

Genetic diversity and phylogenetic analysis of crow species of district Mansehra, Pakistan

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ABSTRACT

Crows are passerine birds of genus *corvus* in family Corvidae. Current study was carried out on three species of crow, jungle crow (*Corvus macrorhynchos*), house crow (*C. splendens*) and jackdaw (*C. monedula*) present in district Mansehra, Pakistan. Crows were trapped for blood sampling. The total genomic DNA was isolated from the blood of each species. The RAPD -PCR analysis of isolated DNA was performed for genetic diversity estimation. All the amplification profiles were observed and genetic distances were estimated. Results of RAPD analysis revealed high level of genetic polymorphism among the three species. The average genetic distance estimates ranged from 50-90%. Phylogenetic relationship was elaborated through dendrogram which supports the genetics distances. The dendrogram showed that house crow and jungle crow share much genetic affinities to each other than to jackdaw. The results also revealed the RAPD markers as effective for such types of studies where an overall picture of genome is required.

Key words: *Corvus macrorhynchos*, *Corvus splendens*, *Corvus monedula*, genetic diversity, phylogeny, RAPD

Introduction

Crows belong to genus *corvus* in family Corvidae. Corvids are most numerous and one of the most widely distributed group of passerines in the world. Family Corvidae consists on more than 100 species and 25 genera [1, 2]. There are uncertainties about the exact evolutionary history of corvids, however much of

available literature [1,3-8] agrees with that the oscine passerine ancestors of corvids had been isolated on Australo–Papuan region of ancient Gondwanaland or Supercontinent. When Australian tectonic plate split from Antarctica in Tertiary period and came close enough to Asia, the oscines passerine migrated into Asia where they evolved into corvids. The corvids then dispersed into

other parts of the world and also re-entered into Australia. In food habit crows are omnivorous. They fly about up to 20 km for feeding around their roosting site. Crows are considered the cleverest and most intelligent among the birds and there are several behaviors of crows in support of their intelligence like: their interaction with predators to lead them to a carcass, presence of guards in flocks which warn the feeding flocks at any sign of danger, the stay of offspring with adult as nest associates, ability of learning various symbols associated with food, cracking the tough nuts by dropping them into heavy traffic, tool making skills and recognizing the individual human from another by facial features [9-11]. The geographical range of corvids contains a variety of climatic zones including the continental as well as isolated islands. Spiridonova et al [12] proposed the jungle crow as a model to study the microevolutionary processes. They picked the idea from restricted populations of jungle crow on isolated islands.

Current study was conducted upon three species of crow, jungle crow (*Corvus macrorhynchos* Wagler, 1927), house crow (*C. splendens* Vieillot, 1817) and jackdaw (*C. monedula* Linnaeus, 1758) present in

district Mansehra, Pakistan. The house crow and jackdaw are found in urban areas of the district while jungle crow is present in mountains. Crows are the serious avian pest in Pakistan [13] and house crow is among the world's worst 100 invasive alien species [14]. Despite their unusual intelligence, fascinating behavioural adaptations and attraction for general biologists, we still lack in adequate knowledge about the evolutionary patterns and phylogenetic relationships of corvids [1]. Several kinds of data are used to measure the genetic variation among the organisms including morphological and biochemical diversity but polymorphism is greater at the DNA level. Avise [15] described a number of genetic markers which have been extensively used by researchers to study the genetic variation in populations and phylogenetic relationships among the taxa. In current study we used the Randomly Amplified Polymorphic DNA (RAPD) analysis to estimate the genetic diversity and to establish DNA based phylogenetic relationship among the three species of crow.

Materials and Methods

Crows were trapped for sampling. Blood samples were obtained from the ulnar superficial vein (wing) and transferred into 4 ml EDTA (Ethylene Diamine Tetra Acetate) tube. Samples were then stored in refrigerator at -20°C . Total genomic DNA from blood was manually extracted in following steps. Three hundred μL blood was taken in an Eppendorf tube and then 300 μL DNA extraction buffer (20% SDS, 100 mM Tris-Cl, 400 mM NaCl, 10 mM EDTA, pH = 8.5) was added to the Eppendorf tube and mixed well with blood. In next step, 300 μL of Phenol: Chloroform: Isoamylalcohol mixture (in ratio of 25: 24: 1) was added (see appendix) and Eppendorf tube were continued to shake until homogenous mixture was obtained. The tubes were then centrifuged at 12000 rpm for 5 minutes. The aqueous phase was transferred into new tubes. In next step 30 μL 3M sodium acetate (pH = 4.8) and 300 μL isopropanol was added and mixed gently. The precipitate of DNA was observed. Tubes were then centrifuged at 12000 rpm for 5 minutes to make the DNA pellet. Supernatant was discarded and DNA pellet was washed with 70% ethanol and dissolved in 50 μL TE buffer. Quality and quantity of the DNA was checked on 1%

