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SEX IDENTIFICATION IN THE CANARY USING DNA TYPING METHODS

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Summary

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In many bird species, the sex determination is very difficult in young birds and many adults. Nowadays, sex identification of animals throughout their lives is possible by molecular genetic techniques. In the present study, the sex identification of canaries by PCR methods based on chromo helicase DNA binding protein CHD-Z and CHD-W genes were explained. Genomic DNA was extracted from feather bulb (old and young) of canary. PCR reaction was performed by a set of novel primers. The results showed two amplified fragments, about 345 and 306 bp for female, and only one 306 bp amplified PCR product for male canaries. This result was shown for each sample. This sex identification system which presented for the first time, especially in canaries. We have established a fast, safe, accurate and inexpensive procedure for sex typing of canary using DNA extracted from feathers.

Key words: canary (*Serinus canaria*), PCR, sex identification

INTRODUCTION

Bird species are considered to be sexually monomorphic, so it is very difficult to distinguish between males and females based on an analysis of their external morphology, especially in young birds (Takagi *et al.*, 1972; Ogawa *et al.*, 1998; Jensen *et al.*, 2003). DNA should provide a versatile way to discriminate between male and female birds. Unfortunately, the selection of a suitable sex-linked marker has proved to be difficult (Griffiths *et al.*, 1998). The constitution of sex chromosomes in vertebrates is either XX (female)/XY (male), as in mammals, or ZW (female)/ZZ (male), as in birds and some reptiles (Gubbay *et al.*, 1990). Most birds have two chromo helicase DNA binding protein (CHD) sex-linked genes, one W-linked (CHD-W) and one Z-linked (CHD-Z) (Bermúdez-Humarán *et al.*, 2002). Many studies exploit the difference in length between introns in the CHD-Z and CHD-W genes. Therefore they can be used for sex determination and differentiation between male and female birds (Caetano & Ramos, 2008).

Most techniques presently used for sex determination of monomorphic birds include avian laparoscopy and laparotomy, cloacal examination, biochemical analysis, surgical examination, and cytogenetic analysis. All of these methods have inconveniences, such as being time-consuming, costly, with low sensibility, or sometimes even harming the organism (Bermúdez-Humarán *et al.*, 2002). Molecular methods like polymerase chain reaction (PCR) have proved to be a versa-

tile and valuable tool in wildlife conservation and for behaviour, sex allocation and genetic studies (Griffiths *et al.*, 1995; Ellegren *et al.*, 1997). The aim of this study was to utilize the CHD gene for sex determination in the canary (*Serinus canaria*) by PCR method and to present a novel set of primers for sex determination. Therefore, in this work, the CHD marker was first characterized in *Serinus canaria* and successfully used for sexing of canary birds.

MATERIALS AND METHODS

A group of young canaries was subjected to sex typing by a molecular technique. For analysis, 5 male and 5 female individuals were selected. Their sex was also identified by conventional techniques. After setting the molecular sex typing in adult birds, 30 young canaries were analysed by this method.

The genomic DNA was purified by DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions from one to three feather bulb of each canary. The isolated DNA was quantified spectrophotometrically and also on the agarose gel prior to dilution to a final working solution of 20 ng/µL.

Since there was not any sequence of CHD-W gene of canary in Genbank database, primers were designed on the basis of the sequence of the CHD-W gene (GenBank- AF288510) of *Hemispingus frontalis* (a member of Fringillidae family) that was closely related to the canary on the phylogenetic tree.

Sex typing was determined by amplification with the following primers: CH-F: 5-GGATGAGGAACTGTGCAAAAC-3 (forward) and CH-R: 5-AATAGTTCGC GGTCTTCCAC-3 (reverse), amplifying 345 bp for CHD-W and 306 bp for CHD-Z genes.

The PCR reaction (30 µL) mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.01 mg bovine serum albumin (BSA), 50 ng of each primer (CH-F and CH-R), 1.5 units of *Taq* polymerase (Fermentas, Germany) and about 100 ng of genomic DNA. An initial denaturing step of 94 ^{o}C for 5 min was followed by 32 cycles of 94 ^{o}C for 1 min, 58 ^{o}C for 1 min, and 72 ^{o}C for 1 min and a final extension of 72 ^{o}C for 5 min.

Ten microlitres of each PCR product were subjected to gel electrophoresis on 1.5 % agarose gels.

RESULTS

Genomic DNA was extracted from feather bulb of old and young canaries, with approximately 1 µg of DNA obtained from each feather bulb (final concentration about 20 ng/µL).

PCR was performed using a specific pair of primers designed to detect the CHD-W gene of *Hemispingus frontalis* which is close to the canary.

Analysis of PCR products of canary CHD-W gene on agarose gel revealed two fragments of about 345 and 306 bp for female canaries, but only a single fragment of 306 bp for male canaries (Fig. 1).

These band patterns were reproducible for all young and old canaries with known sexes.

The greater intensity of some bands in males, as well as the presence of female-specific bands, suggested that the primer set for CHD-W genes reported here was linked to the Z and W chromosomes in canary birds.

