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Microsatellites analysis in Tripureswari duck of Tripura

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Abstract

The Tripureswari duck, a native duck breed of Tripura, has been recognized for its well adaptability and resilience in climatic condition of Tripura. Exploring genetic variability is essential for genetic improvement and preservation of indigenous germplasm. Hence, it was aimed to estimate microsatellite based genetic diversity in native duck of Tripura. Genomic DNA samples from randomly 36 Tripureswari ducks were analysed at 12 duck-specific microsatellite loci. Separation of microsatellite alleles and determination of sizes of allele made through 3.4% MetaPhore™ agarose gel electrophoresis and Image Lab 6 software of Geldoc™ EZ system, respectively. POPGENE version 1.31 was used for analysis of allelic data. Microsatellite analysis documented total 46 alleles varied from two to seven across 12 loci with an average of 3.83 ± 0.46 . The effective number of alleles ranged from 1.38 (SMO010) to 5.31 (APL080) and was less than the observed number of alleles at each locus. The significant results of Chi-square and G-square statistics revealed that all the microsatellite loci maintained Hardy-Weinberg disequilibrium in the population. The average heterozygosity and PIC estimates were 0.60 ± 0.04 and 0.54 ± 0.05 , respectively, where locus SMO010 revealed their lowest estimates and APL080 revealed the highest estimates. Moderate to high level of polymorphism observed at all the microsatellite loci showed that these markers might be helpful to explore genetic variability and exploit optimum genetic potentiality of the Tripureswari duck of Tripura.

Keywords: Genetic diversity, heterozygosity, microsatellite, polymorphism, Tripureswari duck

Introduction

Ducks are considered as viable livelihood option for farmers community and the second most important poultry species just after chicken in rural India for its high resistance ability to disease, more production year length, easy to train, large sized egg, early morning egg laying character and natural foraging inclination (Padhi and Giri 2024) ^[15]. In addition, people in North-East India favour native duck meat and eggs over chicken. In 2020, India ranked fifth amongst the world with the duck population of 33.51 million and the states of Assam, West Bengal, Andhra Pradesh, Orissa, Bihar, Tamil Nadu, and Kerala were home to majority of duck population of the country (Debnath *et al.*, 2023; Padhi and Giri 2024) ^[7, 15]. As per 20th Livestock census (2019) ^[1], about 97% of the total duck population of the country is reared under backyard poultry and Assam, Manipur and Tripura are the leading states in duck population in the North-Eastern region of India. Tripura ranks in 3rd position amongst North-Eastern states with a population of 8.5 lacs duck (Debnath *et al.*, 2023) ^[7]. In India, the production of egg in different desi ducks under backyard and commercial farms vary highly indicating huge scope of duck population improvement in the country (Padhi and Giri 2024) ^[15]. Native ducks are raised by farmers using a traditional free-range system, and they produce a significant amount of meat and eggs with relatively little outside assistance (Phookan *et al.*, 2018) ^[18]. In addition of native ducks, other duck genetic groups, such as Pati Hans, Khaki Campbell, Vigova Super M, Nageswari, etc. are commonly seen in Tripura (Debnath, 2022) ^[8]. Most of the Indian duck populations are of indigenous origin as reported by Padhi (2014). Native duck population of Tripura constitutes 81% of all the ducks in the state and raised by majority of local farmers of Tripura (Das and Rahman 2019) ^[6]. Indigenous duck of Tripura has been recognized as the Tripureswari duck breed of India (Kumar and Dwivedi, 2025) ^[12]. Research on native ducks is still in its infancy, despite the species' significant population and ability to improve the socioeconomic status of small and

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marginal farmers. In addition, duck populations have also been sharply dropping over time, falling from 27.6 million in 2007 to 23.5 million in 2012 (Kaushik *et al.* 2023) [11]. Consequently, it is imperative to increase duck production by better breeding, nutrition, illness control, and other management techniques. Information about genetic variability of Tripureswari ducks is important to frame an effective improvement and conservation strategies. Microsatellite profiling is the most suitable among other different approaches for genetic variability analysis. Microsatellites are the simple sequence repeats (SSR) or short tandem repeats of 1-6 bases, which are highly variable, ubiquitous, polymorphic, repeatable, evenly dispersed across the genome (Alyethodi and Kumar, 2010; Huang *et al.*, 2005) [3, 9] and inherited co-dominantly (Susanti *et al.*, 2021) [20]. Therefore, the present studies were undertaken on estimating genetic diversity of the local duck population of Tripura using microsatellite markers.

