumar (2010) observed moderate PIC value in Moti ducks (0.45) with the same set of markers. Whereas, higher PIC value of more than 0.5 was observed at most of the loci in Chinese ducks (Li *et al*., 2006; Wu *et al*., 2008; Su and Chen, 2009) and Indian ducks by Kumar *et al*. (2011).

The differences in PIC of various microsatellite loci may be due to genetic differences in the population analysed. This indicated that the selected microsatellite loci had high diversity which can reflect the genetic relationship among different populations at molecular level and these loci are highly informative.

Heterozygosity

Genetic diversity can be measured as the amount of actual or potential heterozygosity. Heterozygosity is one of the indices used to assay the genetic variation of each population. The mean observed heterozygosity was 0.4444 ± 0.06 and the mean expected heterozygosity was 0.5113 ± 0.04. Among different loci analysed, the locus CAUD031 had the highest expected heterozygosity as 0.7160 and the locus CAUD025 had lowest value of 0.000 in Assam duck populations. Heterozygosity value of less than 0.6 was observed by Gaur *et al*. (2009), Alyethodi and Kumar (2010), Kumar *et al*. (2011) in various duck breeds. While mean heterozygosity value of more than 0.6 in Chinese and Indonesian ducks were reported by Li *et al*. (2006), Ying Su *et al*. (2007) and Wu *et al*. (2008) in different duck populations.

Generally, a marker to be considered useful for measuring genetic variation in a population should have a heterozygosity value of 0.3 to 0.8. Hence, the markers used in this study are quite suitable for assessing the genetic diversity in duck populations as the range of heterozygosity found in this study fit well within the specified range. This indicates that genetic diversity of this breed is high and there are enough gene resources in duck populations.

Hardy-Weinberg equilibrium

The results revealed that a total of 10 loci were found to depart from Hardy-Weinberg equilibrium proportions. In general, 50 per cent of loci were not in Hardy-Weinberg equilibrium in the duck population. Similarly, in a study conducted by Wu *et al*. (2008), they reported that all the six Chinese duck populations were not in Hardy-Weinberg equilibrium. Among the Indian native ducks, Kumar *et al*. (2011) noticed that the Moti duck population was not in Hardy-Weinberg equilibrium while Indian Runner was in Hardy-Weinberg equilibrium for some markers (CAUD05, CAUD01, CAUD16 and CAUD35). The Hardy-Weinberg equilibrium was used in testing whether the genotypes were maintained in balance or deviated from balance. In this study, most of the loci were not in Hardy-Weinberg equilibria. This result showed that the population structure of this duck population have become unstable. Selection, non-random mating and inbreeding could be the main reasons which caused the disequilibria. In addition, other reasons such as the excursion caused by mutation, genetic drift, etc. could have caused the disequilibria, in the duck populations studied.

The current study revealed that the markers used were highly polymorphic with better heterozygosity and will be used to explore genetic variation in the population which could be exploited for their improvement. The high polymorphism of the markers indicates that they can be used effectively for genetic diversity and phylogenetic analyses of ducks. The high genetic diversity of the duck population could be exploited for improving productivity.

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