Phylogenetic analysis based studies on genetic variation of Cytochrome b gene of Indian peafowl (Pavo cristatus) in Pakistan

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ABSTRACT

This study was designed to analyze genetic variation between Indian peafowl available at different locations by targeting the Cytochrome b gene. A total of ten birds (n=10) were selected randomly for sample collection. Five birds were selected from government sector and five from private breeding farms. DNA was extracted, purified and measured by using Nano drop. Extracted DNA was amplified using universal primers targeting Cytochrome b gene on polymerase chain reaction (PCR). PCR product was run on a gel for the desired DNA bands. DNA from gel was eluted and sent for sequencing. The sequences were compared with a reference reported sequence of cyt b gene of Indian peafowl with Accession No. L08379.1 to find out the genetic diversity. Indian peafowl of government sector showed more similarity ≥95% rather than bird of private sector with ≥90% homology with reference Accession No. More genetic variation, which is the guarantee of resistance to disease and environmental fitness among the Indian peafowl at private sector, might be due to random reproductive behavior.

Key words: Cytochrome b gene, Genetic variation, Indian Peafowl.

INTRODUCTION

The Indian peafowl (Pavo cristatus) is a native bird of Asia and present in different countries including India, Pakistan, Sri Lanka and Nepal etc. The fan shaped crest of spatula-tipped wire-like feathers together with the brilliant glistening blue neck and breast, and the sweeping metallic bronze-green train, boldly oscillated with purplish black-centered coppery discs or eyespots, make the cock unmistakable (Alonso et al., 2004; Krebs, 1998; Vincze, 2016). Across the world, the blue or Indian peacock is recognized and regarded as a most glamorous bird. The Indian peafowl has three bird species in the genera Pavo and Afropavo of the pheasant family, Phasianidae. Apart from the wonderful brilliant plumage and extraordinary performance in raising its long train in a remarkable display, this is a bird of distinctive character (Brown and Van Tuinen, 2011; Simons et al., 2015). The most impressive characteristic of the blue and green peacocks is their ability to lift their train plumes into an enormous curve, 1.9–2.2 metres wide, and walk round with this display, shimmering and rattling the feathers. It takes three years for a peacock to attain its train with full length. Peacock is recognized as a symbol of religious belief of Hinduism and Buddhism. For thousands of years, these magnificent birds have had a close association with man in diverse ways (Clayton et al., 2010; -Hoyo et al., 1993; Wilson Jr., 1999). Progresses in molecular techniques, especially the advancement of the polymerase chain reaction, have made investigations of vertebrate genomes progressively realistic, and have removed the necessity for completely prepared molecular biology laboratories (Baig, 2006; Klaassen, 1996; Mouritsen and Larsen, 2001). To date, a hefty portion of these studies have concentrated on the mitochondrial genome, basically at the level of DNA succession determination (Livezey and Zusi, 2007). An endeavor is made to outline likenesses and contrasts between the mitochondrial genomes of Indian peafowl available at government and private sector in Pakistan to bring up how some of these distinctions have given exceptional chances to test profound phylogenetic inquiries.

MATERIALS AND METHODS

In this study, the blood samples of Indian Peafowl were collected from government and private sectors. Five varieties of Indian Peafowl were selected which are commonly present in central Punjab. Birds were named (PC1-PC5) of government sector, while (PC6-PC10) of private sector.

Blood sample collection: Blood of Indian peafowl was taken from wing vein in anticoagulant containing vacutainers and...
stored in ice chambers and transferred to lab and stored at -20 °C for further analysis.

**DNA extraction and amplification:** Blood was handled carefully and DNA was extracted using the kit (GE Health Care-Amersham). To assay the DNA yield and to check DNA purity, Nanodrop (Thermo Scientific NanoDrop™ 2000/2000c Spectrophotometer) was used. Absorbance ratios were measured at 260 nm/280 nm. For amplification of cytochrome b gene of extracted DNA, Universal Primers (Table 1.1) were used.

**Table 1.1:** Universal Primer sequences for cytochrome b amplification.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’———3’)</th>
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<tr>
<td>mcb398</td>
<td>TACCATAGGACAAATATCATTCTG</td>
</tr>
<tr>
<td>mcb869</td>
<td>CCTCTAGTTTGTTAGGGA TTGA TCG</td>
</tr>
</tbody>
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**Polymerase Chain Reaction (PCR):** The amplification of gene was carried out according to the following program, denaturation at 94°C for 45 sec., annealing at 54 °C for 1 min, first extension was done at 72°C for 2 min and last extension at 72°C for 10 min for 35 cycles.

**Sequencing and analysis:** To identify the genetic diversity between the *Pavo cristatus* available at different sites, DNA sequences of PCR amplicon targeted cytochrome b gene universal primers were blasted. The acquired DNA sequence was used for the homology examination through NCBI-BLAST and the relative investigation of the obtained DNA sequence with the effectively reported data accessible in NCBI Data Bank was done.

**Statistical analysis:** The data were analyzed by using One-Way Analysis of Variance (ANOVA) by utilizing Tukey’s t-test to check the homology between the bird’s genetic makeup with confidence interval of 95%. Data was analyzed on Statistical Package of Social Sciences (IBM, SPSS v 20.0).

**RESULTS AND DISCUSSION**

Samples were amplified (Fig 1.1) using PCR, a significant difference regarding the variation in *cyt b* gene was noted among the birds after sequencing. It was observed that birds of government sector were more homologous to referenced gene sequence (1143 bp) with Accession No. L08379.1, while the birds of private sector were less similar to reference sequence.

**Genetic diversity:** Most homologous Indian peafowl (PC1) was 97% similar to referenced sequence while least was PC6 with only 85% similarities (Table 1.2).

