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Genetic Characterization of Indigenous Duck of North-East Region using Microsatellite Markers

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ABSTRACT

Background: Duck farming plays a significant role, next to chicken in the socio-economic uplift men of the rural farmers of North-East India. Pati duck is the most common duck breed in the Brahmaputra valley of Assam and the other common variety reared in North-East India is Chara-Chambeli, however it originatein Kerala. Genetic characterization plays a significant role for formulation of breeding strategies for improvement of any breed. Microsatellites are codominant in nature and are highly polymorphic. High level of allelic variation, co-dominant mode of inheritance and potential for automated analysis make them an excellent tool for genotyping, mapping and genetic characterization. Pati duck is the most common duck breed in the Brahmaputra valley of Assam and the other common variety reared in North-East India is Chara-Chambeli however its origin in Kerela. Therefore, the present investigation was carried out to characterize these two duck breeds using microsatellite markers to evaluate the genetic diversity in these two duck population.

Methods: For the present study, Blood sample were collected from 50 Patiducks and 50 Chara-Chambeli ducks from different parts of North-East India. Assessment of genetic characterization of duck breeds of north-east region were carried out using 16 microsatellite markers and population genetics analysis were done by POPGENE software.

Result: In the present study, all the studied loci were highly polymorphic. Analysis generated a total of 41 microsatellite alleles. The number of observed alleles (N_a) with an overall mean of 1.93±0.258. However, the effective number of alleles (N_e) with a mean of 1.6933±0.2712. The Shannon's information index was found to a mean value of 0.5685±0.1693. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.2889±0.2477 and 0.5289±0.0853, respectively. The chi-square (χ^2) test for Hardy-Weinberg equilibrium revealed that all the loci are in within Hardy-Weinberg equilibrium.

Key words: Duck, Heterozygosity, Microsatellite, Polymorphism.

INTRODUCTION

North-East India is rich repository of watershed and potential place for duck farming. Duck farming is a major component in integrated farming system and plays a significant role in women empowerment and uplift ment of socio-economic status of the farming community of North-East India. Due to hot - humid climatic condition of the region along with extensive availability of resources like ponds, river, marshy wet lands etc. provides suitable natural habitats for duck rearing in rural areas of the states. Duck eggs and meat are widely accepted by the different sections of the society and provide nutritional security.

To exploit the genetic potential of native duck breeds genetic characterization is a pre-requisite. DNA based markers, particularly microsatellites are the marker of choice for such studies and alsowell recommended by FAO (FAO, 2007). The hyper variability, co-dominant nature, uniform and pervasive nature in genome, easiness of detection and reliability and possibility of analysis of a number of microsatellites simultaneously make it more popular than minisatellites which are otherwise difficult to detect and not distributed uniformly. Reports on the genetic characterization of ducks using microsatellite markers are slowly accumulating across the globe in recent years but are scanty in the Indian native duck breeds (Maak *et al.,* 2000; Huang *et al.,* 2005; Wu *et al.,* 2008). Pati duck is native breed of Assam, squat in posture with solid brown plumage in ducks and dark brown plumage and greyish black head in drakes. In the neck white ring may or may not be seen in both sexes. The beak, shank and feet are predominantly yellow in colour. The age at sexual maturity is between 220-235 days with a mean body weight of 1.56kg. The annual egg production ranges from 75 to 90 with the average weight of 60.5g (Kalita *et al.*, 2009; Mahanta *et al.*, 1993). Another duck variety Chara- Chambeli originated in Kerela and is commonly reared in North-East India. Reports on the genetic characterization of ducks using microsatellite markers are slowly accumulating across the globe in recent years but are scanty in the Indian native duck breeds (Maak *et al.,* 2000; Huang *et al.,* 2005; Wu *et al.,* 2008). Therefore, the

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present investigation was carried out to characterize these two duck breeds using microsatellite markers to evaluate the genetic diversity in these two duck population.

MATERIALS AND METHODS

A total of 100 (50 nos. of Patiducks and 50 nos. of Chara-Chambeli) ducks from different parts of North –East India region were selected for the present study. Approximately 1 ml of venous blood was collected aseptically from the wing vein of the birds in vaccutainer tube containing EDTA (2.7%). The samples were properly labelled and transported immediately in ice box to the Department of ILFC, C.V.Sc, A.A.U, Khanapara and later stored in deep freezer at -20°C temperature till the isolation of genomic DNA. Genomic DNA was isolated from 100il of whole blood. The concentration of genomic DNA was estimated by Picodrop spectrophotometer (Model no. PICOPET01). The concentration of the genomic DNA was estimated at UV-VIS Spectrophotometer at 260 nm Optical Density (OD). The ratio of OD values at 260 nm and 280 nm was used as a criterion to check the purity of the extracted genomic DNA.The present study was conducted using sixteen duck microsatellite specific primers for the PCR amplification. The gel preparation was done using 3% Metaphor agarose was slowly added to the chilled 1x TBE buffer with continuous swirling. It was soaked in that cold buffer for about 10-15 minutes before heating in the microwave (to prevent it from foaming and not to over boil the gel). Just prior to pouring the gel, Ethidium bromide (0.3 µg/ml) was mixed to the dissolved agarose. Once the molten gel was solidified after being poured into a cast, it was kept at 4°C for 20 minutes before use to obtain a better resolution and for easier handling of the gel (Anonymous 2004).

