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# Sex Identification in Pigeons Using Chd Based Molecular Markers

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**ABSTRACT**

In many bird species, males and females cannot be discriminated on the basis of external characteristics. The problems associated with reliable sex identification were overcome by introducing the molecular techniques in the 1990s. These techniques are generally based on DNA hybridization or polymerase chain reaction (PCR). This work reviews the sexing techniques, highlighting their advantages. For the present study a total 256 samples were collected from different places of Marathwada region. Among total 256 samples 125 samples were analyzed by CHD marker.

**KEYWORDS** Pigeons, CHD, PCR

**Introduction:**
Pigeons constitute the bird family Columbidae and order columbiformes. Pigeons are distributed everywhere on Earth, except for the driest areas of the Sahara Desert, Antarctica and its surrounding islands, and the high Arctic. Seeds and fruit form the major component of the diets of pigeons and doves. However, males and females of many species have very similar phenotypic traits (sexual monomorphism), so that even experienced ornithologists may have problems with unambiguous sex identification. In nestlings, it is even more difficult than in the adults to distinguish between the two sexes. In birds, females are the heterogametic sex, carrying one copy each of the Z and W sex chromosomes. Males are homogametic (ZZ).

The development of molecular sexing techniques constituted a breakthrough in reliability and rapidity of sex identification in birds. The genetic sex determination in birds has been performed using genomic DNA extracted from feathers (Mundy et al.1997; Eguchi and Eguchi 2000; Bello et al. 2001), tissue (Arnold et al., 2003), and blood samples (Ellegren 1996; Griffiths et al.1996; Tomasulo et al.2002). The molecular level involves techniques such as polymerase chain reaction/random amplified polymorphic DNA (PCR-RAPD), AFLP, amplification of microsatellite loci, RFLP, and simple PCR using primer pairs based on intronic size variations, which can be used to differentiate between the chromo-helicase DNA-binding (CHD) proteins, CHD-Z and CHD-W (Griffiths' et al. 1996, Cavallo et al. 1997, Cheng et al. 2006). This gene is highly conserved and it has been shown that a single pair of PCR primers (P2 and P8) can be used to sex most of the avian species (Griffiths et al., 1996; Griffiths et al., 1998). These primers anneal to conserved exonic regions and amplify across an intron that varies in size between CHD-W and CHD-Z (Griffiths et al., 1998; Ellegren, 1996; Kahn et al., 1998). Molecular sexing (Ellegren and Sheldon1997) based on polymerase chain reaction (PCR) is an attractive option, since PCR is simple to perform, rapid and requires only a minute quantity of DNA, which could be obtained from a single feather or a drop of blood.

This work aims at search for DNA markers for sex determination thereby infer the phylogeny of pigeons in order to document and establish their relationship.

**Materials and Methods:** A total of 125 individuals were sampled for gender identification from Marathawada region. Among a total of 256 samples, there were 184 feather samples and 72 blood samples. Samples were provided by private breeders and fanciers. Feathers were sampled by plucking and quills were cut into 2-5 mm long pieces samples.

**Isolation of genomic DNA from collected species:**
Genomic DNA was extracted from adult feather quills using a sodium hydroxide boiling method (Zhang and Tiersch 1994). DNA was extracted from blood by a standard phenol/chloroform extraction method.

PCR primers were designed based on the common sequence. PCR amplification conditions were 95°C for 3 min, followed by 38 cycles of 95°C for 1 min, 55°C for 2 min, and 72°C for 2.5 min, and a final extension step of 72°C for 10 min. The amplification was conducted in a 25 µl reaction containing 400 µM dNTPs, 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl; BRL Inc.), 2 mM MgCl2, 4 pmol of each primer and 25–100 ng of genomic DNA.

Based on the complete sequence of CSL-W, several PCR primers were designed to amplify portions of the sequence. Some primer pairs amplify a product from both males and females. For example, the combination of primers (5′-GTTACTGATTCGTCTACGAGA-3′) and (5′-ATTGAAATGATCCAGTGCTTG-3) yielded amplification products from both males and females. To detect any contamination, a negative control including all reagents except the DNA sample was used in each set of amplifications.

**Result:**
A total 256 samples were collected from different places of Marathwada region. Among total 256 samples 125 samples were used for further analysis. The sodium hydroxide boiling method extraction procedure yields large amounts of DNA. Random samples were selected for electrophoresis of PCR products, gender was determined by visualization on agarose gel as two bands in females (Z and W) or one band in males (Z) with the difference range of 150 to 250 bp between Z- and W-bands, which made separation and visualization simple and reliable (Fig.1). Primers 2550F/2718R were successfully used for amplification of CHD gene of Pigeon.

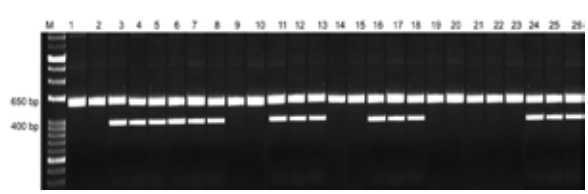


**Fig.1 Agarose gel serration of band of 650 bp and 400bp (M-Marker, 1-26 DNA samples analyzed for presence of CHD markers as indicated in the table**

**Table 1: Differentiation between male and female on the basis of gel image.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 650<br>400 | m | M | f | F | F | f | f | f | m | m | f | f | f | M | m | f | f | f | m | m | m | m | m | f | f | f |
| Sample | fe | Fe | fe | Fe | Fe | fe | bl | bl | fe | fe | fe | fe | fe | Fe | bl | bl | fe | fe | fe | fe | fe | fe | bl | bl | bl | bl |

**(M- male, F- female; FE-feather DNA , BL-blood DNA)**

Gender of sampled birds (125) was successfully identified using feather samples. The results were additionally confirmed using blood samples as well. All types of samples from a single individual gave the same result.

On the basis of gel image out of 26 samples 11 samples were identified as males and 15 were females. In samples identified as 11 male, 9 DNA samples were extracted from feather and 2 DNA samples were from blood. In females 9 were feather and 6 were blood samples.

**Discussion:**

In this study we designed two primers 2550F/2718R for amplification of CHD gene of Pigeons. Sexing various species of parrots was mostly conducted with P2/P8 primer set described by Griffiths et al. (1998). Ong and Vellayan (2008) indicated that 2550F/2718R primer set, which was used in this research, provides a higher confidence level of establishing the sex of birds even without the use of polyacrylamide gels as is required in some bird species with the use of primers P2/P8 and 1237L/1272H (Kahn et al., 1998). In this study, primers of 2550F/2718R enabled successful sex determination in Pigeon. In previous studies (Miyaki et al., 1998; Taylor and Parkin, 2008), sexing various species of parrots was mostly conducted with P2/P8 primer set described by Griffiths et al. (1998).

Molecular techniques for sex identification in birds were revolutionized by discovering in 1995 the first gene located in the W chromosome (Griffiths & Tiwari 1995). A very closely related copy of this gene was soon after discovered also in the Z chromosome (Griffiths & Korn 1997).

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