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Characterization of Microsatellite Markers in Crossbred Ducks

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ABSTRACT

Background: Chicken represents a valuable genetic resource and protein source, it remains a potential threat to human health as they serve as a reservoir for diseases and food borne pathogens. Ducks are promising species suitable for the diversification and will be complimentary with an adaption potential from small to commercial farming. Majority of ducks in the country are desi type with a meagre proportion of improved duck strains. Genetic characterization by developing duck specific microsatellite markers and designing suitable improvement program are required to be undertaken at the earliest. The microsatellite markers are extensively used for assessing genetic structure, diversity and relationships. Information about genetic diversity of indigenous duck breeds is important to design effective improvement and conservation strategies. Therefore, the current studies aim at genetic characterization and evaluation of growth, production and reproduction traits of the crosses of local duck population besides undertaking a suitable duck improvement program.

Methods: Spectrophotometric evaluation of DNA extraction, Electrophoretic evaluation of DNA extraction, microsatellite primers- Presently, a panel of 16 informative duck microsatellite markers were identified from available database and used for the studies. Polymerase chain reaction (PCR), documentation of PCR products by agarose gel electrophoresis, metaphor agarose gel electrophoresis (MAGE)of microsatellites alleles, Determination of molecular size of microsatellite alleles and genotypes, Statistical analysis of population genetics data, Data on genotype of all experimental birds at ten microsatellites were compiled and analyzed using POPGENE® 3.1 software for their population genetics parameters.

Result: The study was conducted at College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India. In this study, the genomic DNA was isolated and characterized microsatellite loci in crossbred duck (*Pati x White Pekin*). The characteristics of the 16 microsatellite loci with allele frequencies were summarized in Table 1 and Table 2 respectively. A total of 16 microsatellites were used to detect polymorphisms in 50 cross bred ducks. A total of 34 nos of alleles were observed and all loci *were* polymorphic.

Key words: Cross bred (Pati duck x White Pekin), Microsatellite markers, PIC, Polymorphism,

INTRODUCTION

Some important traits are quantitative traits which is controlled by polygene. The molecular genetics mapping tools enables the identification of quantitative trait loci in the genome. Application of marker assisted selection for QTL has the potential to enhance the accuracy in animal breeding program, particularly for the traits that are difficult to improve through traditional selection methods. As we know that microsatellites or short tandem repeats (STR), are tandem repeated motifs of 1-6 bases. They were found abundantly and at random throughout most eukaryotic genomes (Stallings *et al*.,1991). A large number of microsatellites have been isolated and widely used for these purposes. In case of duck populations less genetic markers have been established. Thus, we attempted to isolate microsatellite markers for cross bred duck and to investigate their polymorphism. Exploring genetic variability by microsatellite markers is essential for genetic improvement, preservation of indigenous germplasm and production of high-quality offspring (Jowel *et al*., 2023).

Chicken represents a valuable genetic resource and protein source, it remains a potential threat to human health as they serve as a reservoir for diseases and food borne pathogens. Diversification of the poultry production is one of the viable options to enhance the food production with less susceptibility to the threats from the emerging diseases and changing climatic conditions. Ducks are promising species suitable for the diversification and will be complimentary with an adaption potential from small to commercial farming. Majority of ducks in the country are desi type with a meagre proportion of improved duck strains. Recent livestock census indicated that the duck population

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has been drastic decreasing over years from 27.6 million in 2007 to 23.5 million in 2012. Therefore, there is an urgent need to augment the duck production through improved breeding, feeding, disease control and other managemental strategies. Further, fine-tuning of production performance and effective health care management can be achieved with the help of newer technologies. Converged use of various conventional molecular and health care technologies will augment duck egg and meat production. Since majority of ducks are desi or non-descript type having low production potential therefore there is an ample scope of duck improvement. Besides, Limited research and scientific intervention has been paid to characterize them, to improve their productivity and to exploit their unique characteristics. Hence, any attempt to improve the duck farming will have direct bearing on the economically weaker section of the society. Improvement of productivity of ducks through identification of molecular markers could be a method of choice. Therefore, genetic characterization by developing duck specific microsatellite markers and designing suitable improvement program are required to be undertaken at the earliest. The microsatellite markers are extensively used for assessing genetic structure, diversity and relationships. Information about genetic diversity of indigenous duck breeds is important to design effective improvement and conservation strategies. Therefore, the current studies aimed at genetic characterization and evaluation of growth, production and reproduction traits of the crosses of local duck population besides undertaking a suitable duck improvement program.

