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Analysis of Genetic Diversity in Bangladeshi Quail Populations Using RAPD Markers

M.S. Istiak, M.B.R. Mollah, and M.S. Ali
Department of Poultry Science
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
Corresponding Author: M.S. Istiak

Abstract: *To develop strategies for conservation and future utilization of genetic resources of quail it is necessary to understand genetic variability and relatedness at molecular level. The randomly amplified polymorphic DNA (RAPD) markers were used to detect polymorphism among five quail populations i.e. Japanese, Fawn, Dhakaya, White and Rosetta. Six out of 17 random primers screened yielded distinct polymorphic RAPD profiles. Among the six polymorphic primers, three primers (OPC-08, OPL-07 and OPL-18) were used in this study and generated 22 distinct bands (RAPD markers), of which 20 bands (90.91%) were considered as polymorphic. The percentages of polymorphic loci were 90.91, 86.36, 54.55, 72.73, and 90.91 and the Nei's gene diversities were 0.3412, 0.2753, 0.2045, 0.3556, and 0.3200 for Japanese, Fawn, Dhakaya, White and Rosetta, respectively. The values of intra population similarity indices (Si) were higher (0.5515) in white quail populations compared to others. The average within population genetic similarity was 0.4134. The population pair Rosetta and Dhakaya showed higher genetic identity (0.9375) than other population pairs, whereas the White and Fawn pair showed the lowest genetic identity (0.8052). The coefficient of gene differentiation (G_{ST}) and gene flow (N_m) of all the population were calculated to be 0.1762 and 2.3372, respectively representing the genetic divergence among the populations. This study is attempted to use molecular techniques to determine genetic structure of different quail populations available in Bangladesh.*

Keywords: *Quail, RAPD, Genetic similarity, genetic relationship.*

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I. Introduction

In Bangladesh commercial poultry production is dominated by chicken over other species. The single species orientated commercial poultry farming in Bangladesh is quite vulnerable to many challenges. Thus, diversification of poultry industry in terms of species is an emerging need. In recent years quail is becoming an economically valuable poultry species which have potential to compete with other species. By virtue of high acceptability of quail meat and eggs, it has occupied a prominent position. It might be a priority species to introduce in the commercial poultry farming for its several advantages such as: short generation interval, low feed requirement, resistance to many common poultry diseases, low investment requirement etc. Wild type Japanese quail (Coturnix japonica) was introduced in Bangladesh at around 1980 and spread throughout the country. Smaller body size, higher phenotypic and genetic variations for productive and reproductive traits are the critical factors impeding their use in profitable and sustainable commercial production. In order to make quail production profitable and sustainable, it is necessary to harness heterosis for productive traits, especially for egg and meat traits. However, only the Japanese quail is sporadically available in Bangladesh and they are mainly suitable for egg production. Their genetic characterization is still unknown, so studies are required to characterize this quail population and to estimate genetic variability and relatedness among them for selection and breeding.Genetic variability and relatedness is commonly detected through polymorphic markers generated by isozymes, protein electrophoresis pattern, and restriction enzymes. The discovery of the polymerase chain reaction (PCR) had a major impact on the research of eukaryotic genomes and contributed to the development and application of various DNA markers [1]. Molecular markers derived from PCR amplification of genomic DNA are an important part of the toolkit of evolutionary geneticists [2]. By detecting genetic variation, genetic markers may provide useful information at different levels: population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness [3]. A polymorphism assay based on the amplification of random DNA fragments using an arbitrary nucleotide sequenced primer (RAPD) has been developed [4] and [5]. RAPD is a simple and easy method to detect polymorphism based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence [4, 5, 6. 7, 8]. It has several unique advantages such as they do not require the prior knowledge of target sequence, need only small amount of DNA and are simple, fast and less costly [5]. Therefore, the RAPD

may provide a highly polymorphic system of choice, capable of generating polymorphism. After discovery in 1990, it has quickly become a method of choice for genotype identification, population and pedigree analysis, phylogenic studies and genetic mapping.Therefore, the present study was performed to detect polymorphism and to estimate the genetic variability and relatedness among different populations of quails using RAPD markers.

II. Materials And Methods

2.1 Sample collection

The sample was collected from poultry farm of Bangladesh Agricultural University (BAU), Mymensingh. For this experiment, three males and three females were randomly sampled from each of the following quail populations: Japanese (Jp), Fawn (Fn), Dhakaya (Dh, White (Wh) and Rosetta (Ro) those were maintained as random bred non-selected stock. The characteristics of each population are summarized in Table1. The laboratory work was performed in the Poultry Biotechnology Laboratory of Bangladesh Agricultural University, Mymensingh.

