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MOLECULAR SEXING OF CONURES- COMPARATIVE EVALUATION OF THE EFFICACY OF THREE DIFFERENT PRIMER PAIRS

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ABSTRACT

Sexing in birds is important for scientific management, behavioral and ecological studies and also to improve breeding programme in captivity. Sexing by examining external morphology is difficult in more than 50% of the world's bird species. Since maintenance of ideal sex ratio is important for any successful breeding programme pet breeders encounter difficulties with breeding of monomorphic birds. In the present study, DNA was isolated from feathers and polymerase chain reaction was done using three different sets of primers to identify the sex of a popular pet bird, *Pyrrhura molinae* (Green-cheeked conure), which is monomorphic in nature. The primer sets were designed to amplify intron 9 and 16 of *CHDI*gene and intron 16 of NIPBL gene. Both *CHD1* (encoding chromo helicase- DNA-binding protein 1) and NIPBL (Nipped B homolog) is located in both the sex chromosomes, Z and W of birds.Intron 9 of *CHDI* gene was amplified by PCR technique in DNA isolated from the feather. As female birds are heterogametic (ZW) PCR amplification generated fragments of two different sizes- 1100 bp and 600 bp, whereas in male birds (ZZ), only a single fragment of 1100 bp was observed. Two different pattern generated on electrophoresis of amplicons, in male and female birds, helps to identify the sex accurately. The other two primer sets did not consistently yield different patterns in all male and female birds raising doubts in their efficacy in being used for sex identification of conures

KEYWORDS: CHD 1, Conure, NIPBL, *Pyrrhura molinae*, Sexing

INTRODUCTION

In over fifty per cent of the world's bird species, adult males and females appear identical making it difficult and sometimes even impossible to determine sex on the basis of external morphology. Some avian species exhibit marked sexual dimorphism as adults, but lack dimorphism as juveniles (Griffiths *et al.*, 1998).

In birds that are sexually dimorphic, such as the house sparrow, mallard and collared flycatcher, it is very easy to distinguish between males and females based on the secondary sexual characteristics such as body size, plumage color etc. However, morphological identification of sex in birds can often be difficult in monomorphic species and sexually immature individuals. This necessitates specific sexing techniques such as vent sexing, laparoscopy, steroid sexing, karyotyping, molecular sexing etc. for sex identification (Ellegren and Sheldon 1997; Griffiths *et al.,* 1998; Kahn *et al.,* 1998; Fridolfsson and Ellegren, 1999).

Knowledge of the sex of individuals is essential for investigations on evolution and ecology, including sex ratio evolution, sexual selection, parental care strategies, demography and conservation. Also it is important in captive breeding programmes for the survival of endangered birds. In captive breeding programmes sex identification plays an important

role as knowledge of the sex of birds is essential to keep ideal numbers of each sex for the establishment of breeding pairs or breeding flocks. Thus, gender identification is essential for both evolutionary studies and human-assisted breeding of birds for conservation or commerce. Conventional methods of avian sexing are based on behavioral observations, morphometry, differences in the sounds produced, cloacal examination, surgical procedures and cytogenetic analysis. Most of these techniques are either time-consuming, expensive, invasive, have short-time applicability or sometimes harmful. Molecular techniques based largely on chromosome specific markers, exploiting the variations among the heterogametic sex (females with Z & W chromosomes) and homogametic sex (males with ZZ chromosomes) were developed and successfully applied in avian sexing.

SEXING OF GREEN CHEEKED CONURES

Conure parrots have been kept as pets for over a hundred years or possibly longer. The first Conures were found throughout Latin America from Mexico, through the Caribbean, to southern Chile.The Green-cheeked Parakeet or Green-cheeked Conure is a small parrot of the genus *Pyrrhura*, which is part of a long-tailed group of the New World parrot subfamily Arinae which comes under the family Psittacidae. The Green-cheeked conure(*Pyrrhura molinae*) is typically 26 cm (10 inch) long and weighs 60 to 80g. It is mainly green, with a brown/black/grey crown, white peri- ophthalmic rings, green cheeks, blue primary wing feathers, a grey beak, with its long pointed tail, which is mostly maroon. It has short transverse striations on its breast and a red abdominal area. Males and females have an identical external appearance both as juveniles and as adults. It normally measures around 10 inches in length from the beak to the tip of the tail feathers.Green-cheeked Parakeet has six subspecies which is again categorized on the basis of their colour. Though a native of South America, the bird is reared by pet owners throughout the world. Commercial breeders of this bird often encounter problems in sexing due to lack of distinct morphological features in male and female.

