



Research Article

Isolation of DNA from fallen feathers and the impact of environmental factors on its polymerase chain reaction (PCR) amplifiability

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Abstract

This study aimed to isolate genomic DNA from fallen feathers collected from various avian species and locations, examining the influence of collection time and location on DNA quality and quantity. The DNA quality and quantity were assessed using a nanodrop spectrophotometer, and PCR amplification was conducted using universal GAPDH primers to evaluate the samples suitability for downstream applications. This study showed DNA concentrations ranging from 15 to 254 ng/μl with OD260/280 values between 1.38 and 1.94. Higher DNA yields were linked to samples collected from nearby, low-traffic areas, indicating that transit time and environmental exposure affect DNA quality. Pigeons and parrots had higher PCR amplification success compared to Kadaknath and desi chickens. It was concluded that feathers as a viable source of DNA for avian species, emphasizing the importance of optimal collection and storage conditions. A multi-sampling approach is recommended to counteract environmental impacts on DNA quality.

Keywords birds, DNA quality, fallen feathers, feather DNA

Introduction

Isolation of genomic DNA is one of the most basic and important technique in molecular biology. A good quality and quantity of DNA is the foremost requirement for most of the downstream processes. Although obtaining samples for DNA isolation is relatively easy in domestic animals, the task becomes challenging in wild animals, especially if the target species is of the avian family. In wild and domestic birds, there are continuous efforts to standardize and adopt non-invasive sampling methods to reduce the stress of animal handling [1]. Some of the non-invasive sampling methods include the utilization of urine-soaked soil, skin pieces, hairs, post-mortem materials, and feathers in avians [2]. However, in the case of avian species, feathers seem to be the most appropriate sample for obtaining DNA for downstream processes. Numerous species of birds are rare or threatened or sensitive and there is a need for conservation and non-invasive methods of isolating DNA from feathers. As feathers can be collected in the field and cause no disturbance to the bird because there is no need for contact and it can be difficult to get permission for invasive methods of DNA isolation from protected birds [3-4]. DNA from cryptic or rare species can be collected using non-invasive methods and can be used for species identification [5], analysis of population structure, dispersal patterns [6], etc.

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The isolation of DNA from a feather of a bird is a simplified and easy task rather than from blood or tissue as it doesn't cause any pain or stress that can be caused by taking blood or making incisions for tissues. Using non-invasive (feathers, shed skin, or environmental DNA (eDNA),) methods are less risky and the sampling is easier than the invasive methods, as due to the small blood vessels of the small and young birds like finches and parrots, make the blood extraction difficult, likewise taking sample from large birds like ostrich can prove to be very difficult [7]. Feathers are made up of a central shaft (rachis) that comprises numerous barbs and a calamus that extends to the central shaft [8]. The calamus part of the feather are mostly used for DNA isolation but extraction of DNA can also be done by other parts of the feathers [9-11]. However, getting an intact or fresh sample is difficult in using non-invasive sample collection, as the sample can be exposed to various environmental conditions and decay. The likelihood of effective DNA extraction is also influenced by the type of feather used. Primary, secondary, and tail feathers of a bird are more advantageous for acquiring viable genetic material than plumulaceous feathers [1].

Although the implementation of a non-invasive method appears a direct and simplified strategy for obtaining DNA from wild/domestic avian species, it has got its own set of constrains. Sometimes the morphological assessment of feathers is not possible due to similar structures between different species, exposure to the environment and local temperature & humidity may affect the total yield [11]. Samples lying in humidity and heat may not yield the proper quantity and quality of DNA for downstream application [12]. Feather samples are often soiled, moist, and thriving with microbial and fungal load, thus making too much interference in isolating pure target species DNA. Thus, this study was planned to isolate genomic DNA from fallen feather samples and the effect of location as well as time of collection on the quality and quantity of DNA. Further, we also assessed the PCR amplifiability of the samples using universal primers to ascertain their downstream utility.