Table 1. Description of quail populations used in this study

Population	Abbreviation	Source	Features
Japanese	Jp	Dept. of Poultry Science, BAU, Mymensingh	Body weight (42 days): 105-110 gm Hen Day Egg Production: 60-65 %
Fawn	Fn	-do-	Body weight (42 days): 95-100 gm Hen Day Egg Production: 60-65%
Dhakaya	Dh	-do-	Body weight (42 days): 105-110 gm Hen Day Egg Production: 70-75 %
White	Wh	-do-	Body weight (42 days): 115-120 gm Hen Day Egg Production: 70-75 %
Rosetta	Ro	-do-	Body weight (42 days): 115-120 gm Hen Day Egg Production: 65-70 %

2.2 Blood collection and genomic DNA extraction

Blood was collected and prepared for DNA isolation by using the procedure suggested by [9]. Genomic DNA was extracted from blood cell using the Phenol: Chloroform: Isoamyl method. Approximately 10 µl of clotted blood cells were taken in an eppendorf tube containing 250 µl of tail buffer (0.3M Sodium Acetate; 10mM Tris-HCl, pH=7.9; 1% SDS). Then 10 µl of Proteinase K was added in the solution and the mixture was incubated at 55°C overnight for digestion. DNA was purified by successive extraction with 250µl phenol: chloroform: Isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1), respectively. DNA was precipitated by adding two volumes of ice-cold ethanol and peletted by centrifugation. The pellets were then washed with 70% ethanol, air-dried and finally re-suspended in 50µl of TE (pH = 8.0) buffer. The quality of DNA was checked by electrophoresis in a minigel and quantified by using a spectrophotometer (Spectronic® GENESIS^{TM} 5).

2.3 Primer selection

Seventeen deca-mer primers of random sequences were used in this study .These primers were screened on sub samples of one male and one female form each population to test their suitability for amplifying DNA sequence. These primers were evaluated on the basis of intensity, resolution of bands and repeatability of markers, presence of smearing and potential for population discrimination. After that a final subset of three primers, out of seventeen were retained for further analysis (Table 2).

Table2: Parameters of primers used in RAPD analysis

Primers	Nucleotide length (bp)	Sequence	(G+C)%
OPC-08	10	TGGACCGGTG	70
OPL-07	10	AGGCGGGAAC	70
OPL-18	10	ACCACCCACC	70

2.4 PCR amplification and electrophoresis

The amplification conditions used in this study was similar to [7] with slight modifications. The polymerase chain reaction was carried out in 10 µl reaction mix containing 1.0 µl PCR Buffer (20 mM Tris-HCl pH 8·3, 50 mM KCl), 0.05 U of Taq DNA polymerase (Takara, Japan), 0.25 µl dNTPs (2.5mM each), 0.75 µl of random primer and 75 ng of genomic DNA and dH_2O. The final reaction volume of 10 µl was placed in a DNA thermal cycler (ABI GeneAmp PCR System 2700). The PCR program included an initial denaturation step at 94°C for 3 min followed by 40 cycles with 94°C for 1 min for DNA denaturation, 34°C for 1 min for primer annealing and 72°C for 2 min for primer extension. Final extension was at 72°C for 7 min. Then the samples were cooled at 4°C. The amplified DNA fragments were separated on 2% agarose gel and stained with

ethidium bromide. The amplified fragments were visualized on a UV Transilluminator and photographed by using Bimetra Gel Documentation System (Germany). A molecular weight marker DNA (Loading Quick 100bp Ladder, Japan) was electrophorsed alongside the RAPD reactions.

2.5 Genetic data analysis

All distinct bands or fragments were identified according to size and scored visually on the basis of their presence (1) or absence (0). For RAPD data analysis scores obtained by using primers were pooled to create a single data matrix. This was used for estimating the proportion of polymorphic loci, [10] gene diversity (h), gene flow (N_M), coefficient of gene differentiation (G_{ST}), [11] genetic distance and construction of a UPGMA (Unweighted Pair Group Method of Arithmatic Means) dendrogram among populations with 1000 simulated samples were carried out by using POPGENE (Version 1.31) [12] computer program. Band sharing based genetic similarity indices (Si) were calculated by using following formula:Similarity Index (Si) = $2N_{AB}/(N_A+N_B)$Where, N_{AB} is the total number of RAPD band shared by individuals A and B. N_A and N_B are the numbers of fragments scored for each individual respectively [13].

III. Results

Among the seventeen primers tested three retained for RAPD analysis produced varied number of bands. The primers generated 22 distinct bands (RAPD markers), of which 20 bands (90.91%) were considered as polymorphic (either occurring in or absent in less than 95% individuals).The characteristics of the fragments generated by these three primers are summarized in Table 2. Among the primers, primer OPL-18 gave DNA profiles with more bands than OPC-08 and OPL-07. Examples of the varying degree of polymorphism generated with the three primers are shown in Fig. 1a-c.