Materials and Methods

Experimental birds and sampling procedures

The Tripureswari ducks are mostly found in West Tripura, South Tripura, Gomati, Sepahijala, Kowai and Dhalai district of Tripura and traditionally raised by small and marginal farmers, were utilized for this present study. Approximately, 0.5 ml venous blood samples were randomly collected from thirty-six numbers of the Tripureswari ducks distributed in different areas of the breeding tract and genomic DNA was extracted from each blood sample by Phenol-Chloroform-Isoamyl alcohol method (Kagami *et al.*, 1990) [10]. Quality of the DNA was assessed on 0.8% horizontal submarine agarose gel electrophoresis, followed by its purity checking and quantity determination by Eppendorf Bio-spectrophotometer. DNA samples with intact bands and OD (260/280 nm) between 1.7 and 1.9 were used in subsequent experiments. PCR-ready DNA samples were prepared in a concentration of 50 ng/μl.

Scanning birds for microsatellite genotypes

The present investigation employed a panel of twelve duck-

specific microsatellite markers that were described by Magpantay *et al.* (2019) [13] and primers for these markers were synthesised from GCC Biotech, Joychandipur, West Bengal (India). PCR conditions were optimized for each of the primer pairs as per Magpantay *et al.* (2019) [13]. Table 1 shows the primer sequences and optimal annealing temperatures (T_a). PCR reaction mixture was prepared for each primer pair in 0.2 ml nuclease-free maxiamp flat cap PCR tubes (Tarsons Products Pvt. Ltd., India) in a volume of 25 μl into nuclease-free water containing 50 ng of template DNA, 2.5 μl 10X PCR buffer, 2.5 μl $MgCl_2$, 0.5 μl 10 mM dNTPs, 10 pM of each primer, and 1U Taq DNA polymerase. The PCR amplification was performed in a programmable thermal cycler (Eppendorf nexus gradient master cycler). The PCR program included initial denaturation at 94 °C for 5 min, thereafter 30 cycles of (i) denaturation at 94 °C for 1 min, (ii) optimised annealing temperature (°C) for each primer pair for 1 min, and (iii) extension at 72 °C for 1 min, followed by final extension at 72 °C for 5 min and 4 °C forever. The amplified PCR products were run on 2% horizontal submarine agarose gel electrophoresis for 60 min at 2-5 volts/cm, and subsequently, checked for the DNA bands developed onto the gel by viewing under UV light in GelDoc™ EZ System (Biorad Laboratories Inc., USA). The molecular sizes of the amplified products were determined by loading around 10 μl of the PCR product and 3 μl of a 50 bp DNA ladder onto an agarose gel so as to identify the appropriate bands. The verified amplified products were run onto horizontal 3.4% Metaphore™ agarose gel electrophoresis (MAGE) along with 50 bp DNA ladder in parallel @ 6-8 V/cm for 2 h 30 min. Thereafter, the gel was visualized in the GelDoc system and the allelic patterns of the microsatellites were documented for further genotyping. The molecular sizes (bp) of the alleles were estimated by comparing with the standard ladder DNA marker under Image Lab 6 software of the GelDoc System. At each microsatellite locus, the number of detected alleles with a likely genotype was noted for each sample.

Table 1: A panel of duck-specific microsatellite markers along with their primer sequences and optimized annealing temperature (T_a).

