MOLECULAR ANALYSIS OF BIRDS FEATHER BY USING RAPD ANALYSIS

Flight affords the utmost in mobility and has made possible the evolution of avian migration as a means of exploiting distant food resources and avoiding the physiological stress associated with cold weather.

Feathers , the trade mark of Aves, provide the insulation necessary to maintain a high " Engine"(body) temperature, ranging from 107 to113˚F across species . Additionally ,the long feathers of the wings acts as air foils which helps to generate the lift necessary for the flight.

Well-developed pectoral muscle power the flapping motion of the wings. They are light but very strong, and they are flexible but very tough. Birds take a lot of time caring for their feathers. This is called preening. They use their feet and beaks to arrange their feathers.

Avians are warm-blooded. This means that their body temperature remains the same even in differing temperatures. There are 8600 species of birds in the world today. They play a vital role in balancing the nature. They nibble each feather from the base of the tip.

Feathers grow only in certain areas in birds called "Feather Tracks". A streamlined body shape and a lightweight skeleton composed of hollow bones minimize air resistance and reduce the amount of energy necessary to become and remain airborne.

All birds have wings, but not all birds fly. Feathers , protect the bird's skin and insulate them. Feathers can be fluffed up in the winter or squeezed down in the summer. Feathers are also used to line nests. Feathers are made of a tough and a flexible material called 'Keratin'. The spine down the middle, called 'Shaft' which is hollow.

The vanes are the two halves of the feather.The vanes are made of thousand of branches called 'Barbs'. Feather grows from a bump in the skin. Growth begins with

the tip of the feather. When a bird hatches , the tip separates and looks like fuzz on the baby bird. This is soft towards the down.

The tip forms a tough, protective sheath. As the tip continues to grow, the downy fuzz is pushed ahead of it. Each feather is rolled inside the sheather. It is called a pin feather at this stage.

Feathers are used for pillows, quilts and clothing. A Birds survival depends upon the condition of its feather. Birds have between 1,000 - 25,000 feathers . The bird's wing is the basic structure for flight. It is the shape of the wing that allows a bird to fly .

The use of feathers in veterinary clinical practice simplifies the sampling of avian genomic DNA, especially when blood extraction is difficult because of the age or the size of the bird.

A rapid and accurate protocol was used to isolate high-quality genomic DNA from feathers. Purification of the genomic DNA is performed with phenol: chloroform: isoamyl alcohol extraction and phenol precipitation. This protocol consistently provided significant amounts of high-quality genomic DNA from more than 800 birds belonging to 120 different species.

Genomic DNA isolated with this method was used for southern blotting and also in several polymerase chain reaction systems devoted to sex determination and paternity testing(W.Duan, P.A.Furest, april 3,2001) .

Previously , chicken sexing has been an integral part in the breeder, broiler and layer industry since 1935. There are several primers used both in the case of cranes and chicken for sex determination.

Feathers can be divided into six major categories:

Contour Feathers

Semiplume Feathers

Down Feathers

Filoplume Feathers

Bristle Feathers

Powder-Down Feathers

Not all birds have all the types of feathers. It depends on what type of bird it is. But all birds have feathers on their wings. The shape of the wing is made by the feathers.

Feathers are a difficult material to isolate DNA from, since only the feather tip contains DNA. Attached to the outside are old skin cells. While inside are old blood cells, from when the feather was still growing .

However, feathers often advantages compared to the blood samples because they can be collected much earlier from young chicks and DNA analysis can be performed at a very early stage (Mervi Honkatukia,Johanna Kulmala,2009). Isolation of DNA is done from five different bird feathers such as

- Chicken
- Rosy Pelican
- Emu
- Spot-billed pelican
- Cormorant.

CHICKEN: (*Gallus gallus domesticus*)

Convervation status : Domesticated

Scientific classification:

Kingdom : Animalia

Phylum : Chordate

Class : Aves

Order : Galliformes

Family : Phasianidae

Genus : Gallus

Species : Gallus gallus

Sub- species: *Gallus gallus domesticus (Linnaeus,1758)*

The chicken (*Gallus gallus domesticus)* is a domesticated fowl, a sub-species of red jungle fowl. As one of the most common and widespread domestic animal, and with a population of more than 24 billion in 2003.

ROSY PELICAN: *(Pelecanus conspicillatus)*

Kingdom : Animalia

Phylum : Chordate

Class : Aves

Order : pelecaniformes

Family : Pellicanidae

Genus : Pelecanus

Species : *Pelecanus conspicillatus*

Binomial name : *Pelecanus consicillatus* (Temminck,1824)

The Australian pelican (*Pelecanus consicillatus*) is a large water bird, widespread on inland and coastal waters of Australia and new guinea, and Indonesia.