**Table 1.2:** Homology % of different sequence of Mitochondrion cytochrome b with reference sequence Accession No. L08379.1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Homology (%)</th>
</tr>
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<tbody>
<tr>
<td>PC1</td>
<td>Government Zoological Gardens</td>
<td>(1116/1145) 97%</td>
</tr>
<tr>
<td>PC2</td>
<td>Government Zoological Gardens</td>
<td>(1103/1151) 96%</td>
</tr>
<tr>
<td>PC3</td>
<td>Government Zoological Gardens</td>
<td>(1065/1153) 92%</td>
</tr>
<tr>
<td>PC4</td>
<td>Government Zoological Gardens</td>
<td>(1040/1161) 90%</td>
</tr>
<tr>
<td>PC5</td>
<td>Government Zoological Gardens</td>
<td>(1082/1147) 94%</td>
</tr>
<tr>
<td>PC6</td>
<td>Private Sectors</td>
<td>(999/1177) 85%</td>
</tr>
<tr>
<td>PC7</td>
<td>Private Sectors</td>
<td>(667/777) 86%</td>
</tr>
<tr>
<td>PC8</td>
<td>Private Sectors</td>
<td>(1059/1159) 91%</td>
</tr>
<tr>
<td>PC9</td>
<td>Private Sectors</td>
<td>(1059/1158) 91%</td>
</tr>
<tr>
<td>PC10</td>
<td>Private Sectors</td>
<td>(1030/1161) 89%</td>
</tr>
</tbody>
</table>

**Genetic Variation between Indian Peafowl at government and private sectors:** After comparison, the genomic sequences of Indian peafowl mitochondrion cytochrome b gene, we found that there is significant difference (p<0.05) of genetic diversity observed between the two sectors (Table 1.3).

**Table 1.3:** Genetic Homology Percentage between Indian peafowl at different locations

<table>
<thead>
<tr>
<th>Sector</th>
<th>Homology % (Mean ± S.E.)</th>
<th>p-value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Government</td>
<td>93.80 ± 1.28</td>
<td>0.017</td>
</tr>
<tr>
<td>Private</td>
<td>88.40 ± 1.24</td>
<td></td>
</tr>
</tbody>
</table>

Graphical representation (Graph 1.3) indicated that three birds of Government sector were ≥95% similar to referenced sequence while three birds of private sector were ≥90% similar, while other were <90% homologous.

**Phylogenetic analysis:** Phylogenetic tree clearly demonstrated that Mitochondrion cytochrome b gene sequence of reference model with accession no. L08379.1 clustered with sample sequences (Fig 1.3).

To perform phylogenetic analysis, mitochondrial DNA has widely been used in certain animals (Dash et al., 2013). *Pavo cristatus* mitochondrial DNA (mt-DNA) is approximately 16,686 bp length that comprises of 13 genes encoding for proteins, 22 tRNA genes and 2 rRNA genes along with a displacement loop (D-loop) (Zhou et al., 2015).

**Fig-1.1:** The PCR product photograph of samples from Govt. zoological Gardens and private sector with standard marker (M)
In the present study phylogenetic relationship and genetic diversity was estimated by using cyt b marker. The same study was conducted by Briolay et al., (1998) and reported the same results and found the interesting phylogenetic relationships within different 29 European cyprinids by using cyt b mitochondrial genes. The finding reported in our study also supported by Podsiadlowski et al., (2017) and stated that farm bred animals may lead to inbreeding resulting in genetic flaws while hybrids are genetically more flexible and can tolerate disease, may combat severe environmental conditions and are more fit in diverse habitats. It is also reported that mt-DNA has also been used to estimate the reproductive success and to identify the breeding pair (Arnold et al., 2017).

Indian peafowl has various reported varieties. These varieties are mostly based on color etc. and the current study provides us a phylogenetic relationship between different varieties based on color polymorphism. The study is supported by the findings of Boonkhaw et al., (2017) when he established a relationship in 16 polymorphic pelage color varieties of Finlayson’s squirrel by using the cyt b DNA. The genetic diversity of govt. sector was lower because mostly the birds were inbred but the genetic diversity of private sector was higher. The same findings were reported by Koju et al., (2017) when they reported the phylogeny of living pikas (Ochotonidae, Ochotona) from China by using two mitochondrial (CYT B and COI) and five nuclear gene segments (RAG1, RAG2, TTN, OXAIL and ILIRAPLI). Mitochondrial DNA in animals causes the five to ten times greater rate of evolution than in nuclear genome, that is why it is hypothesized to be ideal marker to study divergence between domestic and wild animals (Saikia et al., 2015). In the following study, diversity among the India peafowls at
government and private sectors was investigated by sequence analysis of cytochrome \( b \) gene to elucidate the genetic structure and degree of differentiation between the birds at different locations.

Indian peafowl (\textit{Pavo cristatus}), the largest pheasant and national bird of India does found throughout the world but since last decade their populations has undergone the massive decline due to changes in cropping and poaching pattern, human interference and pesticides issues etc. These alarming problems may lead to extinction of definite species (Jain and Rana 2013). There is no specific mechanism to conserve the certain breeds of Indian peafowl. It is supposed that in spite of developed areas, none of them have Indian peafowl with good progeny status, but government institutions may have the specific species with their know ancestors (Rana and Jain 2013). This statement is according to our results that government sector has Indian peafowl with good progeny transcripts rather than the birds available at private sector.

**CONCLUSION**

It is concluded that the different varieties of Indian peafowl are closely related. Random breeding guarantees the more reproductive success and the birds are more capable to tolerate the changing environmental conditions hence make them the fittest. More the heterozygosity in various genes makes the population more stable. It is recommended that animal zoo keepers and private animal breeders must ensure the random breeding to enhance the genetic heterozygosity.

**REFERENCES**