MS Loci	Accession Number	Forward (F) and reverse (R) primer sequences	Optimised T_a (°C)	Reported T_a (°C) (Magpantay <i>et al.</i> , 2019)
APL002	AY498540	F 5'-GATTCAACCTTAGCTATCAGTCTCC-3' R 5'-CGCTCTTGGCAAATGTC3'	60	62
APL011	AY498541	F 5'-AACTACAGGGCACCTTATTTCC-3' R 5'-TTGCATCAGGGTCTGTATTTTC-3'	60	62
APL012	AY498542	F 5'-AGTTGACCCTAATGTCAGCATC-3' R 5'-AAGAGACACTGAGAAGTGCTATTG-3'	60	62
APL077	AJ272577	F 5'-TCACTTGCTCTTCACTTTCTTT-3' R 5'-GTATGACAGCAGACACGGTAA-3'	55	56
APL078	AJ272578	F 5'-AACCAAGACAGAATAATCCTTA-3' R 5'-GAACACAACCTGCTTTGCTA-3'	55	52
APL079	AJ272579	F 5'-ACATCTTTGGCATTGAA-3' R 5'-CATCCACTAGAACACAGACATT-3'	55	50
APL080	AJ272580	F 5'-GGATGTTGCCCCACATATTT-3' R 5'-TTGCCTTGTTTATGAGCCATTA-3'	58	58
APL081	AJ272581	F 5'-ATTAGAGCAGGAGTTAGGAGAC-3' R 5'-GCAAGAAGTGGCTTTTTTC-3'	58	58
APL083	AJ272583	F 5'-GAATAAAGTAACGGGCTTCTCT-3' R 5'-CTGCTTGGTTTGGAAAGT-3'	55	58
SMO007	AJ427847	F 5'-TTTTACCCAGTTCACCTCAGCC-3' R 5'-GATTCAAATTTGCCGAGGATTA-3'	55	60
SMO010	AJ427850	F 5'-TCCTAGCGACAGCAATTCTAATG-3' R 5'-CATTGTTCAATTGTTCTTCTCA-3'	55	54
SMO013	AJ427853	F 5'-ACCATCTTCCTTTCCTCCCAACC-3'	58	60

R 5'-GGGCTTGAGGCATACACTCCCTA-3'

Statistical analysis

All DNA samples' genotypic data at twelve microsatellite loci were compiled and analysed using POPGENE® 1.31 software, following Yeh *et al.* (1999) [22] instructions for population genetic parameters such as observed and effective numbers of alleles, allele frequencies, Shannon's information index, goodness of fit (Chi-square), and likelihood ratio (G-square) statistics.

Assuming that the population was under Hardy-Weinberg equilibrium, average heterozygosity at each microsatellite locus was computed as per Nei (1978) [14] using the following formula:

$$H_i = \frac{2N}{2N-1} \left(1 - \sum_{j=1}^k p_j^2 \right)$$

Where,

p_j is the frequency of the j^{th} allele at i^{th} locus with k number of alleles in a population and N is the number of individuals. Polymorphic information content (PIC) at each microsatellite locus was calculated as per Botstein *et al.* (1980) [4] with the help of following formula:

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

Where,

p_i and p_j are the frequencies of i^{th} and j^{th} alleles, respectively at a locus with k number of alleles in the population.

Results and Discussion

The present investigation documented a profile of microsatellite alleles (Figure 1) and resolved overall 46 distinct alleles at 12 microsatellite loci in Tripureswari duck of Tripura and the results are presented in Table 2. The observed number of alleles ranged from 2 (SMO010 locus) to 7 (APL080 locus) at different loci with an average number of 3.83 ± 0.46 per locus. Molecular size of the alleles ranged from 105 bp (SMO010) to 263 bp (APL079). The overall allelic frequencies ranged from 0.01 (APL079) to 0.83 (SMO010). Out of total 46 (73.91%) alleles, 34 had a level of more than 10% frequencies (Table 2). The corresponding means ($\pm$ S.E) of the observed (N_a) and effective (N_e) number of alleles and Shannon's information index (I) were 3.83 ± 0.46 , 2.89 ± 0.34 and 1.09 ± 0.11 . The effective number of alleles at each locus was less than the observed number of alleles and ranged from 1.38 (SMO010) to 5.31 (APL080) (Table 3).