EMU: *(Dromaius novaehollandiae)*

Kingdom : Animalia

Phylum : Chordate

Class : Aves

Order : Struthioniformes (or casuariforms)

Family : Casuariidae

Genus : Dromaiius

Species : *Dromaius novaehollandiae*

Binomial name: *Dromaius novaehollandiae* (Lantham, 1790)

The Emu (*Dromaius novaehollandiae)* is the largest bird native to Austraslia and the only extant member of the genus Dromaius. It's the Third- largest extant bird in the world by height, after its ratitie relative, the Ostrich.

CORMORANT: *(Phalacrocorax melanoteucos)*

Kingdom : Animalia

Phylum : Chordate

Class : Aves

Sub – class : Neorinthes

Infra-class : Neoaves

Order : Pelecaniformes

Sub-order : Sulae

Family : Phalacrocoracidae (Reichenbach,1850)

Genus : Phalacrocorax (Brisson,1760)

Species : *Phalacrocorax melanoleucos*

The bird family Phalacrocoracidae is represented by some 40 species of cormorants and shags. They are medium to large sea birds. The bill is long , thin and sharply hooked.

SPOT-BILLED PELICAN: (Pellicanus Philippensis)

Kingdom : Animalia

Phylum : Chordate

Class : Aves

Order : Pelecaniformes

Family : Pellicanidae

Genus : Pelecanus

Species : *Pelecanus philippensis*

Binomial name : Pelecanus philippensis (Gmelin ,1789)

The Spot-billed Pelican or Grey Pelican *(Pelecanus philippensis)*is a members of the pelican family. It breeds in southern Asia from southern Pakistan across India east to Indonesia. It is a bird of large inland and coastal waters, especially large lakes. At a distance they are difficult to differentiate from other pelicans in the region although it is smaller but at close range the spots on the upper mandible, the lack of bright colours and the greyer plumage are distinctive. In some areas these birds nest in large colonies close to human habitations.

DNA FINGERPRINTING (RAPD) ANALYSIS:

Random amplification of polymorphic DNA (RAPD) is the common molecular approach employed Genotypic differentiation , Gene linkage analysis, Assigning evolutionary and taxonomic affinities and DNA Fingerprint analysis.

In the field of biotechnology there are several DNA isolation method with variant principle protocols (Doyle and Doyle.1987). RAPD analysis is simpler and faster to perform other than DNA fingerprinting techniques. This technique requires no sequence information prior to analysis and only a minute amount of DNA is needed (Williams et.al., 1990).

DNA quality is to be considered as the main factor that may affect the results. On the other hand, recent results indicate that RAPD fingerprints may also be influenced by differentiation and has been successfully used in a variety of taxonomic and Genetic diversity studies.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The use of feathers in veterinary clinical practice simplifies the sampling of avian genomic DNA, especially when blood extraction is difficult because of the age or the size of the bird. A rapid and accurate protocol was used to isolate high-quality genomic DNA from feathers.(Natalia Bello, et.al., 1999)

The protocol consistently provided significant amounts of high-quality genomic DNA from more than 800 birds belonging to 120 different species. Genomic DNA isolated with this method was used for southern blotting and also in several polymerase chain reaction systems devoted to sex determination and paternity testing. (Natalia Bello, et.al., 1999)

The use of genetic material as a diagnostic tool for disease becomes a necessity. First, purification of DNA with the classical methods is difficult and time consuming. In addition, the use of reagents in the classical method can be dangerous to researchers because of the use of organic compounds are harmful and increase the risk of transmission of DNA from sample to sample so that false positive results can also occur (Bernade, et.al., 2010)

The arrival of humans and their associated mammalian species in New Zealand at approximately AD 1280 (Wilmshurst *et al*. 2008) resulted in the extinction of 41 per cent of New Zealand's breeding bird species (Tennyson & Martinson 2006). These species are relatively well known osteologically because of New Zealand's rich late Quaternary avifaunal fossil record (Worthy & Holdaway 2002).

While most are known only from their bones, a few partially mummified remains have also been found (Anderson 1989*;*Worthy 1989*;* Vickers-Rich *et al*. 1995), while isolated feathers have been recovered from a range of late Holocene rockshelter sediments (Wood 2008*;* Wood *et al*. 2008).