Methodology

Samples collection

Feather samples (n= 36) were collected from various locations and time period, which included Animal Biotechnology Centre (ABT), JNKVV Campus, Adhartal, Jabalpur (approximate location 23° 12' 58'' N & 79° 57' 43'' E), Poultry Farm (PFN), NDVSU, Adhartal, Jabalpur (approximate location 23° 12' 52'' N & 79° 57' 17'' E), Nayagaon Jabalpur (NGJ) (approximate location 23° 02' 59'' N & 79° 48' 56'' E), Jabalpur city (JC) (approximate location 23° 10' 13'' N & 79° 56' 00'' E), Bhopal city (BC) (approximate location 23° 12' 58'' N & 77° 24' 56'' E) and Ramnagar, Mandla (RNM) (approximate location 22° 36' 41'' N & 80° 30' 30'' E). Fallen feathers were collected and labelled in plastic zip lock bags and brought to the lab for further processing; care was taken to select only complete, undamaged & morphologically intact, and identifiable feathers.

Isolation of DNA

DNA was isolated from feather samples using the protocol described by Bello et al., [7] with certain modifications as per lab standardized protocol. In brief, a 1 cm piece was cut from the base of the feather, cleaned with 500 µl of phosphate-buffered saline (pH ~ 7.4) (if required), the piece was cut into smaller manageable pieces and 500 µl of lysis buffer (50 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2% SDS) was added to it with 20 µl proteinase K (20 mg/ml). The samples were incubated overnight at 40 oC. The next day, the samples were vortexed vigorously for 15 minutes and then centrifuged at 10,000 rpm for 15 minutes. The upper phase was separated and washed with phenol: chloroform: isoamyl alcohol (25:24:1). The aqueous phase was taken and DNA was precipitated using 5 µl sodium acetate (3M) and 0.6 times 100% isopropanol. The samples were incubated overnight at -20°C and then centrifuged at 10,000 rpm for 10 min at 4°C to make a DNA pellet. The DNA pellet was washed in 70% ethanol and resuspended in 30 µl of nuclease-free water.

The quality and quantity parameters were ascertained using a nanodrop spectrophotometer (ND-2000).

PCR amplification

To check the integrity of DNA for downstream applications, it was used as a template in polymerase chain reaction (PCR) using designed primers of housekeeping gene GAPDH (F:5'-ACATGGCATCCAAGGAGTGAG-3', R: 5'-GGGGAGACAGAAGGGAACAGA-3'). A typical PCR reaction mix consisted of 12.5 µl of PCR master mix (Thermo-Fisher), 10 pmol of both forward and reverse primers, 90-120 ng of template DNA, and nuclease-free water to make a total reaction volume of 25 µl. The reaction mix constituents were gently thawed and briefly centrifuged; each reaction tube was prepared with appropriate markings. The PCR programme used for the amplification of targeted loci consisted of initial denaturation of 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, cycles repeated for 35 times, followed by final extension at 72°C for 5 min and hold at 4°C. For separation and visualization of products, 1.5% horizontal agarose gel electrophoresis was used, and the gel was visualized under the gel doc system.

Statistical analysis

The effect of various factors on DNA quantity and quality parameters was ascertained using an appropriate model in IBM SPSS v 24. The graphical representation of data was done by SPSS v 24 internal package and flourish data visualization software.

Results and Discussion

Quality, and quantity of isolated DNA

DNA was isolated from feather samples of all the species. A total of 36 samples were processed, the maximum from ABT (Nos. 14) and a maximum number of samples were collected during May (Nos. 12). A qualitative detail of samples collected for the current study, their species, location, and month of collection is given in Figure 1.