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Table 3: Total number and percentage of polymorphic loci, gene diversity and intra-population similarity indices (Si) of seven populations of quail

Populations	No. of polymorphic loci	Polymorphic loci (%)	Gene diversity (ME ± SD)	Similarity %
Japanese	20	90.91	0.3412±0.1619	45.16
Fawn	19	86.36	0.3556±0.1808	45.90
Dhakaya	12	54.55	0.2045±0.2074	23.43
White	16	72.73	0.2753±0.1868	55.15
Rosetta	20	90.91	0.3200±0.1550	37.05
Overall	20	90.91%	0.17	-

The number of polymorphic loci, percentage of polymorphic loci, gene diversity and intra- population similarity indices of five populations of quail are shown in Table 3. The overall number of polymorphic loci, percentage of polymorphic loci and gene diversity were 20, 90.91% and 0.17, respectively.The numbers of polymorphic loci were higher in Japanese (20) and Rosetta (20) quail population and lower in Dhakaya (12) quail population. The overall frequencies of RAPD markers across primers OPC-08, OPL-07 and OPL-18 were 0.7164, 0.6809 and 0.6946, respectively. The value of Nei's gene diversity [11] of Fawn population was found to be higher (0.3556) than those of all other populations, whereas the lowest value in Dhakaya (0.2045 Analysis of Nei's gene diversity in subdivided populations estimated the gene flow (N_m) value of 2.3372 and the proportion of total genetic diversity (G_{ST}) attributed to subpopulation differentiation was 0.1762.The values of intra population similarity indices (Si) were higher (0.5515) in white quail populations compared to others. The Si value of Dhakaya population was found to be the lowest (0.2343).The results of the Nei's original measures of genetic identity and genetic distance [11] among population pairs are shown in Table 4 and the corresponding UPGMA dendrogram in Fig.2.The highest genetic distance pair values were found between White and Fawn (0.2167) followed by White and Japanese (0.2088), and White and Dhakaya (0.1714). The lowest genetic distance was found in case of Dhakaya and Rosetta population pair (0.0646). The population pair Rosetta and Dhakaya showed higher genetic identity (0.9375) than other population pairs, whereas the White and Fawn pair showed the lowest genetic identity (0.8052).

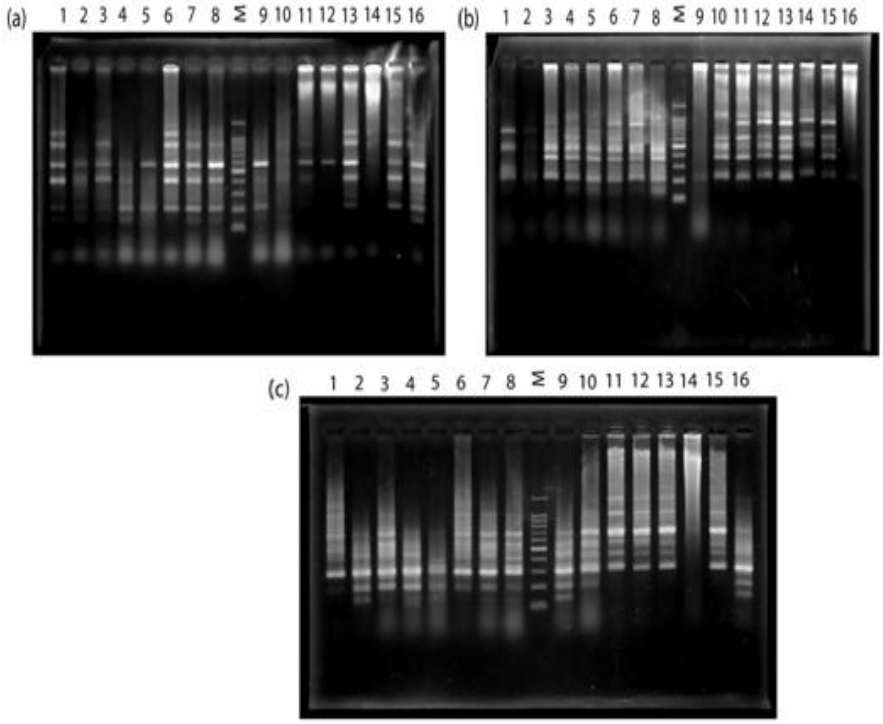

Fig 1: RAPD Profile of different quail population generated by different primers; (a) OPC 08 (b) OPL 07 and (c) OPL 18: Line 1-6= Japanese; Line 7-9= Fawn; Line 10= Dhakaya; Line 11-13= White and Line 14-16= Rosetta. M: Molecular weight marker (100 bp ladder).