The majority of subfossil feathers found in New Zealand have been attributed to the extinct palaeognathus (ratite) moa (Aves: Dinornithiformes), although none has been confirmed genetically. If DNA could be recovered routinely from subfossil feathers, it would create many opportunities for genetic studies of extinct taxa and populations, as well as providing an important insight into the appearance of extinct species. *(Nicholas J. Rawlence et al.2009)*

DNA has been extracted previously from the base, or calamus, of feathers from modern and historical museum specimens (Payne & Sorenson 2002*;*Sefc *et al*. 2003*;* Horváth *et al*. 2005*)*. Extraction protocols have generally ignored the distal components of the feather, such as the rachis and barbs (including barbules and barbicels), which comprises the bulk of the feather structure, because it has been thought that there is no amplifiable DNA in these structures.

The distribution of mitochondrial DNA (mtDNA) throughout paralogous structures like hair (Gilbert *et al*. 2007) and reptilian scales (Fetzner 1999*;* Feldman & Spicer 2002) raises the possibility that mtDNA might also be present in all parts of the feather structure. Ancient DNA can be a powerful tool when reconstructing the phenotype of extinct and extant species.

It has been used previously to reconstruct mammoth and horse coat colour (Rompler *et al*. 2006*;* Ludwig *et al*. 2009) and to suggest that Neanderthals had differing degrees of skin and hair pigmentation (Lalueza-Fox *et al*. 2007*).*These studies used ancient DNA to identify colour genes, including the melanocortin-1 receptor (*MCR1*) gene, rather than directly linking hair samples of known colour to an extinct species of unknown external appearance.

Recently, it has been demonstrated that even fossilized feathers from the Cretaceous and Eocene (Vinther *et al*. 2008) can be preserved as carbonaceous traces of melanosomes (pigment containing organelles). When compared with modern taxa, such fossils provide information on the colour pattern of the original feather (Vinther *et al*. 2008*)*.

However, reconstructions of specific taxa using this methodology are only possible if the fossilized feathers can be identified positively to species. When there is no fossil feather information, reconstruction attempts have relied on the phenotype of closely related extant taxa (Vickers-Rich *et al*. 1985*;* Gill & Martinson 1991*;*Flannery & Schouten 2001*;* Murray & Vickers-Rich 2003*;* Tennyson & Martinson 2006*).*

In the rare cases in which subfossil feathers have been preserved, it has generally been assumed that the colours reflect accurately their original appearance (White 1885*;* Hamilton 1894). However, historical museum and subfossil feathers are prone to fading from exposure to sunlight or other factors (Oliver 1955*; G. Pohland 2007*), and it is conceivable that feathers excavated from cave sediment may have also altered.

One approach to investigating this issue is to use standardized Munsell colour chips *(*Villafuerte & Negro 1998*)* to compare subfossil and modern feathers from the same species and to quantify colour fading for a given site and horizon.

The molecular weight of the extracted DNA was higher than the conventional method and the yield of DNA was increased by more than one hundred fold. The DNA extracted by this method is applied to various samples, for instance, extracting DNA from bird feather in general. (*Tomoko Eguchi,2000*).PCR is a useful method for analysis of DNA and mRNA (Saiki et al.,1988).

The sequencing of mitochondrial DNA and other genes using the PCR technique has been reported for a wide variety of species (Olivo et al. 1983, Kocher et al.1989, Irwin et al.1990). usually , the template DNA for PCR is extracted from blood , liver, or other tissues.

Moa have been the focus of considerable palaeontological *(*Worthy & Holdaway 2002), palaeoecological (Wood *et al*. 2008*)* and evolutionary research *(*Cooper *et al*. 1992*;* Bunce *et al*. 2003*;* Huynen *et al*. 2003*;* Baker*et al*. 2005*;* Lambert *et al*. 2005*).* However, relatively little is known about their external phenotype or interspecific plumage variation.

Most mummified moa specimens, where skin is present, have just the bases of feathers preserved (Hutton & Coughtrey 1875; Forrest 1987). As a consequence, it has been difficult to assign isolated moa feathers to species.Ancient DNA analysis offers the potential to link these feathers to known fossil taxa and provides insight into their plumage and appearance.

To better understand where DNA is distributed in different parts of a feather, we also tested whether amplifiable DNA was detectable in the rachis and barbs of 10 moa feathers from one of the sites, as well as three modern emu feathers (Jamie R. Wood,2009).

To investigate the location of DNA in feathers, the rachis and barbs from a further 10 moa feathers from Sawers' rockshelter were examined in a later series of extractions. The two sets of samples were collected at different times from different excavations. Naturally shed modern emu (*Dromaius novaehollandiae*) feathers were also used as a positive control.(*Kyle N. Amstrong,2009).*

To determine whether DNA could be extracted from subfossil feathers, the calamus from the first nine moa feathers was cut in half longitudinally and further diced with a sterile scalpel blade to help facilitate enzymatic digestion.

