Inter-generic relatedness of pheasants (Aves: Galliformes: Phasianidae) based on RAPD using non-invasive samples

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Abstract— Molecular tools play an important role in conservation genetics. RAPD, the simplest among all PCR techniques, has been used in the present study to reveal the inter-generic relatedness among pheasants such as Indian Peafowl (*Pavo cristatus*), Silver pheasant (*Lophura nycthemera*), Domesticated Turkey (*Meleagris gallopavo*) and Domesticated fowl (*Gallus gallus*). Among 38 bands amplified by three primers, 34 (89.4%) were highly polymorphic. The bands produced were checked for reproducibility and further statistical analyses were made with Jaccard's and UPGMA method for genetic distance and developing dendrogram. The results clearly revealed the clade divergence of *Pavo cristatus* and *Gallus gallus* with that of *Lophura nycthemera* and *Meleagris gallopavo*.

Keywords: RAPD, inter-generic relatedness, genetic distance, polymorphism

1 INTRODUCTION

Birds are important for maintaining healthy ecosystem and hence it is vital that they are protected and conserved. Pheasants are well known for their colorful plumage and attractive nature. They are classified under family Phasianidae (Aves: Galliformes). Among pheasants, chicken, turkey and quail are domesticated species. Pheasants like peacock, grey jungle fowl, red jungle fowl and other species which live in wild, are susceptible to various threats like poaching and habitat destruction. In such cases, identification of sample is a challenging task, which could be analyzed by molecular tools.

Genetic techniques can be used instead of traditional means for studying wild life. Non-invasively collected samples could be used for genetic studies of species that are threatened and endangered. Naturally shed feathers are increasingly used to study avian demography and behavior. The advances in DNA techniques have a great impact in addressing problems in many aspects of Biology.

Molecular markers derived from PCR amplification of genomic DNA are an important tool kit of evolutionary geneticist [1]. RAPD marker analysis can show high levels of polymorphism even among closely related species. Detecting genetic variation using genetic markers may provide useful information at different levels like population structure, level of gene flow, phylogenetic relationship, patterns of historical biogeography and the analysis of parentage and relatedness. RAPD has also been reported in sexing birds [2], population genetics [3]; [4], genetic diversity [5]; [6]; [7]. The feasibility of RAPD analysis using feather samples has been reported [8]. Galliformes are reported in poaching for flesh and feathers for ornamental articles. Finding the origin of related species is still a challenge and solution to this rely on molecular techniques.

The present study was undertaken to analyze the genetic relatedness of the birds belonging to the family Phasianidae of Galliformes and developing RAPD based marker for identification of species using shed feathers.

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2 MATERIALS AND METHOD

2.1 DNA extraction and amplification

Naturally shed Feathers were collected from Indian Peafowl (*Pavo cristatus*), Silver pheasant (*Lophura nycthemera*), Domesticated Turkey (*Meleagris gallopavo*) and Domesticated fowl (*Gallus gallus*) belonging to the family Phasianidae of the order Galliformes. The first two were collected from Arignar Anna Zoological Park, Vandalur and the later were collected from Livestock Research Station, Kattuppakkam. DNA extraction from shed feathers was carried out with modified protocol of [9] and [10]. Final DNA extracted was dissolved in TE buffer for further use.

DNA was amplified using 11 random primers of 10 nucleotide length (decamers). The amplification was carried out in a thermal cycler (Bio-Rad) with following reaction conditions: 95°c for 2 min, followed by 40 cycles of 94°C for 1 min, 45 °C for 1 min, 72 °C for 2 min; and final extension at 72 °C for 5 min. The products were separated and visualized in 2.5% agarose gel.

2.2 Data analysis

The bands obtained were scored visually by binary method making presence as '1' and absence as '0'. Binary data obtained was used as input for analysis of Jaccard's similarity index and genetic distance calculation using DendroUPGMA [11]. Based on Jaccard's matrix, dendrogram was developed by neighbor-joining method using BioNJ [12].

