MOLECULAR SEXING OF BIRDS AT THE NATIONAL ZOOLOGICAL GARDENS IN DEHIWELA

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INTRODUCTION

Information about the sex of a bird is important in studies of ecology, evolutionary biology and especially in captive breeding and conservation. However, in more than 50% of bird species it is very difficult to distinguish between the males and females based on an analysis of their external morphology. The rate of error, when sexed through conventional techniques, is estimated to be around 40%, especially when they are young. Moreover, the conventional techniques, which identify bird sex involve invasive techniques; thus present problems such as stress for the bird. Currently molecular methods are being used to establish the sex of monomorphic birds in developed countries (1,2,3). These molecular methods are found to be accurate, safe and fast, when compared to conventional techniques.

The molecular methods that determine the sex of a bird are based on the Polymerase Chain Reaction (PCR) technology. The genomic DNA extracted from male and female birds are amplified using several different pairs of conserved exon PCR primers (1, 2,3, 4). In birds, the females are heterogametic ((WZ) and males are homogametic (ZZ). Thus, the PCR tests are based on the identification of a sex-chromosome specific gene locus of the chromo-helicase DNA binding protein (CHD). The CHD gene is present as a pair of duplicated gene loci on the W and Z chromosomes. CHD–W and CHD-Z have conserved exons but differ in their intron lengths. Hence, when the PCR products are visualized by gel electrophoresis in an agarose gel stained with ethidium bromide, it is possible to differentiate between males and females based on the banding pattern and their sizes.

Objectives of the study

- To develop a PCR based molecular assay to sex different species of birds
- To determine the sex of birds in the Dehiwela Zoological Gardens using the assay developed

METHODS

***Birds**:* Blood was collected from about 50 birds present at the National Zoological Gardens, Dehiwela for the study. At the preliminary stage pairs of birds of known sex from about seven different species were selected for the development and optimization of the PCR assay. Once the assay was established the other species at the Zoological Gardens (of unknown sex) were tested to determine their sex.

***DNA Extraction**:* Bird DNA was extracted separately from blood using Chelex 100 and Phenol Chloroform techniques (2,3). The extracted DNA was stored at –20°C or at -70°C till analyzed by PCR.

***Polymerase Chain Reaction (PCR) and visualization**:* The assay used the primers described by Fridolfsson and Ellegren, 1999 (3F/3R), Griffith's *et al*., 1998 (4F/4R), and Malago *et al*., 2002 (1F/1R, 2F/2R). The amplification was carried out as in the literature with modifications (1,2, 4). The PCR products were visualized by 2% agrose gel electrophoresis stained with ethidium bromide.

The PCR reaction conditions, PCR programme, gel electrophoresis conditions, etc., were optimized with each species of birds in order obtain the best resolution.

FINDINGS

i) Optimization of the PCR assay using birds of known sex:

It was possible to correctly resolve the sex of birds belonging to 7 different species (where sex was known) using either 3F/3R or 4F/4R primers (Table 1).

With 3F/3R two bands were given for female birds around 650 (CHD-Z) and 450 bp (CHD-W), whilst the males gave only one band at 650 bp, specific for the CHD-Z gene. With 4F/4R primers, the birds yielded a single or double band in males and females respectively in the 200-500 bp range. The multiplex PCR carried out with 1F/1R (female specific) and 2F/2R (internal control) gave one band around 650 bp for males and an additional band around 230 bp for females.

Both Phenol chloroform and Chelex were found to be suitable for the extraction of DNA from bird blood.

ii) Determination of bird sex using the PCR assay developed

The PCR assays carried out with chelex extractions of bird DNA and 4F/4R primer pairs it was possible to determine the sex of 8 species as shown in Table 2.

Table 1: Summary of results of PCR assays done with birds of known sex

Bird	Sex	DNA Extraction Method	3F/3R Primers		4F/4R Primers	
			Z-600bp	W-450bp	Z-400bp	W-350bp
Silver Pheasant *Lahore nycthemera*	Male	Phenol chloroform/ Chelex	√	-	√	-
	Female	Phenol chloroform/ Chelex	√	√	√	√
Golden Pheasant *Chrysolophus pictus*	Male	Phenol chloroform/ Chelex	√	-	√	-
	Female	Phenol chloroform/ Chelex	√	√	√	√
Peacock *Pavo cristatus*	Male	Phenol chloroform	√	-		
	Female	Phenol chloroform	√	√		
Chinese Pheasant *Phasianus decollatus*	Female	Chelex	-	√		
Ostrich *Struthio camelus*	Male	Chelex			√	-
Ring-necked pheasant *Phasianus colchicus*	Male	Chelex			√	-
Macaw *Ara sp.*	Female	Chelex			√	√

Table 2: Results of PCR assays – where the sex of bird was unknown

Bird Sp.	***Primer pair and DNA Ext. Method***	***Z / W band (bp)***	***Sex Analysed***
Cockato - *Cacatua sp.*	4F/4R; Chelex	400	Male
Sulphur Crested Cockatoo *Cacatua galerita galerita*	4F/4R; Chelex	400	Male
Galah Cockatoo *Cacatua roseicapillus*	4F/4R; Chelex	400	Male
Toucan *Ramphastos tucanus cuvieri*	4F/4R; Chelex	400	Male
Budgerigar 1 *Melopsittacus undulatus*	4F/4R; Chelex	400	Male
Budgerigar 2	4F/4R; Chelex	400,350	Female
Grey Horn Bill *Buceros bicornis*	4F/4R; Chelex	400	Male
Argus Pheasant *Argusianus argus*	4F/4R; Chelex	400,350	Female
Cassowary 1- *Casuarius* sp.	4F/4R; Chelex	400	Male
Emu *Dromaius novaehollandiae*	4F/4R; Chelex	400,350	Female

CONCLUSIONS

- The PCR assay developed accurately resolved the sex of 16 bird species.
- Both Chelex and Phenol Chloroform were found to be suitable for the extraction of bird DNA from blood. However, Chelex is recommended as this methods is quicker and cheaper - without compromising accuracy and reliability
- Depending on the bird species, suitable primers and PCR programmes should be used. The PCR assay needs further optimization to be used as a universal technique for all bird species.
- This method will facilitate breeding of rare and monomorphic birds in the Zoological Gardens, Dehiwela

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