



Genetic analysis of four indigenous duck populations of north-east India using microsatellite markers

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Received: 10 August 2018; Accepted: 20 August 2018

Key words: Duck, Microsatellite, North-east India

The indigenous ducks (*Anas platyrhynchos*) of north eastern region of India have innate potential to produce eggs and meat with less input and are good sources of protein. There are various duck populations in this region reared by farmers under traditional systems. One of such duck populations, called 'Pati' duck, is most common in the Brahmaputra valley. Another important unique egg-type native variety of duck known as 'Nageswari' is presently confined to a few areas of the Cachar and Karimganj districts of Barak Valley of Asom. The other parts of north eastern region like Manipur and Tripura also have some indigenous duck populations which play an important role in the rural farming system. However, information on these duck populations, their physical and production characteristics are very scanty and there have been very few systematic studies of these ducks.

Among the native poultry genetic resources, the duck has witnessed a rapid decrease in the population of the north eastern region. Least attention towards these native ducks by the scientific community as well as with the introduction of various improved varieties/breeds to bridge the gap of duck production resulted in the decrease of native duck populations. All these indigenous ducks being our invaluable genetic resources need to be genetically characterized.

Microsatellites are repetitive DNA sequences motifs composed of 1 to 6 nucleotide repeats and often with a high degree of polymorphism for different numbers of repeats (Pajuelo *et al.* 2015). These are abundant in copy number and have been successfully used as a powerful marker for

genetic mapping and genome analysis (Su Y and Chen 2009) and also one of the most useful tools for population genetic studies, linkage mapping, parentage determination and QTL analysis (Hsiao *et al.* 2008). Therefore, the present study was undertaken for molecular characterization of these ducks using microsatellite markers.

A total of 200 blood samples were collected randomly from apparently healthy indigenous ducks of north-east India, including Tripura, Manipur, Nageswari ducks of Barak valley and Pati ducks of Brahmaputra valley of Assam. Blood 1 ml from each bird was aseptically collected from the wing vein in vacutainer tube containing EDTA (2.7%) as anticoagulant and samples were transported and stored at 4°C prior to isolation of DNA. Genomic DNA was isolated from 20 µl of whole blood using DNeasy blood and tissue Kit (QIAGEN).

Twenty five microsatellite loci (CAUD001, CAUD002, CAUD003, CAUD004, CAUD005, CAUD006, CAUD007, CAUD009, CAUD010, CAUD011, CAUD012, CAUD013, CAUD014, CAUD019, CAUD023, CAUD024, CAUD025, CAUD026, CAUD027, CAUD031, CAUD033, CAUD035, CAUD038, CAUD049, CAUD069) were selected from published data. The forward primer for each marker was fluorescently labeled with FAM, ROX, TAM or HEX dye. The PCR conditions were optimized for all of the 25 Microsatellite primers for the amplification in 4 duck populations. The amplification was carried out in Thermal Cycler (BIO-RAD, Model-S100) at suitable annealing temperatures of respective primers. The PCR products were checked for amplification by loading on 2% agarose gel. A 50 bp ladder was loaded alongside as molecular size marker, and checked for amplification on Gel Documentation System (Molecular Imager, Gel DOC™ XR+, BIO-RAD).

Amplicons were sized by fragment analysis on ABI automated DNA sequencer and typing of the individual bird at 25 microsatellite loci was carried out. The post PCR multiplexing was used to simultaneously genotype 3 or 4 loci depending upon the size and dye label of the PCR product. The sizing and allele calling was performed using Genotyper ver. 3.0 software (Applied Biosystems).

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The allele data thus generated was used for further statistical analysis. The statistical analysis was carried out using POPGENE 3.2 software. Intra-population genetic variation of the microsatellites was quantified using the allele frequencies, observed and effective number of alleles (Nei 1987). The heterozygosity measures were calculated using the formulae given by Levene (1949). The F statistics values F_{IS} , F_{IT} and F_{ST} were estimated (Nei 1987). Nei's unbiased measures of genetic identity and genetic distances were estimated following the method of Nei (1978).

The total number of alleles in 25 microsatellite loci of four duck populations was 77. The observed and effective numbers of alleles across all the populations are given in Table 1. The mean observed numbers of alleles (N_a) were 2.720, 2.760, 2.520 and 2.680 in Tripura, Manipur, Nageswari and Pati ducks, respectively. The effective number of alleles (N_e) was the highest in Manipur ducks (2.045) and the lowest in Nageswari ducks (1.851). The mean N_a and N_e was found to be 2.670 and 1.949 respectively in all the four duck populations over 25 loci. The mean N_e was less than the mean N_a in all the four populations. Thus, more genetic diversity was found in Manipur ducks followed by Tripura ducks, Pati ducks and Nageswari ducks, respectively. Kumar *et al.* (2009) also found the similar observations and reported that the mean N_a and N_e at 9 loci were 3.22 and 2.27 in Moti and 2.78 and 2.07 in Indian Runner ducks respectively.