Polymorphic patterns were observed in all the loci (100%). The highest polymorphism was recorded at APL080 locus with seven alleles and their molecular size ranged from 107 to 137 bp. Polymorphic information content (PIC) values indicated that all the loci were moderate to highly polymorphic. The PIC values varied from 0.24 (SMO010) to 0.79 (APL080) with a mean of 0.54 ± 0.05 . APL002, APL011, APL012, APL079, APL080, SMO07 and SMO013 had the PIC value more than 0.50 (Table 3). Average heterozygosity was approximated as 0.60 ± 0.04 with a range from 0.28 (SMO010) to 0.81 (APL080). The results of Chi-square and G-square statistics were found significant at all the microsatellite loci and revealed that all the loci were maintaining Hardy-Weinberg disequilibrium in the duck population (Table 3).

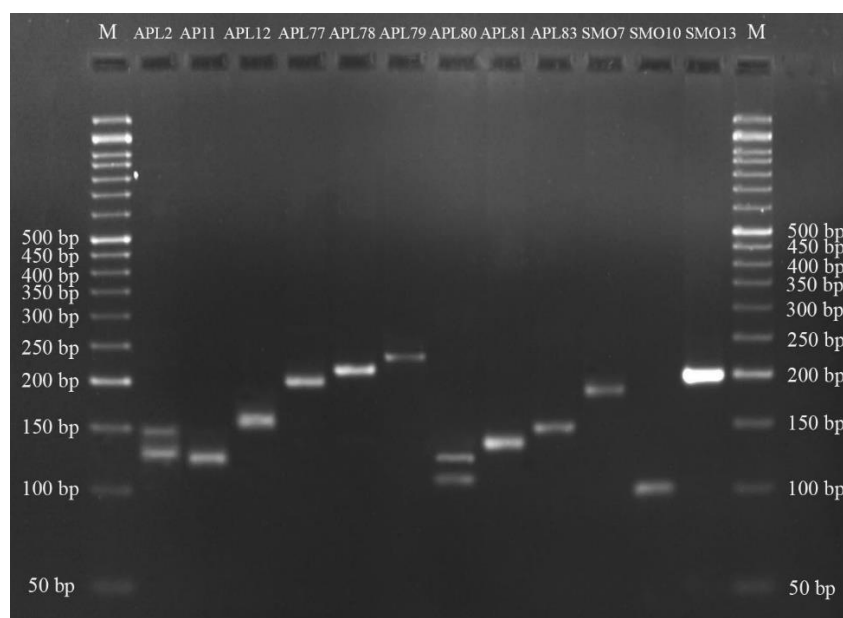


Fig 1: MAGE photograph of alleles resolved at 12 microsatellite loci in representative DNA samples of indigenous ducks of Tripura.

Table 2: Microsatellite allelic profile of Tripureswari duck of Tripura.

Microsatellite Loci	No. of alleles	Allele sizes (bp) and respective allele frequencies in the parentheses
APL002	6	116 (0.11), 123 (0.08), 128 (0.21), 133 (0.20), 141 (0.26), 147 (0.07)
APL011	4	122 (0.40), 26 (0.39), 133 (0.14), 139 (0.07)
APL012	5	142 (0.08), 160 (0.43), 165 (0.32), 176 (0.7), 193 (0.10)
APL077	3	203 (0.60), 206 (0.30), 213 (0.10)
APL078	3	214 (0.28), 28 (0.67), 248 (0.06)
APL079	5	205 (0.01), 227 (0.47), 232 (0.35), 243 (0.03), 263 (0.14)
APL080	7	107 (0.17), 112 (0.14), 115 (0.30), 117 (0.17), 128 (0.08), 133 (0.11), 137 (0.03)
APL081	3	139 (0.33), 141 (0.56), 144 (0.11)
APL083	2	149 (0.33), 153 (0.67)
SMO007	3	178 (0.28), 184 (0.35), 194 (0.38)
SMO010	2	105 (0.83), 111 (0.17)
SMO013	3	201 (0.33), 205 (0.36), 209 (0.30)

Table 3: The measures of heterozygosity and diversity statistics, Chi-square (χ^2) and likelihood ratio (G-square) test for Hardy-Weinberg equilibrium at various duck specific microsatellite loci in Tripureswari duck of Tripura.