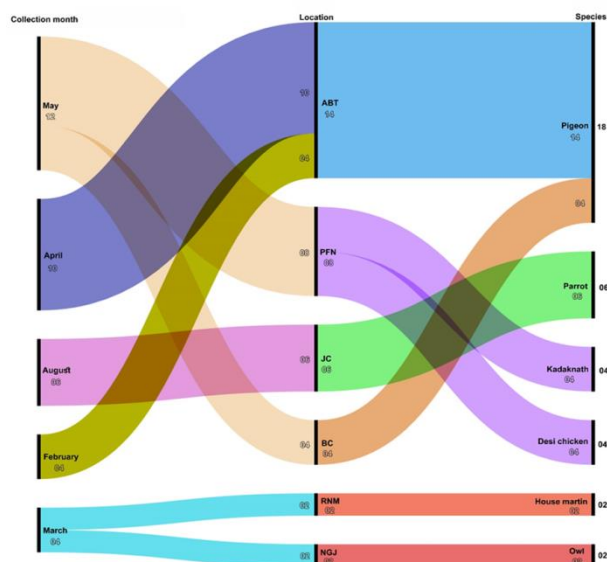


Figure 1. Qualitative details of samples collected as per their geographical distribution, time of collection, and species. (The black vertical bars indicate divisions/ classes viz., collection month, location, and species. Numbers on the right of the bars indicate the total number in the respective class, and numbers to the left indicate the breakup as per the previous class)

The black vertical bars indicate divisions/ classes viz., collection month, location, and species. Numbers on the right of the bars indicate the total number in the respective class, and numbers to the left indicate breakup as per the previous class (Figure 1). Evidently, the maximum number of samples obtained was of pigeons, mainly from ABT, in the month of April. The DNA quality and quantity were measured

using a nanodrop ND-2000. The overall concentration values were then mapped against OD_{260/280} to ascertain the general trend. The DNA concentrations were varying between 15 to 254 ng/μl, and the O.D values were varying between 1.38 to 1.94. The major clustering was observed between concentrations 20 to 40 ng/μl, and OD 1.65 to 1.75 (Figure 2).

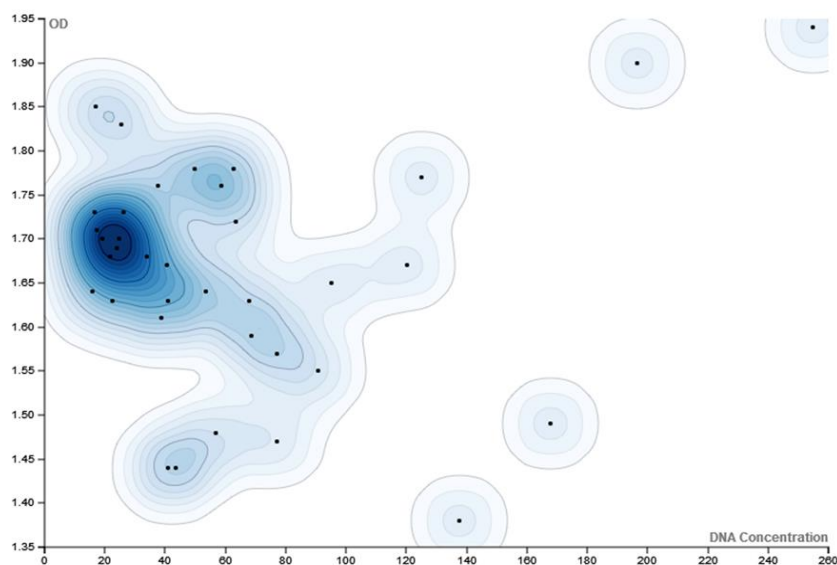


Figure 2. Distribution of DNA concentrations and O.D among studied samples

Bello et al., [7] reported DNA yield to be varying from 1 to 100 μg in 120 different species of birds. De Volo et al., [13] standardized a protocol for DNA isolation in moulted feathers and reported an average yield of 24 ng/μl, they also reported that tail feathers yielded more DNA as compared to feathers from other parts. With the aim of utilizing DNA for conservation studies, Peters et al., [1] optimized the protocol for DNA isolation from moulted feathers. Utilizing a commercial kit but optimizing the protocol by user-developed method, they were able to increase mean DNA yield from 4.11 to 39.75 ng/μl.

PCR amplifiability using housekeeping primers

PCR amplifiability has been one of the most important parts for downstream DNA analysis, and it has been used for analysis of DNA from un-common tissue samples [14]. Studies related to the conservation of species mostly target mitochondrial DNA or microsatellite loci for genetic analysis [13]. The present study tested the PCR amplification efficiency of isolated DNA samples with respect to GAPDH gene (found in both, cytosol, and mitochondria). The primers were able to successfully amplify a region of 152 bp of the GAPDH gene in the current study (Figure 3). There were a total of 22 positive amplifications, and the rest 14 were negative. The positive amplifications mostly consisted of pigeon samples, collected during February, April, and May, that too from nearby locations (ABT and BC). Other samples included; feathers of parrots (n=6) collected from a nearby locality during August month. Interestingly all the parrot samples were positively amplified. The negative samples predominantly comprised kadaknath and desi chickens. In house martin and pigeon, the quality and quantity both were very low and hence these samples did not show GAPDH amplification. Apart from