To asses the value of molted feathers as a non invasive source of DNA for genetic studies of northern goshawks(accipter genilis), they isolated and quantified DNA from molted feathers and compared yields across five feathers types. They also compared PCR success across the same five feather types using five microsatellite genetic markers of varying size. In addition , they compared DNA yields from a commonly used extraction method versus one they modified to increase DNA yield.

All DNA extractions and the setup of PCR reactions were performed in the physically remote, isolated and dedicated ancient DNA facility using appropriate logistical and methodological procedures *(*Cooper & Poinar 2000*).* PCR amplifications targeted 31, 180 or 205 bp of the moa mtDNA control region (excluding primers) using the primer pairs 262F/294R (31 bp), 262F/419R (180 bp) and 185F/294R (205 bp; from Cooper *et al*. 2001*;* Bunce*et al*. 2003*;* Wood *et al*. 2008*)*

To test whether amplifiable DNA was present in the distal portion of both modern and subfossil feathers, an additional 10 moa and 3 modern emu feathers were divided into sections for separate DNA extractions. The entire distal portion (rachis and barbs) of the moa feathers was examined, while for emus, separate DNA extractions were performed on subsamples comprising: the calamus; the distal; and proximal halves of the rachis (only); and all of the barbs (Jamie R.Wood,2009).

A similar possibility exists for analogous studies of ancient DNA from mammoth *(*Gilbert *et al*. 2007*)* and thylacine hair *(*Miller *et al*. 2009*),* and historical and modern DNA from reptile *(*Fetzner 1999*;* Feldman & Spicer 2002*)* and fish scales *(*Yue & Orban 2001*).* In addition to DNA from subfossil bone *(*Baker *et al*. 2005*),* coprolites *(*Poinar*et al*. 1998*;* Hofreiter *et al*. 2000*)*, hair *(*Gilbert *et al*. 2007*)* and sediment *(*Willerslev *et al*. 2003*),* ancient DNA from subfossil feathers offer insights into the presence and absence of species on temporal scales.

For example, the taxonomic information from moa coprolites *(*Wood *et al*. 2008*)* and subfossil feathers from the same horizons in Roxburgh Gorge B and Sawers' rockshelters indicate that multiple species of moa are present, raising the possibility of competition between moa for prime rockshelter and nesting sites.

Interestingly, the success rate for extraction of amplifiable ancient DNA from subfossil moa feathers is much higher than coprolites excavated from the same horizons (4 of 21 coprolites versus 11 of 19 feathers from Central Otago rockshelters had amplifiable ancient DNA; Wood *et al*. 2008*).*

Furthermore, the size range of amplifiable mtDNA fragments from subfossil feathers is comparable to hair (60–130 bp; Gilbert *et al*. 2007*)* and coprolites *(*Poinar *et al*. 1998*;* Hofreiter *et al*. 2000*;* Wood *et al*. 2008*)*, depending on the age and preservation conditions of samples.

Agarose gel electrophoresis is a method used to separate DNA based on large molecules. The separation of molecules occurs through the movement of the negatively charged nucleic acids that penetrate the agarose matrix in the electrophoresis tank. Molecules with lower molecular weight will move faster.

After making gel electrophoresis, DNA can be run but first needed to be loading buffer (bromophenol blue) to provide the sample weight and as a marker until when electrophoresis can be stopped.*(Bernade,2010)*

The monomorphism of newly hatched chicks poses a problem for farmers who need to separate them. Currently, the most widely used technique is the cloacal sexing method which has a low accuracy rate. Thus, a PCR based gender determination protocol was established to obtain a higher accuracy rate than conventional sexing methods.

Feathers of a day up to a week old chicks were collected for the molecular analysis using the PCR targeting the CHD-W and CHD-Z genes in the chicken's chromosome Z and chromosome W, respectively.

The results of this study showed that the PCR based gender determination protocol was a sensitive and accurate method for determining the gender of monomorphic chicks as compared to the conventional sexing method. Richard Teh Swee Aun and Jayaraj Vijaya Kumaran330 Pertanika J. Trop.T.

To amplify both CHD-W and CHD-Z simultaneously with different product sizes for both sex chromosome linked genes in non-ratite avian. This results in females having double bands and males with a single band of PCR products after agarose gel electrophoresis due to the amplification of both targeted genes. Thus, an attempt was done in this study to test the application of the existing universal primers, described by Griffith et al. (1998).