MS Loci	df	Nei'H (%)	PIC	N _a	N _e	I	Chi-square	G-square
APL002	15	0.79	0.76	6	4.86	1.67	60.09***	57.96***
APL011	6	0.66	0.60	4	2.96	1.19	15.88*	16.44*
APL012	10	0.69	0.64	5	3.24	1.35	33.21***	35.97***
APL077	3	0.54	0.47	3	2.18	0.90	66.53***	58.88***
APL078	3	0.48	0.40	3	1.91	0.79	37.75***	43.82***
APL079	10	0.64	0.57	5	2.75	1.15	33.65***	44.11***
APL080	21	0.81	0.79	7	5.31	1.78	80.47***	75.91***
APL081	3	0.57	0.49	3	2.31	0.94	78.03***	69.55***
APL083	1	0.44	0.35	2	1.80	0.64	37.30***	46.85***
SMO007	3	0.66	0.59	3	2.96	1.09	69.74***	72.05***
SMO010	1	0.28	0.24	2	1.38	0.45	38.83***	33.49***
SMO013	3	0.67	0.59	3	2.99	1.10	75.15***	80.99***
Mean±SE		0.60±0.04	0.54±0.05	3.83±0.46	2.89±0.34	1.09±0.11		

MS: microsatellite, df: degree of freedom, H: heterozygosity, PIC: polymorphic information content, N_a: observed number of alleles, N_e: effective number of alleles, I: Shannon's information index; * $p \leq 0.05$, *** $p \leq 0.001$

The present microsatellite allelic profile was developed exploring APL002, APL011, APL012, APL077, APL078, APL079, APL080, APL081, APL083, SMO07, SMO010 and SMO013 loci in an indigenous duck population native to Tripura and such type of reports were not available in Indian native ducks to compare or contrast with the present findings. However, outside India, Philippine mallard duck was investigated (Magpantay *et al.*, 2019) [13] exploring 22 microsatellite loci including the present panel of markers using Fragment Analyzer. SMO013 locus had quite same number of observed alleles while other loci contained variable sizes with number of observed alleles. Likewise, Shaoxing duck population was also reported (Chepiha *et al.*, 2018) [5] to contain quite similar number of observed alleles at SMO013 locus and dissimilar sizes with observed allelic numbers at APL077, APL078, APL079, SMO07 and SMO010 loci. Agatep *et al.* (2016) [2] reported quite comparable number of observed alleles at APL011, APL083 and SMO07 but variable sizes with number of observed alleles at APL02, APL012, APL077, APL078, APL079 and APL081 in three genetic groups of Philippine Mallard, Khaki Campbell and Pekin ducks. The inscribed differences in allele sizes and numbers in different studies might be due to the attributed variation in different duck population or breeds along with adopted methodologies for resolution and molecular size estimation.

In context to the distribution of allelic frequency, Debnath *et al.* (2023) [7] and Susanti *et al.* (2021) [20] reported that the alleles with frequency about 10% levels might be more suitable to tag as specific population. The present study revealed that alleles with more than 10% level of frequency