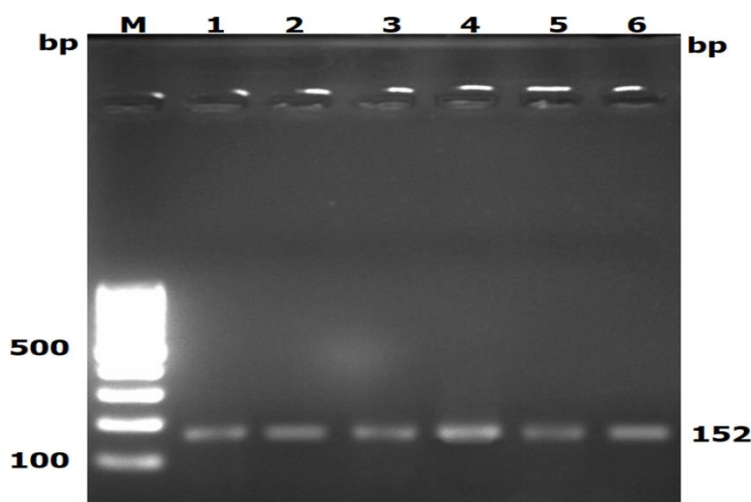


Figure 3. Gel doc image of PCR amplification of GAPDH primers in different DNA samples.
Where, M – 100 bp marker; Lane 1,2 – Pigeon DNA; Lane 3,4 – Parrot DNA; Lane 5,6 – Owl DNA

negative pigeons, both house martin samples were collected from far areas, extending beyond 100 km, hence there are fair chances of sample quality degradation was also visible during morphological observations. For *Kadaknath* and *desi* chicken, the GAPDH amplification was negative, irrespective of quality and quantity, the same was expected as the GAPDH primers available for the study, were inefficiently binding to the target species i.e., poultry (*Gallus domesticus*) during in silico analysis (Figure 4).

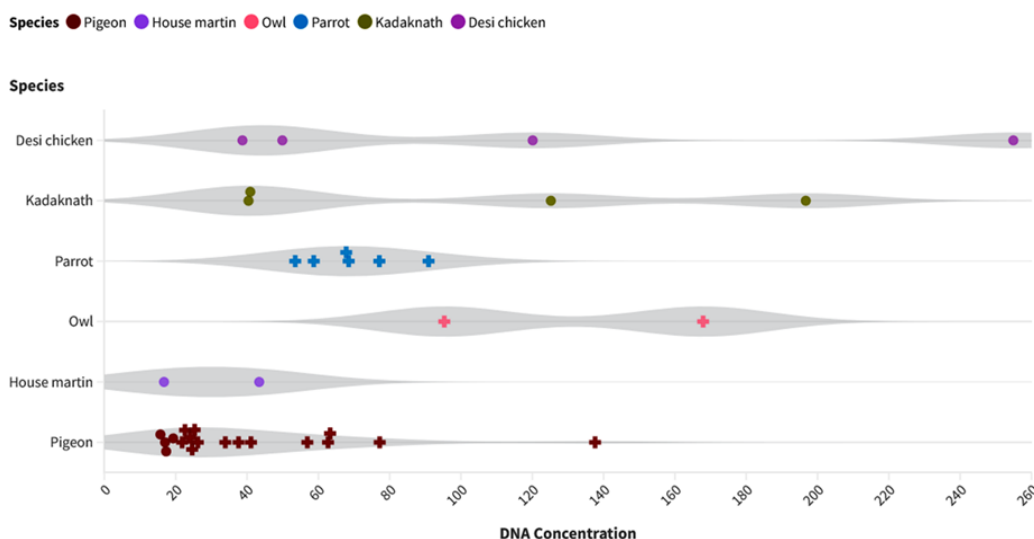


Figure 4. Amplification of GAPDH gene as across DNA concentration in various studied species.
(A plus indicates positive amplification and round indicates negative amplification)