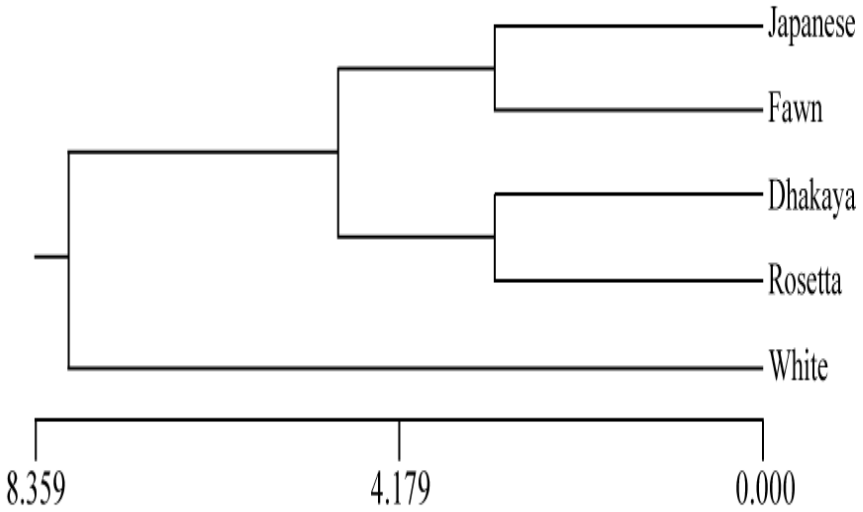

Fig 2: UPGMA dendrogarm for five quail populations based on Nei's genetic distance.

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among five populations of quails

Population	Japanese	Fawn	Dhakaya	White	Rosetta
Japanese	****	0.9347	0.8925	0.8116	0.9105
Fawn	0.0675	****	0.9104	0.8052	0.9012
Dhakaya	0.1138	0.0939	****	0.8425	0.9375
White	0.2088	0.2167	0.1714	****	0.9307
Rosetta	0.0938	0.1040	0.0646	0.0719	****

IV. Discussion

In recent years, different marker system have been developed and applied to a wide range of livestock species. The randomly amplified polymorphic DNA (RAPD) markers were used to detect polymorphism among five populations of quails i.e. Japanese, Fawn, Dhakaya, White and Rosetta. Of the total 22 fragments amplified from three primers, about 90.91% of them showed polymorphism. The average number of bands per primer was ranged from 7 to 8. Since the amplification from these arbitrary sequenced primers depends upon the presence of annealing site on template DNA, different primers are expected to give different numbers of amplicons. The results agreed well with the findings of [14, 15, 16, 7, 17]. Intra-population similarity or within population similarity indices (Si) for White quail population was the highest that was followed by Fawn, Japanese, Rosetta and Dhakaya, respectively. Higher within population genetic similarity in White, Fawn and Japanese populations might be due to differences in population structure and selection history. Since the White population is a mutant line and kept as small population with selective mating thus low amount of genetic variability is expected in this population. The findings of [18] regarding within population similarity of highly inbred Japanese quail lines HBW and KLQ was from 0·726 to 0·836, respectively is comparable with the findings of the present study. The Nei's gene diversity (h) value of Fawn population was found to be higher (0.3556) than those of all other populations, whereas the lowest value in Dhakaya (0.2045). Analysis of Nei's gene diversity in subdivided populations estimated the gene flow (N_m) value of 2.3372 and the proportion of total genetic diversity (G_{ST}) attributed to subpopulation differentiation was 0.1762.The high level N_m and low level of G_{ST} representing the presence of subdivided population with lower genetic differentiation and with possible migration.o measure genetic identity and genetic distance [11] among population pairs test were performed. The highest genetic distance pairs were between White and Fawn (0.2167) followed by White and Japanese (0.2088), and White and Dhakaya (0.1714). Therefore it is evident that White is the most differentiated of all the samples studied. The population pair Rosetta and Dhakaya showed higher genetic identity (0.9375) than other population pairs, whereas the White and Fawn pair showed the lowest genetic identity (0.8052). Since White populations is in under intense inbreeding and selection for plumage color and conformation, low amount of genetic diversity is expected in this population.The findings of this study will provide a baseline data on the genetic structure of quail populations available in Bangladesh which will further help to take initiative in improving their productivity. However to know the genetic variation more concisely further studies are required with a large number of sample and more RAPD markers.

V. Conclusion

The findings of this study will provide a baseline data on the genetic structure of quail populations available in Bangladesh which will further help to take initiative in improving their productivity. In this study the numbers of sample and primer were few, which reduced the chance to know the genetic variation more concisely. In future, additional individuals per populations and more primers need to be included in the analysis to make conclusive remarks about the genetic structure of different quail populations available in Bangladesh.

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