agarose/TBE gel. A total of 15 RAPD primers (obtained from Gene Link, Inc, 10532, NY, USA) were screened and among them only 5 gave the good, reproducible and polymorphic bands. These primers were: GLB-14, GLC-11, GLC-15, GLC-16 and GLC-19 (Table 1). Thermo cycling was carried out by using Applied Biosystem Thermal Cycler (model 2720) and consisted of three steps: (i) Denaturation of double stranded genomic DNA template at 95°C ; (ii) Annealing of randomly amplified polymorphic DNA (RAPD) primer with the template DNA at 32°C and (iii) Extension of primer at 72°C . The Cycling was continued for 40 cycles. Amplicons were separated on 2% agarose gel stained with ethidium bromide. Genetic diversity among the genotypes was estimated by using unweighted pair group of arithmetic means (UPGMA) as described by Nei and Li [16]. Dendrogram was constructed with the help of "Popgene ver 3.2" [17].

List of the reagents used for analyzing crow genome

DNA extraction Buffer

Tris Pure	1.21 gm
NaCl	0.52 gm

EDTA Na ₂ salt	0.32 gm	Tris (base)	4.8 gm
SDS	1.0 gm	Boric Acid	2.7 gm
Distilled water	100 ml	EDTA 0.5 M solution	2 ml
Adjust pH to 8.5 using HCl and stored at room temperature		Distilled water	450 ml

Phenol: Chloroform: Isoamylalcohol

Phenol	
Chloroform	96 ml
Isoamylalcohol	4 ml

Stored at 4°C

3M Sodium Acetate

Sodium Acetate	40.8 gm
Distilled water	100 ml

Adjust pH to 4.8 using acetic acid and stored at room temperature.

1XTBE Buffer

Loading Dye

Bromophenol blue	0.6 gm
Glycerol	15 ml
5X TBE	20 ml
Distilled water	65 ml

Stored at room temperature

Ethidium bromide

Ethidium Bromide	10 mg
Distilled water	100 ml

Stored at room temperature.

Table 1. List of primers used in the present study

S. No	Oligo name	Sequence(5'–3')	Mol. w.t	% GC
1	GLB-14	TCCGCTCTGG	2,995	70
2	GLC-11	AAAGCTGCGG	3,013	60
3	GLC-15	GACGGATCAG	3,077	70
4	GLC-16	CACACTCCAG	2,957	60
5	GLC-19	GTTGCCAGCC	3,004	70

Results and Discussion

The generated RAPD profiles were observed and analyzed. An example of such amplification profile is represented in Figure 1. Data from these profiles was used to assess similarities among the crow species by genetic distances (Table 2) and dendrogram (Fig. 2). A total of 31 alleles were amplified in 3 genotypes by using 5 RAPD primers giving an average of 6.2 alleles per primer. The size of amplicons was estimated by using 1 kb DNA ladder. The amplified fragments ranged in size from >200 to >1000 bp. Relatively little amplification was observed from PCR amplification profile of RAPD primer GLC-19, as 5 alleles were amplified in three genotypes having an average of 1.6 alleles per genotype. Genetic diversity estimates

from the five RAPD primers ranged from 20–100%. The average minimum genetic diversity was found 50% between jungle crow and house crow and the average maximum genetic diversity was observed 90% in two comparisons, one between jungle crow and jackdaw and other between house crow and jackdaw (Table 2). The phylogenetic relationship among the three species of crow was studied by constructing the dendrogram (Fig. 2) in computer software “Popgene 3.2”. The genetics distances are supported by the dendrogram which consists of two main groups. In one group the jungle crow and house crow are clustered together while in remaining one the jackdaw is placed.