PCR based methods for sex identification is based on the amplification by PCR of Z and W alleles using specific primers designed to screen the intron variations (length and nucleotide sequence) in sex-linked genes, and subsequent electrophoresis analysis. In theory, the amplified products should migrate as a single band in males (being homogametic - ZZ) and as two bands in females (being heterogametic - ZW). In the present work the efficacy of PCR amplification ofthree regions of sex chromosomes- intron 9 and intron 16 ofCHD1 and intron 16 of NIPBL genes-of green cheeked conures in distinguishing the sexes was ascertained.DNA isolated from feather was utilized for the purpose.

MATERIALS AND METHODS

Feathers were collected from the breast region of twenty five birds hygienically into High Density Polyethylene pouches. Around 1-3 mm long pieces were cut from the base of the feathers and processed for the extraction of genomic DNA using DNeasy blood and tissue kitaccording to the instructions of the manufacturer. Extracted DNA was stored at -20ºC till further use. PCR was done with primers for CHD1 intron 9, CHD1 intron 16 and NIPBL intron 16. Primers used were as follows:

Gene		Primer	Reference
CHD 1			
Intron 9	F	5'CAGCAGAAATCAATCCAAGAC3'	Suh *et al.,* 2011
	R	5'CAGCCCATTTAACTGATAATCTC3'	
Intron 16	F	5'GTCCTGATTTTCTCACAGATGG3'	
	R	5'ATGATCCAGTGCTTGTTTCC3'	
NIPBL			
Intron 16	F	5'TTGTCAGAGTTGCTGGAGATAC3'	Suh *et al.,* 2011
	R	5'AATTTGATGGCACATAACTGTAG3'	

During PCR, initial denaturation at 94°C for 120s was followed by 35 cycles of denaturation at 94°Cfor 30 s, annealing at 52°Cfor 30 s and extension at 72°Cfor 80 s. Following amplification PCR products were visualized on ethidium bromide stained agarose gel in a Gel Documentation System.

RESULTS

The quantity of extracted DNA ranged from 4.0 to 35.0 ng/µl and the ratio of OD260 and OD280 were between 1.78 and 2.0 in all samples. PCR amplification using specific primers for intron 9 region of CHD1 showed a single band corresponding to 1100 bp region in male birds and two bands at 600 bp and 1100 bp regions in female birds (Figure 1). BLAST analysis of sequence of the products amplified of intron 9 of CHD1 Z showed 77 per cent identity with intron 9 of CHD1 Z chromosome of Columba palumbus with 98 per cent query cover whereas CHD1W showed 88% identity with intron 9 of CHD1 W chromosome of Columba palumbus with 67% query cover. Results obtained with this set of primers were consistent.

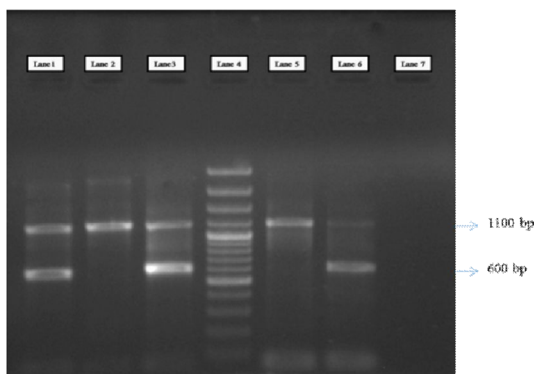

Figure 1: PCR Products Resolved in 1.5% Agarose Gel. Lanes 1,2,3,5,6: Samples; Lane 4: DNA Ladder, Lane 7: NTC

PCR amplification of intron 16 of CHD1 was also under conditions similar to that of intron 9, but it showed variable results. Two amplicons of sizes 400 bp and 600 bp were obtained for three birds (male) and one amplicon of 600 bp was obtained for two birds (female) analysed. Twenty five samples failed to produce any amplicons under the same conditions.

PCR amplification of intron 16 of NIPBL produced a single amplicon of 1100 bp in female birds and two amplicons of size 1100 bp and 500 bp in male birds. But, as with intron 16 of CHD1, several samples failed to produce any product. Failure of PCR might be due to polymorphism at the primer binding region which prevents the primer annealing process.

Results obtained in the present study has revealed that PCR based method with genomic DNA isolated from feathers using primers for intron 9 region of CHD1 gene is a simple, non-invasive and quick method for unambiguous sex identification in green cheeked conure.

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