3 RESULTS

As Among 11 primers screened, 5 were selected based on amplification and further, based on their reproducibility, P1, P4 and P11 were selected. The overall polymorphism observed using these primers was 89.4% (Table-1). These were used to determine the genetic diversity of the selected pheasant species.

The genetic similarity and distance between species was found using Jaccard's Similarity index (Table-2) and distance matrix (Table-3). The dendrogram was developed using BioNJ, a bioinformatics tool(Figure-1).

4 DISSCUSION

RAPD based molecular study has been widely reported in many bird species such as Rufous-Vented Prinia [3], see-see partridge [4], common myna [5], Francolin [6], ground parrot [13], Conopophaga [14], Manchurian pheasant (Kulikova et al, 2002). Our findings on inter-generic relatedness based on RAPD-dendrogram analysis, matched with [16] and [17],. This study revealed the clade divergence of *Pavo cristatus* and *Gallus gallus* with that of *Lophura nycthemera* and *Meleagris gallopavo*. And the results were in agreement with [16] and [17].

5 CONCLUSION

In conclusion, our study revealed the inter-generic relatedness of *Pavo cristatus*, *Gallus gallus*, *Lophura nycthemera* and *Meleagris gallopavo* by RAPD.

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TABLE 1

PRIMERS AND THEIR AMPLIFIED PRODUCTS WITH PERCENTAGE POLYMORPHISM

Primer	Sequence (5′-3′)	No. of bands	No. of Polymorphic bands	Product Range (bp)	Polymorphism (%)
P-01	TTCCCCCCAG	13	12	175-1500	92.3
P-04	GGTGCTCCGT	11	9	100-1200	81.8
P-11	TCTGCCATCC	14	13	200-1400	92.8
		38	34		89.4

TABLE 2

GENETIC SIMILARITY BASED ON JACCARD'S COEFFICIENT

	G.gallus	L.nycthemera	P.cristatus	M.gallopavo
G.gallus	1	0.389	0.261	0.304
L.nycthemera	0.389	1	0.304	0.348
P.cristatus	0.261	0.304	1	0.167
M.gallopavo	0.304	0.348	0.167	1

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TABLE 3

GENETIC DISTANCE MATRIX BASED ON JACCARD'S COEFFICIENT

	G.gallus	L.nycthemera	P.cristatus	M.gallopavo
G.gallus	1	0.389	0.261	0.304
L.nycthemera	0.389	1	0.304	0.348
P.cristatus	0.261	0.304	1	0.167
M.gallopavo	0.304	0.348	0.167	1

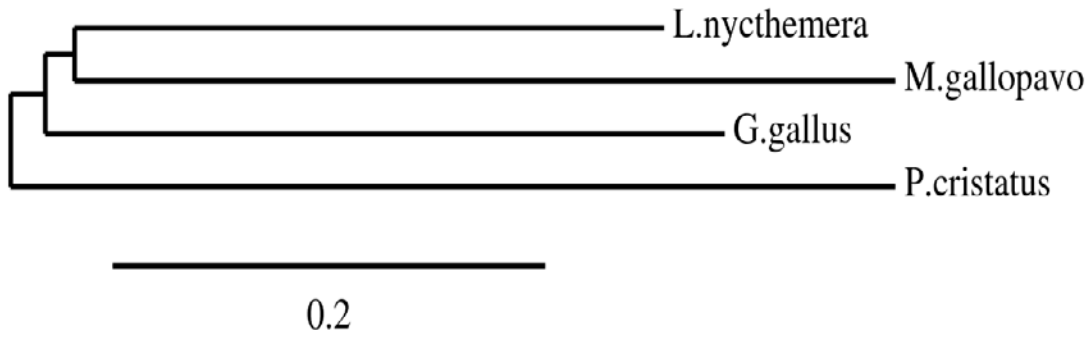

Fig. 1. RAPD based dendrogram of selected pheasant species. The Tree clearly shows the clade divergence *Pavo cristatus* and *Gallus gallus* with that of *Lophura nycthemera* and *Meleagris gallopavo*.

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