In determining the gender of a day up to a week old chicks, as well as to compare the accuracy rate of this PCR-based sexing method with the conventional sexing method. Subsequently, two sets of primers were also designed to confirm the results obtained from the primers described by Griffith et al. (1998) and Richard et al. (2009).

To assess the value of molted feathers as a noninvasive source of DNA for genetic studies of Northern Goshawks (*Accipiter gentilis*), we isolated and quantified DNA from molted feathers and compared yields across five feather types. We also compared PCR success across the same five feather types using five microsatellite genetic markers of varying size.

In addition, we compared DNA yields from a commonly used extraction method versus one we modified to increase DNA yield. Results indicated molted feathers provided on average 24 ng µl-1 of DNA, which is a relatively high DNA yield compared to other noninvasive tissue sources.

Tail feathers yielded significantly more DNA than primary, secondary, and smaller feathers, yet all feather sizes produced equally high rates of PCR success. Although our modified extraction method increased the time required for processing feathers, it resulted in significantly higher yields of DNA as compared to the unmodified protocol.(Bayard De Volo, et.al., 2008)

On the other hand, conventional techniques to identify the sex of ostriches present significant problems: the animals may suffer stress, since they are subjected to invasive procedures such as endoscopy and cloaca touch. Furthermore, the bird's sex can be identified only in the adult stage, and even then only with a low rate of efficiency (in juvenile birds the rate of error may reach up to around 40%).

In the last few years, two reports described PCR-based methods to sex-type ostrichs]. Both these methods involved the use of DNA extracted from blood and they were not yet adapted for large-scale analysis. This paper presents an improved protocol, beginning with DNA extracted from feathers bulbs, which can provide sex-identification in the first days of nestling life. Furthermore, we used different control primers to determine the best sets for sex typing and to avoid any kind of spurious band. Finally, we adapted the procedure for use with 96 wells PCR plates, providing a large-scale method.(Flavio et.al., 2001)

METHODOLOGY

3. METHODOLOGY

ISOLATION OF DNA:

Collection of Feathers:

The feathers of five different kinds of birds were collected from a Zoo. The feathers are collected in such a manner that the tip is not damaged in either way.

PROTOCOL:

1. Feathers were plucked so that some cells / tissue from the feather follicle remained attached to the tip (Calamus).
2. The tip of the feathers are been diced up and 5 gram of the sample is taken in a mortar and pestle and grind them with 5 microlitre of Extraction buffer.
3. Transfer the mixture to a centrifuge tube (1.5ml) and incubate at -20 degree Celsius for 10-15 minutes.
4. Centrifuge the tube at 6,000 rpm for 5 minutes.
5. Discard the supernatant and to the pellet add 500 microlitres of Suspension buffer and 20 microlitres of 20% Sodium dodecyl sulphate, invert the tube and mix well.
6. Incubate at 62-65 degree Celsius for 20-30 minutes.
7. Then add 200 microlitres of 7.5M ammonium acetate and keep it at -20 degree Celsius for 10 minutes.
8. Centrifuge at 10,000 rpm for 10 minutes.
9. Transfer the supernatant to a new microcentrifuge tube and add equal amount of Phenol: Chloroform : Isoamyl alcohol (25:24:1) to precipitate the nucleic acid.
10. Mix well and centrifuge for 15 minutes at 1000 rpm.
11. Then transfer the aqeuous layer to a new centrifuge tube and add 2M Sodium chloride in equal amount.
12. Then centrifuge at 12,000 rpm for 10 minutes.
13. Wash the pellet with 75% ethanol, air dry and resuspend in 1X TE buffer.
14. To remove RNA 5 microlitres of DNAase free RNase A (10 milligram/ millilitre) was added to DNA.

DIALYSIS:

Dialysis is a process by which small molecules are selectively removed from a sample containing mixture of both small and large molecules. Dialysis is effectively accomplished using a special type of membrane known as semi-permeable membrane.

PREPARATION OF DIALYSIS TUBE:

- Cut the membrane tube into pieces (3-4cm length wise)
- Boil for 10 minutes in 2% Sodium carbonate and 10mM EDTA.
- Wash both sides with sterile double distilled water.
- Keep in 40% Ethanol and store it in refridgerator.