In all populations, the H_o of Tripura ducks was the highest (0.601), followed by Manipur ducks (0.560), whereas Nageswari ducks was the lowest (0.477). The average H_o of all populations for all the loci was 0.546. Among all the populations, the H_e of Manipur ducks was the highest (0.443), followed by Tripura ducks (0.435), whereas Pati ducks was the lowest (0.396). The average H_o and H_e of all populations for all the loci was 0.546 and 0.420 respectively. This indicate that there is non existence of population structure and breeding is uncontrolled. High genetic diversity was also observed in Chinese ducks by Huang *et al.* (2005), Su *et al.* (2007), Wu *et al.* (2008), Xiao *et al.* (12) and in Indian ducks by Gaur *et al.* (2009).

The F_{IS} value in all four populations were negative again indicating out-breeding and no deficiency of heterozygotes.

Table 1. Mean N_a , N_e , H_o , H_e , PIC and F_{IS} for 25 microsatellite loci in four duck populations

Mean	Tripura	Manipur	Nageswari	Pati	Mean
N_a	2.720	2.760	2.520	2.680	2.670
N_e	1.983	2.045	1.851	1.903	1.949
H_o	0.601	0.560	0.477	0.546	0.546
H_e	0.435	0.443	0.405	0.396	0.420
PIC	0.382	0.407	0.333	0.340	0.366
F_{IS}	-0.315	-0.223	-0.090	-0.274	-0.225

N_a , Observed number of alleles; N_e , Effective number of alleles; H_o , Observed heterozygosity; H_e , Expected heterozygosity; PIC, Polymorphism information content; F_{IS} , Heterozygote deficiency.

Nei's genetic identity and genetic distances among populations are presented in Table 2. Nei's genetic distance (Nei 1978) was utilized for construction of phylogenetic tree by UPGMA for finding out the relationship among four duck populations as shown in Fig. 1. The genetic distance between Tripura ducks and Manipur ducks was the highest (0.2495) followed by Manipur and Nageswari ducks (0.2090) and, Nageswari and Pati ducks (0.1790) in the

Table 2. Nei's unbiased measures of genetic identity and genetic distances

Distance	Identity			
	Tripura ducks	Manipur ducks	Nageswari ducks	Pati ducks
Tripura ducks	–	0.7792	0.8687	0.9000
Manipur ducks	0.2495	–	0.8114	0.8605
Nageswari ducks	0.1408	0.2090	–	0.8361
Pati ducks	0.1054	0.1502	0.1790	–

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

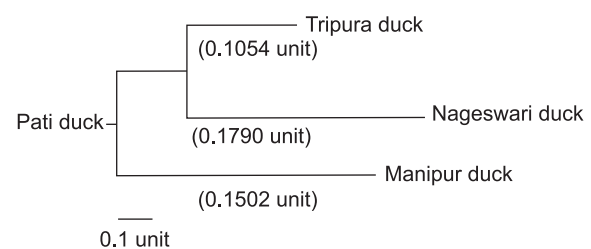


Fig. 1. Phylogenetic tree.

present study. The highest genetic distance between Tripura and Manipur ducks may be due to physical barriers between two states. Gaur *et al.* (2010) revealed that the maximum distance was between West Bengal and Khaki Campbell ducks (0.78) while the minimum genetic distance was found between Tamil Nadu and Orissa ducks (0.07). Higher genetic distance (0.64) was observed between Assam and Uttarakhand ducks and lesser genetic distance (0.06) between Assam and West Bengal ducks by Mukesh *et al.* (2011). The result of Nei's genetic distance between six duck populations revealed the longest genetic distance of Muscovy and White Pekin ducks with other Indian duck varieties (Veeramani *et al.* 2014). They also observed higher genetic distance (0.9270) between these two exotic duck varieties.

The resultant phylogenetic tree, showing the branch length 0.1 proportional to distance from origin. Pati ducks and Tripura ducks are the closest relatives followed by Pati ducks and Manipur ducks, and Pati ducks and Nageswari ducks.

SUMMARY

The genetic diversity and phylogenetic relationship of four duck populations, viz. Pati, Nageswari, Manipur and Tripura ducks of north eastern region of India were

investigated by employing genetic polymorphisms of 25 microsatellites. The mean observed and effective number of alleles were found to be 2.670 and 1.949 respectively in all the four duck populations over 25 loci. The mean expected heterozygosity (H_e) was lower than the mean observed heterozygosity (H_o). Among all the populations, the H_e of Manipur ducks was the highest (0.443), followed by Tripura ducks (0.435), whereas Pati ducks was the lowest (0.396). The average H_e of all populations for all the loci was 0.420. The average PIC of all sites and populations was 0.366. However, CAUD007 in Manipur, Nageswari and Pati ducks, CAUD009 in Pati ducks, CAUD069 in Tripura ducks, and CAUD012 in all the four duck populations were found to be monomorphic. The test of Hardy-Weinberg Equilibrium showed that most of the loci in all the four populations were in Hardy-Weinberg disequilibrium. The F_{ST} value ranged between 0.000(CAUD012) and 0.512 (CAUD009) for each locus individually. The mean F_{ST} was 0.120. The genetic distance between Tripura ducks and Manipur ducks was the longest (0.2495) followed by Manipur and Nageswari ducks (0.2090) and, Nageswari and Pati ducks (0.1790).

ACKNOWLEDGEMENT

The authors are thankful to ICAR for providing funding to conduct this study. Help received from the respective State Animal Husbandry Departments during sample collection is also duly acknowledged.

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