MATERIALS AND METHODS

DNA isolation protocol

About 1.5 mL of distilled water was added to the duck blood (35-50 µL) that was placed in the anticoagulation tube. The content was mixed well and transferred into a 1.5 mL micro centrifuge tube and centrifuged for 2 min at 13500 rpm in a micro centrifuge. The supernatant was discarded and the repeated the steps. The pellet was resuspended in a a 55°C pre warmed 1 mL of WBC lysis buffer (10 mM Tris-Cl pH 7.7, 1.5 M NaCl, 2 mM EDTA, 0.5% SDS) and then the whole suspension was mixed well and micro centrifuged at 13500 rpm for 4 min. The supernatant containing DNA thread mass was picked up with a micropipette equipped with a wide orifice and placed in a new tube. To the supernatant, 1 ml of absolute ethanol was added and the tube was inverted several times. The DNA threads were picked up and placed in a new micro centrifuge tube containing 1 mL of ice cold 70% ethanol and mixed well. Micro centrifugation was performed at 13500 rpm for 4 min. The supernatant was discarded. The DNA was resuspended in 0.5 mL of TE buffer.

Spectrophotometric evaluation of DNA extraction

The spectrophotometric evaluation of the concentration and and purity of DNA was carried out by spectrophotometer. DNA concentration was evaluated. DNA purity with regards to protein and salt contaminants was based on the A 260/280 and A 260/230 absorbance ratios respectively.

The electrophoretic evaluation of DNA extraction

The genomic DNA integrity was checked electrophoretically in agarose gel.

Microsatellite primers

Presently, a panel of 16 informative duck microsatellite markers (Table 1) were identified from database as reported by Alyethodi and Kumar (2010) and Huang *et al*. (2006) and used for the studies. The synthesized primer pairs were obtained in lyophilized form and were reconstituted with nuclease-free water as per manufacturer's instructions. A stock of 100 µM was prepared and from this working primer solution of 10 pM was prepared and used in PCR.

Polymerase chain reaction (PCR)

Each PCR assay was carried out in a total of 25 µL containing 12.5 µL PCR master mix(2X), 5.5 µL nuclease free water, 5 µL template DNA, 1 µL each of reverse and forward primer. Initial denaturation was done at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at optimized temperature for 1 min and extension at 72°C for 1 min and then final extension at 72°C for 5 min. The PCR products were analyzed by Agarose gel electrophoresis.

Following PCR cycling conditions were optimized for 16 microsatellite loci

Heat inactivation at 95°C for 5 minutes 30 cycles of

a. Denaturation at 94°C for 1 minute.
b. Annealing at T_a°C (T_a=optimized annealing temperature for each Microsatellite primer pair)for 45 seconds.
c. Extension at 72°C for 45 Seconds.

- Final extension at72°C for 5minutes.
- 4°C forever.

The PCR products were kept a- 20°C until further analysis

Documentation of PCR products by Agarose Gel Electrophoresis

Approximately, 10 µl of PCR product was added with 2 µl Bromophenol blue dye (6X loading dye, GCC Biotech, India Pvt. Ltd.) for loading in the gel. Samples were loaded into wells of 2% agarose gel containing ethidium bromide (5 µl per 100 ml of 1X TBE buffer) along with 5 µl of 100 bp DNA ladder (GCC Biotech, India Pvt. Ltd.) as molecular size marker for identification of the desired product. The electrophoresis was done at 2-5 volts/cm. The products were examined under UV light in Gel Documentation system (Bio rad Laboratories,USA) and documented.

Metaphor agarose gel electrophoresis (MAGE)of microsatellites alleles

The confirmed amplification of all the samples, the amplicons were run on 3% metaphor agarose gel electrophoresis

(MAGE) to resolve microsatellite alleles for further genotyping.

Determination of molecular size of microsatellite alleles and genotypes

The molecular sizes (in bp) of all the alleles at sixteen studied microsatellites were determined with the help of Image Lab software (Bio-Rad Laboratories Inc., U.S.A.) through Gel Doc system. Genotypes of all the birds were determined on the basis of presence of microsatellite alleles.