ELECTROELUTION:

- Wash the bag in TE buffer thoroughly (do not let it dry).
- Put the clip on one side of the bag.
- Put the “Cut gel piece” inside , add some TE buffer , put the clip on the other side of the bag(air bubble should not be trapped).
- Put the assembly in gel tank, run for one hour at 100V(tank buffer- 1X TAE buffer)
- Run at 100V, reverse polarity, for 1 minute to release the DNA from the wall of the bag.
- Open one side of the bag, drain (pipette out) the buffer containing the DNA.
- Give a water saturated Butanol wash (add equal amount, invert 5-10 times , keep undisturbed for 3 minutes) remove the upper layer containing Ethidium Bromide. Give Chloroform and take upper aqueous layer in new eppendorf .
- Precipitate using $1/10^{th}$ volume of 3M Sodium acetate and Iso propanol. Keep it in -20˚Celsius for O/N precipitation .
- Centrifuge at 12,000 rpm for 10 minutes at 4˚Celsius. Wash the pellet with 70% Ethanol. Dissolve the dried pellet in 20 microlitre TE buffer.

QUANTIFICATION OF DNA:

Materials:

i. DNase/RNase- free water.

ii. Deionised water for dilution of DNA prior to reading.

iii. One sterile 1.5 ml microcentrifuge tube for each sample.

iv. Plastic (for concentrated samples, > 50µg ml-1) or quartz (for low concentration samples, <50 µg ml-1) cuvettes.

v. Spectrophotometer.

vi. Microcentrifuge tubes(1.5 milliliters)

vii. Gloves

viii. Micropipettes and tips

ix. Vortex

Methodology:

Preparing DNA samples:

Remove the samples from the freeze and thaw them on ice or in the refrigerator.

Mix them by tapping the side of the tube with a finger. Do not vortex to mix.

DNA quantification:

- In a separate sterile 1.5 milliliters microcentrifuge tube for each sample, mix 10 microliters of DNA with 990 microliters of deionised water.
- Vortex to mix.
- Let this solution stand for 10 minutes to ensure the complete diffusion of DNA throughout the solution. This represents a 1:100 dilution of DNA samples.

Reading samples:

Inspect the cuvette to make sure that there are no smudges or blemishes that might interfere with the absorbance reading.

Briefly vortex the DNA sample again and transfer the solution to that cuvette being careful not to form bubbled along the wall of the cuvette.

Insert the cuvette into the spectrophotometer. Be sure that the correct face of the cuvette is aligned with the direction of the light beam.

Close the lid and press “Read sample” to begin reading.

An absorbance reading will appear on the screen when the reading is complete.

The read out will provide several important pieces of data:

“A260”- this is the wavelength of light that is absorbed by DNA. This value is used to determine that concentration of DNA in the samples according to the conversion factor (A260 of 1.0 = 50µg ml-1 DNA).

“A280”- the absorbance generated at 280 nm is used in the ratio A260:A280, which determines the purity of the DNA. Samples are considered of adequate purity if A260:A280 >1.5.

To calculate concentration of original solution:

A260 × Conversion factor × Dilution factor = original DNA concentration (µg/ml)

absorbance unit.

The conversion factor for double stranded DNA is 50µg/ml, for single stranded DNA and RNA the conversion factor is 40µg/ml.

DNA fingerprinting (RAPD) analysis:

Materials:

1. Equipment:
2. Thermocycler, Power supply unit.
3. Water:
4. Sterile de-ionized or distilled water should be used for preparing all reagents and pre-mixes.
5. Reaction buffers:
6. Assay buffer for Taq DNA polymerase (supplied by the manufacturer of Taq DNA polymerase).
7. Deoxynucleoside triphosphates(dNTPs):
8. 2.5 mM each of dCTP, dATP, dTTP, dGTP. Readymade solutions of dNTPs are available from many manufacturers. Store at -20°C.
9. Magnesium chloride:

   2.5mM stock and store at -20°C
10. Taq DNA polymerase
11. Genomic DNA 5-25ng/ml stock:

    DNA of sufficient quality can be obtained from feathers by using SDS protocol.

Methods:

Assemble RAPD reaction as follows:

2.5µl	DNA Stock(25ng/µl)
2.5µl	Assay buffer containing 2.5M Magnesium chloride(2.5mM)
1µl	Magnesium chloride stock(1.5mM)
1µl	Primer stock (25pmol)
4µl	dNTP's (400µM)
1µl	Taq polymerase (1U)

Sterile water to make 25µl

1. Wear gloves throughout RAPD reaction preparation procedure. Assay buffer, dNTP's, magnesium chloride and primer solution are thawed from frozen stock. Keep the assembled reaction in thermocycler for amplification.
2. Amplify DNA in thermoccler: cycling conditions may be modified depending on the themocycler used.