could be used as marker of choice for genetic characterization of indigenous duck of Tripura. Debnath *et al.* (2023) [7] reported that heterozygosity and polymorphic information content could be the indicative of genetic variation measures in each population. No reports were available on heterozygosity and polymorphic information content (PIC) in Indian indigenous ducks to compare or contrast with the present investigation. However, lower observed heterozygosity was reported by Magpantay *et al.* (2019) [13] in Philippine mallard duck at APL02, APL011, APL012, APL077, APL078, APL079, APL081, APL083 and SMO07 loci. In contrast to the present findings, Agatep *et al.* (2016) [2] reported quite comparable heterozygosity estimates at APL02 (Khaki Campbell duck), higher estimates at APL02 (Pekin), APL078 (Philippine Mallard) and APL083 (Khaki Campbell and Pekin duck) and lower estimates at APL02, APL011, APL012, APL077, APL083 and SMO07 (Philippine Mallard duck). The variation in the findings might be due to the attributed differences in the different duck population and adopted methodology. Debnath *et al.* (2023) [7] and Veeramani *et al.* (2015) [21] reported that those microsatellite markers with heterozygosity estimates ranged from 0.3 to 0.8 could be suggestive for usefulness of genetic variation and diversity estimation. In the present investigation, the heterozygosity estimates were found within the specified range for all the studied microsatellite markers. Hence, all the markers used in the present study might be quite suitable for assessment of genetic variation in duck population.

Polymorphic information content (PIC) symbolises the degree of informativeness of a marker (Parmar *et al.* 2007)

^[17] and the *PIC* value is a good index for genetic diversity evaluation in the population (Rahim *et al.* 2017) ^[19]. According to Susanti *et al.* (2021) ^[20] and Debnath *et al.* (2023) ^[7], a *PIC* score more than 0.50 specifies high genetic variation, lesser than 0.25 specifies low diversity and between 0.25 and 0.5 indicates moderate degree of polymorphism at a particular microsatellite locus. In present study, seven loci (APL02, APL011, APL012, APL079, APL080, SMO07 and SMO013) showed higher degree of polymorphism, four loci revealed moderate and one marker revealed lower polymorphism. No information was available on the *PIC* values of Indian indigenous duck at the present panel of microsatellites to compare or contrast with the present findings. However, Magpantay *et al.* (2019) ^[13] reported quite comparable *PIC* values at APL011, APL012, APL078 and SMO07, higher *PIC* values at APL077 and lower value at APL02, APL079, APL080, APL081, APL083, SMO07 and SMO013 in Philippine mallard duck. In contrast to the present findings, Chepiha *et al.* (2018) ^[5] reported the SMO010 as monomorphic locus, higher *PIC* values at APL077 and lower values at APL078, APL079, SMO07 and SMO013 loci in Shaoxing duck population. The differences in polymorphic information content of various microsatellite loci might be due to the differences in genetic architecture of the population analysed or probably due to the differences in adopted methodology. In the present study, there was prevalence of heterozygosity in the studied population as the average effective numbers of alleles (2.89 ± 0.34) were lesser than an average observed number of alleles (3.83 ± 0.46) which was in agreement with the earlier report in Tripura-native duck for different microsatellites (Debnath *et al.* 2023) ^[7]. No other reports were available to compare or contrast with the present findings. Debnath *et al.* (2023) ^[7] reported Shannon's information index as a measure of gene diversity. In the present investigation, the estimate of the index was 1.09 ± 0.11 indicating prevalence of heterozygosity in the studied population. The present estimates of observed and effective numbers of alleles and Shannon's information index indicated the prevalence of heterozygosity in the Tripura native ducks constituting a more diverse population, which might be due to the reason that this population was not subjected to selection other than natural. The Hardy-Weinberg equilibrium test was performed to validate whether the genotypes were conserved in equilibrium or deviated from equilibrium (Debnath *et al.* 2023) ^[7]. In the present study, the results of chi-square and G-square statistics were found significant at all the studied microsatellite markers and revealed that all the loci were maintaining Hardy-Weinberg disequilibrium. This result revealed that studied population structures have become unbalanced, which could be due to probable influence of some forces like selection, non-random mating, inbreeding, mutation and genetic drift.

Conclusion

In view of the findings that the 12 studied duck specific microsatellite loci revealed polymorphism in Tripureswari duck and most of the microsatellite loci showed high *PIC* values (>0.50), it may be concluded that the usefulness of studied microsatellite as genetic markers to disentangle the genetic variability in various duck population. Further study of these microsatellites may unravel more vital information for their application in marker assisted selection programme

in duck and may also be employed as marker for genetic characterization study of other indigenous duck populations in India.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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