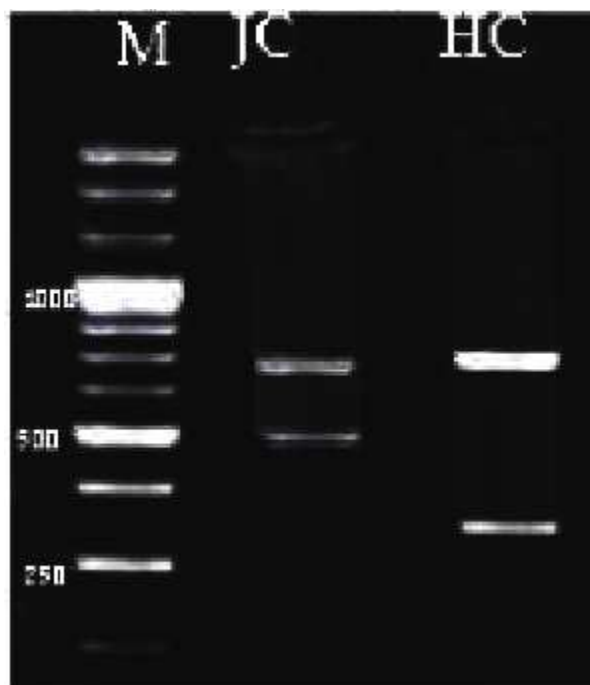


Fig. 1. Polymerase chain reaction (PCR) amplification profile of three spp of crow by using randomly amplified polymorphic DNA (RAPD) primers GLC-19 during present study: jungle crow: JC; house crow: HC; jackdaw: JD

The phylogenetic of corvids has remained controversial among the researchers [2]. Phylogenetic relationships among and within genera of corvids were addressed in several molecular studies [1, 2, 8, 18-20] reported that the study of phylogenetics of corvids demands detailed analysis of molecular characters,

morphological traits and vocalization. In present study we assessed the genetic diversity and phylogenetic relationship among the said three species of crow through RAPD molecular approach [21]. Various techniques in the past were developed and utilized for analyzing DNA. The choice of any one of them for specific use depends upon the material being studied and the nature of question being addressed. The molecular biologists have vigorously been utilized the techniques like restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellites and RAPD, since their development for the molecular characterization of species. The RAPD technique is not only easier, user-friendly and more convenient but also has an added advantage that it doesn't require any previous knowledge about the genome. Hence it is very suitable for characterizing those species on which no work has been done so far. Spiridonova et al [12] have also applied the RAPD technique to analyse the genetic diversity in carrion crow, *C. corone* and jungle crow, *C. macrorhynchos* in Russia. Our results indicate that there is a high amount of genetic diversity ($GD_{max} = 100\%$) present among three species of crow.

It is obvious from phylogenetic analysis that house crow and jungle crow show much genetic affinities to each other than to jackdaw. If we observe the morphological characters or social behavior of three species we would find the jungle crow and house crow more related in general body morphology, food habit, nesting habit and vocalization as compared to jackdaw. Ericson et al [1] presented hypothetical intergeneric relationships among various species of Corvidae based on cytochrome *b*

gene of mitochondrial genome. Their results also clustered the jackdaw into a separate group than other species of the genus. Haring et al [2] also clustered the two species of jackdaw separately than other species of genus *corvus*. The present study also agrees with it that DNA based characterization could be used as useful tool in biosystematics especially for those newly discovered species whose morphological characters are confusing to classify them properly.

Table 2. Average estimates of genetic distances among three *spp* of crow by using five randomly amplified polymorphic DNA (RAPD) primers during present study

	JC*	HC
HC	0.5	-
JD	0.9	0.9

*Jungle crow: JC; house crow: HC; jackdaw: JD

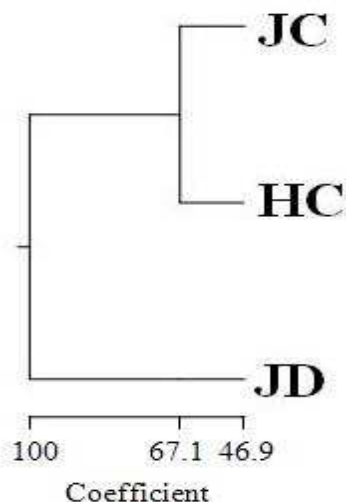


Fig. 2. Dendrogram constructed for three spp of crow based on data obtained by using five randomly amplified polymorphic DNA (RAPD) primers during present study: jungle crow: JC; house crow: HC; jackdaw: JD

We also suggest the mitochondrial genome based analysis as well as inter species genetic diversity estimation of the three

species of crow so that a better picture could emerge regarding phylogentic analysis of the three species.

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