Statistical analysis of population genetics data

Data on genotype of all experimental birds at sixteen microsatellites were compiled and analyzed using POPGENE® 3.1 software (Yeh *et al*., 1999) for their population genetics parameters. The primary data on genotype was subjected to co-dominant marker diploid data analysis to estimate observed and expected genotypic frequencies, Hardy-Weinberg (HW) equilibrium status, allele frequency, observed and effective number of alleles, percentage of polymorphic loci, observed and expected homozygosity and heterozygosity and Shannon index.

Genetic variability analysis

Average heterozygosity per microsatellite marker was calculated according to Nei (1978).

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

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, assuming that the population was under Hardy-Weinberg equilibrium.

Table 1: Primers with their nucleotide sequence.

Primer name	Accession number	Nucleotide sequence of forward (F) and reverse (R) primers	Ta (°C)	Ref.
CAUD001	AY493246	F-GCAGAAAGTGTATTAAGGAAG R-ACAGCTTCAGCAGACTTAGA	54	Alyethodi *et al*., 2010
CAUD002	AY493247	F-CTTCGGTGCCTGTCTTAGC R-AGCTGCCTGGAGAAGGTCT	60	Alyethodi *et al*., 2010
CAUD003	AY493248	F-CCTGGCATTCTGCTAAGTTC R-TGGGTTTGAACAGTGTAGCC	51.4	Hung *et al*., 2005
CAUD004	AY493249	F-TCCACTTGGTAGACCTTGAG R-TGGGATTCAGTGAGAAGCCT	60.8	Hung et al., 2005
CAUD005	AY493250	F-CTGGGTTTGGTGGAGCATAA R-TACTGGCTGCTTCATTGCTG	60	Alyethodi *et al*., 2010
CAUD006	AY493251	F-ATGGTTCTCTGTAGGCAATC R-TTCTGCTTGGGCTCTTGGA	56	Alyethodi *et al*., 2010
CAUD007	AY493252	F-ACTTCTCTTGTAGGCATGTCA R-CACCTGTTGCTCCTGCTGT	60	Alyethodi *et al*., 2010
CAUD009	AY493253	F-AGGGATTTTGGAGCGGAGC R-TGTGCGGCGTTTTCCTCTG	63	Hung *et al*., 2005
CAUD010	AY493254	F-GGATGTGTTTTTCATTATTGAT R-AGAGGCATAAATACTCAGTG	50	Alyethodi *et al*., 2010
CAUD016	AY493261	F: TTT AGG TAA AAC TGT GAA TCA A R: ATC AAA GCA GGG AGC TAA G	56	Hung *et al*., 2005
CAUD023	AY493268	F: CAC ATT AAC TAC ATT TCG GTC T R: CAG CCA AAG AGT TCA ACA GG	51.4	Hung *et al*., 2005
CAUD026	AY493271	F: ACG TCA CAT CAC CCC ACA G R: CAG CCA AAG AGT TCA ACA GG	60.8	Hung *et al*., 2005
CAUD028	AY493273	F: TAC ACC CAA GTT TAT TCT GAG R: ACT CTC CAG GGC ACT AGG	55.5	Hung *et al*., 2005
CAUD029	AY493274	F: GAC CTC AAG AAT TTA CCA C R: ATT ATT TTC TTC TGG F:CAG CA	55.5	Hung *et al*., 2005
CAUD030	AY493275	F: ATT ATT CCT GAT GGC GTG GT R: TCA TGC TGA ATT TGG CTG TT	50.3	Hung *et al*., 2005
CAUD035	AY493280	F: CTT ATC AGA TGG GGC TCG GA R: GGC CTA ACC CTG ATG GAT G	63.5	Hung *et al*., 2005

Polymorphic information Content (PIC) at each microsatellite locus was calculated using the following formula (Botstein *et al*., 1980):

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

Where,

P_i and P_j are the frequencies of i^{th} and j^{th} alleles, respectively at *a* locus with *k* numbers of alleles in the population.