After adding 3 µl 6x loading dye to the 8 µl PCR product, 10 µl of it was loaded on the Metaphor agarose gel submerged in chilled 1x TBE buffer and electrophoresed for about 1-2 hours at 6 Volt/cm using horizontal electrophoresis system. After electrophoresis, the finely resolved products were visualized under UV transilluminator. The data were analysed for probable genotype of each sample at each microsatellite loci and population genetics (Shanan index, effective nos. of alleles, observed and expected heterozygosity, Hardy-Weinberg equilibrium, numbers of alleles per microsatellite marker, allele frequency) analysis were done by POPGENE software v 1.32 (Table 1).

RESULTS AND DISCUSSION

Analysis generated a total of 41 microsatellite alleles across all studied loci by using 16 microsattelites. The study utilized the same set of primers as reported by Alyethodi *et al.* (2010). Highest numbers of alleles 5 (A/B/C/D/E) were recorded at locus CAUD 04 and CAUD 05. The number of alleles at various polymorphic microsatellite loci ranged from 1 to 5 and frequency ranges from 0.04 to 1. The polymorphic patterns were observed in 12 out of 17 microsatellite loci studied in the present study. The average number of alleles per locus is 2.41. Similar studies was reported by Sankhyan, (2007) on Indian Runner and Moti native ducks, where Moti native duck had higher number of alleles (5) than Indian Runner, whereas Huang *et al.* (2005) recorded the highest numbers of alleles (3) at locus CAUD 011. The number of alleles at various polymorphic microsatellite loci ranged from 1 to 3. They used automated DNA sequencer/genotyper and reported higher average allele number with same primer

Table 1: The microsatellite primers and their sequences.

Primer name	Primer sequence 5' to 3'
Locus	**Primer sequence**
CAUD001	GCA GAA AGT GTA TTA AGG AAG
	ACA GCT TCA GCA GAC TTA GA
CAUD002	CTT CGG TGC CTG TCT TAG C
	AGC TGC CTG GAG AAG GTC T
CAUD003	CCT GGC ATT CTG CTA AGT TC
	TGG GAT TCA GTG AGA AGC CT
CAUD004	TCC ACT TGG TAG ACC TTG AG
	TGG GTT TGA ACA GTG TAG CC
CAUD005	CTG GGT TTG GTG GAG CAT AA
	TAC TGG CTG CTT CAT TGC TG
CAUD006	ATG GTT CTC TGT AGG CAA TC
	TTC TGC TTG GGC TCT TGG AG
CAUD007	ACT TCT CTT GTA GGC ATG TCA
	CAC CTG TTG CTC CTG CTG T
CAUD009	AGG GAT TTT GGA GCG GAG C
	ATG TCT GAG TCC TCG GAG C
CAUD010	GGA TGT GTT TTT CAT TAT TGA T
	AGA GGC ATA AAT ACT CAG TG
CAUD013	ACA ATA GAT TCC AGA TGC TGA A
	TGT GCG GCG TTT TCC TCT G
CAUD016	TTT AGG TAA AAC TGT GAA TCA A
	ATC AAA GCA GGG AGC TAA G
CAUD017	AGA AAT ACA CTT ACA GCA CT
	TGT CAT AAA ATG GTT AAT TGC
CAUD018	TTA GAC AAA TGA GGA AAT AGT A
	GTC CAA ACT AAA TGC AGG C
CAUD020	TAG GGT CAA TAG TAA GAA ACA
	TAA CTG TGT GAT AAG GGA GA
CAUD023	CAC ATT AAC TAC ATT TCG GTC T
	CAG CCA AAG AGT TCA ACA GG
CAUD026	ACG TCA CAT CAC CCC ACA G
	CAG CCA AAG AGT TCA ACA GG

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pop ID   1       2
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1        ****    0.7429
2        0.2972  ****
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Fig 1: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

pairs which may be attributed to the sensitivity of instrument/ techniques employed in their studies, besides the genetic differences. In the present study, the frequencies of most of alleles were high and 36 out of 43 (83. 72%) polymorphic alleles had a frequency of more than 10 per cent. Another study indicating that Moti native duck can be tagged as a distinct population from other native Indian ducks based on the microsatellites studies (Rajkumar *et al,* 2008). Polymorphic patterns were observed in 14 out of 16 microsatellite loci studied. The average number of alleles per locus (2) was in accordance with the report of Maak *et al.,* 2000. They estimated the same as 2.9 and 3.5 in Muscovy and Pekin ducks, respectively. Sankhyan (2007) reported an average number of allele as 3.0 in Indian Runner duck. All the loci investigated in the present study were polymorphic in nature. The number of observed alleles (N_a) with an over all mean of 1.93± 0.258. However, the effective number of alleles (N_e) with a mean of 1.6933± 0.2712.Shannon's information index with a mean value of 0.5685±0.1693. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.2889±0.2477 and 0.5289 ± 0.0853 respectively. Similar findings were reported by Sankhyan, 2007 with an average heterozygosity of 0.56±0.02 in Indian Runner native duck. Rajkumar *et al.* (2008) also reported an average heterozygosity at polymorphic microsatellite loci as 0.52±0.02 in Moti ducks. Whereas, Huang *et al.* (2005) recorded average heterozygosity of 0.47±0.01 for the same set of primers. The chi-square (χ^2) test for Hardy-Weinberg equilibrium revealed that all the loci is in within Hardy-Weinberg equilibrium. The Nei's genetic identity and genetic distance has been depicted in Fig 1.

CONCLUSION

The present study may help to explore the genetic diversity of native duck breed namely Pati duck in Assam and it will work as baseline information for these unique duck breed.

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Conflict of interest: There is no conflict of interest.

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