TEMPERATURE PROFILE:

General cycling steps followed are:

Step 1: Initial denaturation at 94˚C for 5.00 min

Step 2: Denaturation at 94˚ C for 1.00 min

Step 3: Primer annealing at 55˚C for 1.00 min

Step 4: Primer extension at 72˚C for 2.00 min

Step 5: Go to 2, 72 times

Step 6: Final extension at 72˚C for 10 min

Step 7: 4˚C for ever

After the reaction , DNA is analysed through gel electrophoresis.

AGAROSE GEL ELECTROPHORESIS:

Agarose gel electrophoresis is a simple and effective method of separating and purifying nucleic acid fragments.it can be used both as an analytical and preparative tool. The voltage applied at the ends of an agarose gel generates an electric field with the strength defined by the length of the gel and potential difference (v/cm) at the ends. Nucleic acid molecules migrates toward anode in the electric field due to negatively charged phosphate group along the back bone.due to similar charge to mass ratio of nucleic acid molecules of different lengths, it is the nucleic acid that determines the role of movement.

Molecules of linear duplex DNA travel through gel matrices at a rate that is inversely proportional to their molecular weight. The molecular weight of a fragment can thus be determined by comparing its mobility to the mobility of fragments of known size (markers). This is the most valuable feature of agarose gel electrophoresis as it provides a reproducible and accurate means of characteristics of nucleic acid fragments by size.

The DNA isolated was run and separated by electrophoresis in 1.4 to 1.8% agarose gel and visualised by staining with Ethidium Bromide and viewed under UV light.

PROTOCOL :

- Prepare the gel tray by placing in the casting tray place the comb and level the tray.
- Boil and prepare 1.4% agarose gel in1X TAE buffer. Cool it to 60˚C and add ethidium bromide of appropriate volume. Pour the gel into the tray and avoid air bubbles. Allow to set for 30-40 minutes.
- Place the gel in electrophoresis tank, fill it with 1X TAE buffer, remove the comb carefully . Pour the buffer till the gel is fully immersed.
- Load the samples carefully. Connect the leads and start electrophoresis, run at constant 60V.
- Stop the run when bromophenol blue dye has travelled less than 2/3rd of the length of gel. View the gel under UV light and photograph the gel.

Results and discussion

4. RESULTS

The bird feathers of five different kinds of bird species were collected from a zoo. They are maintained in a room temperature Feathers are a difficult material to isolate DNA from, since only the feather tip contains DNA. Attached to the outside are old skin cells. While inside are old blood cells, from when the feather was still growing .

A rapid and accurate protocol was used to isolate high-quality genomic DNA from feathers. Purification of the genomic DNA is performed with phenol: chloroform: isoamyl alcohol extraction and phenol precipitation. This protocol consistently provided significant amounts of high-quality genomic DNA.

DNA was been isolated with the usual SDS protocol. The DNA isolated was run and separated by electrophoresis in 1.4 to 1.8% agarose gel and visualised by staining with Ethidium Bromide and viewed under UV light.

In the field of biotechnology there are several DNA isolation method with variant principle protocols (Doyle and Doyle.1987). RAPD analysis is simpler and faster to perform other than DNA fingerprinting techniques. This technique requires no sequence information prior to analysis and only a minute amount of DNA is needed (Williams et.al., 1990).

DNA quality is to be considered as the main factor that may affect the results. On the other hand, recent results indicate that RAPD fingerprints may also be influenced by differentiation and has been successfully used in a variety of taxonomic and Genetic diversity studies.

ISOLATION OF DNA:

DNA was isolated from five different kinds of bird feathers such as Chicken, Rosy Pelican, Spot- Billed Pelican, Sarus Crane, Cormorent using SDS method and they were quantified to check for purity. The purity obtained was A260:A280>1.5. These samples were further used for PCR and RAPD analysis.

DNA FINGERPRINTING(RAPD) ANALYSIS:

The isolated and quantified DNA samples were used for DNA fingerprinting(RAPD) analysis using five sets of primers . the result of this analysis showed that the band pattern of all five samples had variations between them with the use of these primers .