RESULTS AND DISCUSSION

The primers along with their nucleotide sequences were summarised in Table 1. the characteristics of the 16 microsatellite loci with allele frequencies were summarized as Table 2. Table 3 depicts Polymorphic Information Content (PIC), Shannon's index, Number of observed and expected alleles, Nei's heterozygosity and Wright's fixation index, observed and expected heterozygosity, chi-square and G-square value at duck specific microsatellite loci in crossbred duck (F1) generation. A total of 16 microsatellites were used to detect polymorphisms in 50 cross bred ducks. A total of 34 nos of alleles were observed and all loci *were* polymorphic. The number of alleles ranged from 1 to 4 with an average of 2.125±0.07 per microsatellite locus. The observed and expected heterozygosity of these polymorphic makers ranged from 0.00 to 0.42 and 0.41 to 0.84 with an average number of 0.068±0.04 and 0.616±0.02 respectively. Among the polymorphic markers, the observed heterozygosities of loci were less than 0.50. The

Table 2: Number of alleles, their molecular sizes and frequencies at various microsatellites loci in crossbred duck (Crosses between pati and white pekin).

Microsatellite Loci	Allele number	Size	Alleles	Allele frequency
CAUD001	1	148	A	1
CAUD002	1	100	A	1
CAUD003	2	154	A	0.75
		162	B	0.25
CAUD004	2	100	A	0.5385
		118	B	0.4615
CAUD005	3	100	A	05455
		135	B	04242
		140	C	.0303
CAUD006	1	114	A	1
CAUD007	1	64	A	1
CAUD009	1	78	A	1
CAUD010	3	78	A	0.1364
		82	B	0.7272
		98	C	0.1363
CAUD016	3	110	A	0.4706
		120	B	0.4412
		126	C	0.0882
CAUD023	3	80	A	0.1154
		90	B	0.1923
		100	C	0.6923
CAUD 26	4	70	A	0.5151
		80	B	0.2424
		84	C	0.1818
		90	D	0.0607
CAUD 28	1	98	A	1
CAUD 29	2	160	A	0.3
		165	B	0.7
CAUD 30	3	120	A	0.4375
		130	B	0.4062
		140	C	0.1563
CAUD 35	3	100	A	0.3871
		118	B	0.5162
		125	C	0.0967
Mean±SE	2.125±0.07			

Table 3: Polymorphic Information Content(PIC), Shannon's index, Number of observed and expected alleles, Nei's heterozygosity and Wright's fixation index, observed and expected heterozygosity, chi-square and G-square value at duck specific microsatellite loci in crossbred duck (F1) generation.

Primer Name	PIC	I	na	ne	Nei	Fis	Ho	He	Chi square	Probability	Gsquare	Probability
CAUD001	0.53	1.02	3.00	2.69	0.63	1	0.01	0.64	76.75***	0.00001	75.75***	0.000000
CAUD002	0.38	0.71	2.00	1.81	0.49	1	0.00	0.45	38.66***	0.000	46.81***	0.000000
CAUD003	0.57	1.05	3.00	2.65	0.65	1	0.00	0.65	75.76***	0.000	76.74***	0.000000
CAUD004	0.65	1.13	4.00	3.33	0.74	0.80	0.42	0.76	115.04***	0.000012	102.45***	0.000001
CAUD005	0.78	1.52	5.00	4.19	0.80	0.45	0.04	0.82	141.75***	0.000018	107.20***	0.000151
CAUD006	0.33	0.69	2.00	1.68	0.42	1	0.00	0.41	36.66***	0.000	42.55***	0.000000
CAUD007	0.32	0.68	2.00	1.67	0.41	1	0.00	0.41	36.45***	0.000	42.21***	0.000000
CAUD009	0.33	0.68	2.00	1.68	0.42	1	0.00	0.45	36.66***	0.000	42.55***	0.000000
CAUD010	0.33	0.69	2.00	1.67	0.42	1	0.00	0.45	36.66***	0.000	42.55***	0.000000
CAUD016	0.55	1.09	3.00	2.67	0.62	1	0.00	0.66	75.85***	0.000	77.91***	0.000000
CAUD023	0.54	1.07	3.00	2.66	0.61	1	0.01	0.60	75.83***	0.000	77.89**	0.000000
CAUD 26	0.55	1.05	3.00	2.67	0.62	1	0.08	0.68	75.86***	0.0000	77.92**	0.000000
CAUD 28	0.37	0.72	2.00	1.82	0.48	1	0.00	0.46	38.66**	0.000	46.82**	0.000000
CAUD29	0.68	1.15	4.00	3.34	0.75	0.80	0.43	0.76	116.04**	0.000013	102.46***	0.000001
CAUD 30	0.70	1.51	5.00	4.16	0.80	0.49	0.05	0.83	141.78***	0.000019	108.02***	0.000153
CAUD 35	0.71	1.52	5.00	4.17	0.81	0.50	0.05	0.84	141.78***	0.000018	108.03***	0.000154
Mean±SE	0.52±0.03	1.0175±0.05	3.12±0.002	2.678±0.05	0.604±0.04	0.877±0.02	0.068±0.04	0.616±0.02				

H_o= Observed heterozygosity; H_e= Expected heterozygosity; df= Degrees of freedom;***p≤0.001.

polymorphism information content (PIC) of 21 loci ranged from 0.32 to 0.78 with an average of 0.52±0.03.