Due to these reasons, an overall analysis of concentration vs O.D. showed that some samples, even when having optimum O.D. & concentrations, does not show amplification in PCR using GAPDH primers (Figure 5). Additionally, the effect of the month, species and location was ascertained over total DNA yield using SPSS 24.0 software using linear model design. It was observed that among the

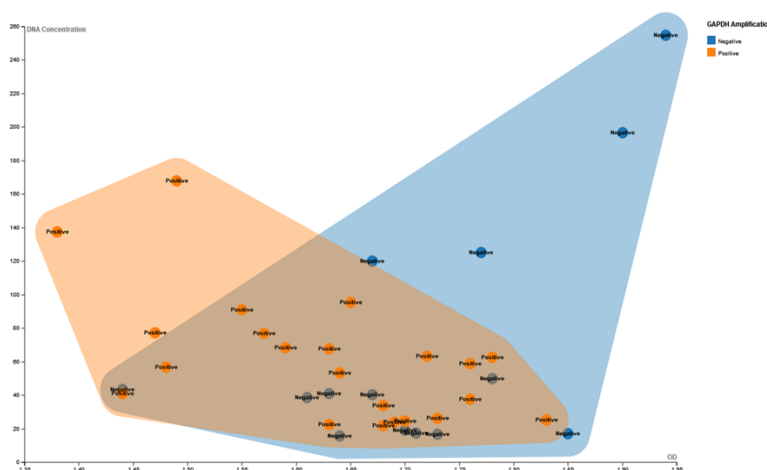


Figure 5. Correlation Between DNA Concentration and OD with GAPDH Amplification Status

(There is a significant overlap between the positive and negative samples in the mid-range of both OD and DNA concentration, indicated by the brown region where blue and orange areas intersect)

months, May had the highest mean DNA yield and April had the lowest which differed statistically but they did not differ from March. Between the species, an owl had highest DNA yield, and house martin had the lowest yield. Among the locations, Nayagaon Jabalpur had the highest yield and Ramnagar (Mandla) had the lowest ($P < 0.01$) (Table 1). Clearly, it was observable that locations that were near the processing lab and had less human traffic (which can damage the feather sample) had better yields as compared to the other ones (Table 1).

Table 1. Mean and SE of concentration DNA obtained for different months, species, and locations of collected samples

Month of DNA isolation	Mean Concentration (ng/μl)
April	33.91 ^a ± 14.79
March	60.35 ^{ab} ± 17.50
May	70.97 ^b ± 12.08
Species	
Desi chicken	115.90 ^{bc} ± 23.39
House martin	30.05 ^a ± 33.08
Kadaknath	100.80 ^{abc} ± 23.39
Owl	131.60 ^c ± 33.08
Parrot	69.41 ^{abc} ± 19.10
Pigeon	40.46 ^{ab} ± 14.79
Location	
Animal Biotechnology Centre	43.50 ^{ab} ± 18.86
Bhopal City	34.37 ^{ab} ± 23.39
Jabalpur City	69.41 ^{abc} ± 19.10
Nayagaon Jabalpur	131.60 ^c ± 33.08
Poultry Farm, NDVSU	108.35 ^{bc} ± 16.52
Ramnagar Mandla	30.05 ^a ± 33.08

*Values having different letter superscripts differ statistically (At $P < 0.05$)

It is reported that generally, the larger feathers yield better quality and quantity of DNA [13]. A similar trend was seen in the present study too. It has also been observed that recently fallen feathers yield better results as compared to the ones that have undergone some level of degradation [13]. Also, Hogan et al., [15] have concluded that feather condition and type of marker may be the limiting factor during genomic DNA isolation from fallen feathers. A similar observation was noted in the present investigation which indicated that a single universal marker may not be suitable and multiple loci and multiple sampling approaches may have to be adopted for better results.

Conclusion

It may be concluded that in tropical conditions, the quality of genomic DNA was influenced by transit time (distance between the sample collection site and laboratory), ambient temperature, and location. Hence, multiple-sampling approach should be adopted to nullify the effect of the environment on the quality parameters of genomic DNA.

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