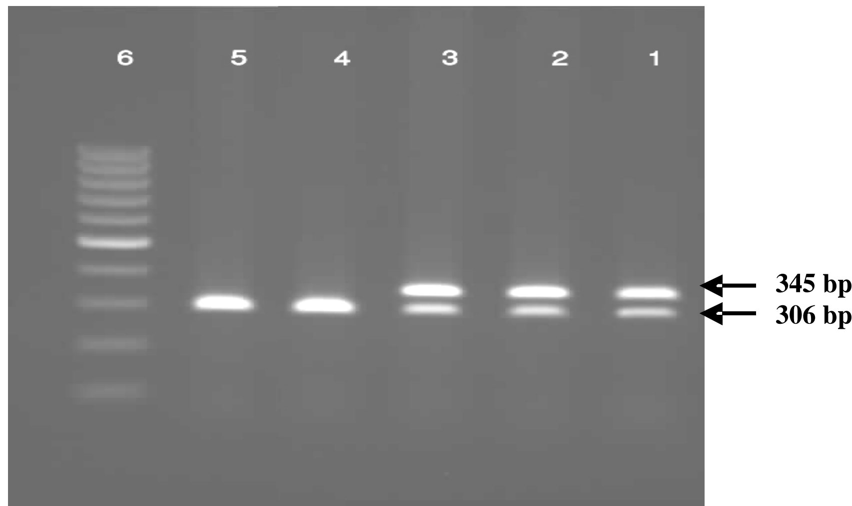

Fig. 1. PCR products amplified by using CH-F and CH-R primers showed banding patterns for female and male *Serinus canaria* – 100 bp marker, three female (1, 2 and 3) and two male (4 and 5) individual band patterns.

DISCUSSION

Many avian species are considered to be sexually monomorphic. Unless breeders are not sure of the sex of the birds, they can not get any newborns from monomorphic birds. The differences in the sale prices and care costs of male and female avian species and the time spent for the reproduction process cause significant financial losses (Cerit & Avanus, 2006).

Unfortunately, about 60% of all bird species are sexually monomorphic. There are no morphological differences between sexes, and therefore the sex identification is very difficult. In birds, females are heterogametic, carrying one copy of each of the Z and W sex chromosomes and males are homogametic (ZZ) (Cerit & Avanus, 2007).

Saitoh *et al.* (1991) were the first to report DNA typing in an avian species. A family of repetitive sequences, designated as the EcoRI family, was found in the DNA of the chicken W chromosome by hybridization with the W chromosome-specific XhoI family probe. From 70% to 90% of the DNA in the chicken W chromosome was shown to be occupied by repetitive sequences (Saitoh *et al.*, 1991).

PCR along with oligonucleotide probe was used for identifying the sex of early chicken embryos by Petitte & Keglemeyer (1992). Brief phylogenetic analyses including CHD-Z and CHD-W have been presented by Fridolfsson *et al.* (1998). Canon *et al.* (2000) identified the sex of three psittacine species (Amazon parrots, budgerigars, and cockatiels) by flow cytometric analyses of nuclear DNA (Canon *et al.*, 2000). Sex was identified in 94.4% of Amazon parrots, 100% of budgerigars, and 51.3% of cockatiels, however sample storage at 4ºC for 48 or 72 hours caused variability in DNA content (Canon *et al.*, 2000).

Karyotyping may be a good choice for the sex typing of birds but, unfortunately, the low divergence of Z and W chromo-

somes makes this approach inappropriate in ostriches and most birds (Malago *et al.*, 2002).

Nesje & Røed (2000) reported microsatellite loci NVH fp102 and fp49 for sex identification in peregrines, gyrfalcons, merlins, kestrels, and hobbies. Amplification of locus NVH fp102 and locus NVH fp49 will easily identify female falcons and peregrines, respectively. The results should be however evaluated carefully, since there is a possibility that some females could be classified as males as a result of failure of the PCR reaction. This problem will be avoided in gyrfalcons, merlins and kestrels, if a radioactively-labelled primer and electrophoresis on acrylamide gel is used for locus NVH fp49 (Nesje *et al.*, 2000).

Birkhead *et al.* (2001) reported that P2 and P8 primer pairs were successfully used for sex identification in the common mure (*Uria aagle*). There was conservation projects aimed to sexing some endangered bird species (Birkhead *et al.*, 2001). Cerit & Avanus (2006) identified the sex of a parrot species *Nymphicus hollandicus* by P2 - P8 primer pairs. Since females are heterogametic, they present two alleles (ZW) and males are homogametic, presenting two copies of the same allele (ZZ) (Cerit & Avanus, 2007).

In this work a pair of primers was designed for CHD-W gene of *Hemispingus frontali* which could amplify canary CHD-W gene. The PCR amplified products gave two distinct band patterns for male and female canaries.

In conclusion, in this report, we have established a fast, safe, accurate and inexpensive procedure for sex typing of the canary using DNA extracted from feathers. Furthermore, the use of canary feathers can be adapted for large-scale analyses being useful for the sex identification of chicks in the first days of nestling life. This new sex identification method could be used for other birds, especially flourish birds.

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