Based on the classification of Hamilton *et al.* (1999), Botstein *et al*. (1980), ten (50%) polymorphic markers were highly informative (PIC>0.50) and rest six (50%) were reasonably informative (0.50>PIC>0.25) which can be comparable to Hsu *et al.* (2003) and Maak *et al.* (2003), Dwi Nur Happy Hariyono *et al.* (2018) and Jowel *et al.* (2023).

CONCLUSION

In conclusion, the identified appropriate microsatellite marker systems for crossbred ducks will provide a good choice for genetic monitoring of the quality and the population genetic diversity of poultry stocks.

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Author's contribution

All the Authors have equally contributed for the research article.

Conflict of interest

The authors declare that there is no Conflict of Interests regarding the publication of this article.

REFERENCES

Alyethodi (2010). Genetic characterization of moti indian native duck using microsatellite markers, Journal of Applied Animal Research. 38(1): 223, DOI:10.1080/09712119. 2010.10539515.

Alyethodi, R.R. and Kumar, S. (2010) Genetic characterization of Moti Indian native duck using microsatellite markers. J. Appl. Anim. Res. 38: 223-227.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Dwi Nur Happy Hariyono, Dyah Maharani, Sunghyun Cho, Prabuddha Manjula, Dongwon Seo, Nuri Choi, Jafendi Hasoloan Purba Sidadolog and Jun-Heon Lee (2018). Genetic diversity and phylogenetic relationship analyzed by microsatellite markers in eight Indonesian local duck populations. Asian-Australas J Anim Sci. 2019 Jan; 32(1): 31-7.Published online 2018 Dec 21. doi: 10.5713/ajas. 18.0055.

Hamilton, M.B., Pincus, E.L., Di Fiore, A. and Fleischer, R.C. (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques. 27: 500-507.

Hsu, Y.C., Severinghaus, L.L., Lin, Y.S. and Li, S.H. (2003). Isolation and characterization of microsatellite DNA markers from the Lanyuscops owl (*Otuselegansbotelensis*). Mol. Ecol. Notes 3: 595-597.

Huang, Y.H., Tu, J. F., Cheng, X.B., Tang, B., Hu, X.X., Liu, Z.L., Feng, J.D., Lou, Y.K., Lin, L., Xu, K., Zhao, Y.L. and Li, N. (2005). Characterization of 35 novel microsatellite DNA markers from the duck (*Anas platyrhynchos*) genome and cross-amplification in other birds. Genet. Sel. Evol. 37: 455-472.

Huang, Y.H., Zhao, Y.H., Haley, C.S., Hu, S.P., Hao, J.P., Wu, C.X. and Li, N. (2006). A Genetic and Cytogenetic Map for the Duck (*Anas platyrhynchos*). Genetics 173: 287-296.

Jowel, D., Debnath, S., Sarkar, D., Debroy, B., Kumar, M., Das, A.K. (2023). Genetic characterisation of indigenous duck of Tripura state in India using microsatellite markers. Tropical Animal Health and Production volume. Article number: 55: 198.

Maak, S., Wimmers, K., Weigend, S. and Neumann, K. (2003). Isolation and characterization of 18 microsatellites in the Peking duck (*Anas platyrhynchos*) and their application in other waterfowl species. Mol. Ecol. Notes 3: 224-227.

Nei, M.1. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89(3): 583-90. doi: 10.1093/genetics/89.3.583.

Stallings, R.L., Ford, A.F., Nelson, D., Torney, D.C., Hildebrand, C.E., Moyzis, R.K. (1991). Evolution and distribution of $(GT)_n$ repetitive sequences in mammalian genomes, Genomics. 10(3): 807-815.

Yeh, F.C., Yang, R.C. and Boyle, T. (1999). POPGENE Version 1.32: Microsoft Window-Based Freeware for Population Genetics Analysis. University of Alberta, Edmonton.