DNA isolated from five different bird species

Lane 1,3,4 & 8 : Chicken

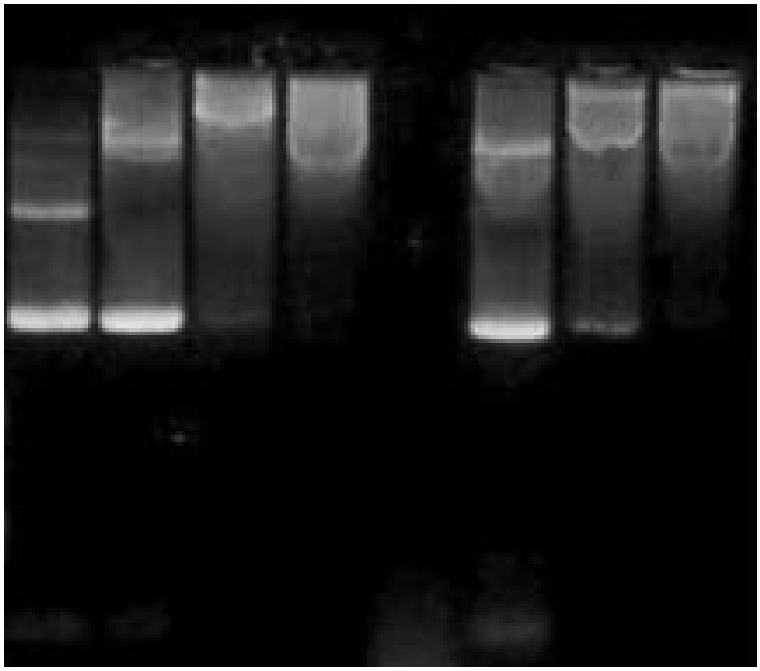
Lane 2 : Rosy Pelican

Lane 5 : Cormorant

Lane 6: White Pelican

Lane 7: Emu

L1 L2 L3 L4 L5 L6 L7 L8



Lane 1 : Cormorant

Lane 2 : Chicken

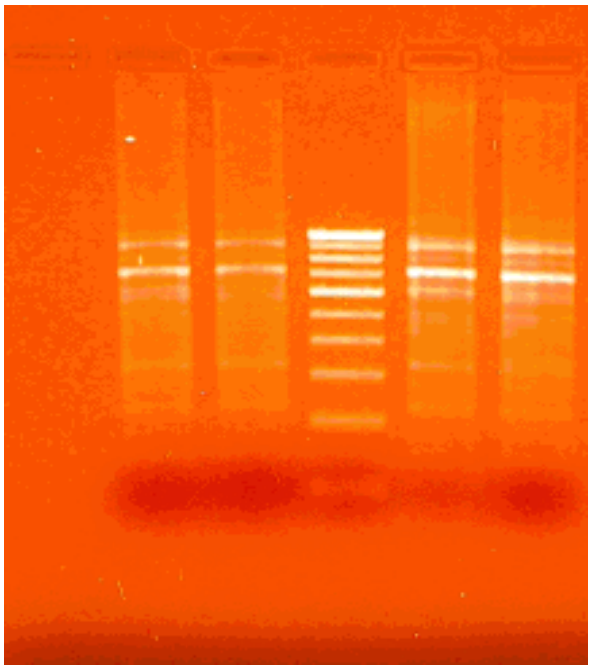
Lane 3 : Saras Crane

Lane 4 : Molecular Weight Marker DNA

Lane 5 : Rosy Pelican

Lane 6 : Spot- Billed Pelican

L1 L2 L3 L4 L5 L6



DISCUSSION

The collected feathers were been kept in room temperature and DNA is thus been isolated from five different samples. The protocol used to isolate the DNA is SDS method. Followed by the quantification and the dialysis of the DNA.

Molecules of linear duplex DNA travel through gel matrices at a rate that is inversely proportional to their molecular weight. The molecular weight of a fragment can thus be determined by comparing its mobility to the mobility of fragments of known size (markers). This is the most valuable feature of agarose gel electrophoresis as it provides a reproducible and accurate means of characteristics of nucleic acid fragments by size. And this work will be very useful even to identify the relations between different kinds of birds even their relationship with extinct birds.

Agarose gel electrophoresis is a simple and effective method of separating and purifying nucleic acid fragments.it can be used both as an analytical and preparative tool. The voltage applied at the ends of an agarose gel generates an electric field with the strength defined by the length of the gel and potential difference (v/cm) at the ends. Nucleic acid molecules migrates toward anode in the electric field due to negatively charged phosphate group along the back bone.due to similar charge to mass ratio of nucleic acid molecules of different lengths, it is the nucleic acid that determines the role of movement.

The isolated DNA was amplified using the PCR and then they were analysed for RAPD analysis using sets of primers ann there is a genetic variation found in these different bird species. Thus with this polymorphism they can further do some archaeological researches.

SUMMARY

1. SUMMARY

DNA was isolated from five different bird species, and their polymorphism was been analysed with the help of RAPD.

And this work will be very useful even to identify the relations between different kinds of birds even their relationship with extinct birds.

The SDS method was used for DNA isolation. The amplification of DNA was done with the help of PCR followed by the RAPD , the polymorphic relationship between these five different birds.

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