Molecular study for the sex identification in Japanese quails (*Coturnix Japonica*)

Nasrollah Vali1[1] and Abbas Doosti[2]

[1]Department of Animal Sciences, Faculty of Agriculture, Islamic Azad University, Shahrekord Branch, Shahrekord, Postal box, 166. Post code, 8813733395, Iran. Nasrollah.vali@gmail.com

[2]Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. Abbasdoosti@yahoo.com

Abstract

In many birds' species such as Japanese quail (*Coturnix japonica*), sex determination in young and many adult birds is very difficult. Nowadays, sex identification of animals throughout their lives is possible by molecular genetic techniques such as polymerase chain reaction (PCR). The aim of this study was to determine the sex of Japanese quail by DNA analysis. Chromo helicase DNA (CHD) genes are preserved within avian Z and W sex chromosomes. The intron regions of the CHDW and CHDZ genes vary between male (ZZ) and female (ZW) individuals. The method used in this study was based on this difference. Genomic DNA was extracted from feathers instead of blood. The intron regions of CHDW and CHDZ genes were amplified by sex specific primers (Coja-F and Coja-R). PCR products were screened by agarose gel electrophoresis. These results show single (ZW) amplified fragments, about 320 bp for female, and no amplified PCR product for male (ZZ) Japanese quails. These results of this study show that CHD gene amplification is a convenient, inexpensive, safe, and simple technique for sex typing of Japanese quails and other avian species in the wild.

Key words: Japanese quail (*Coturnix japonica*), polymerase chain reaction, chromo helicase DNA gene, sex identification.

INTRODUCTION

Sex identification of quails is important for their rearing especially in many commercial units, because of its need for different rearing goals. *Coturnix japonica* (Japanese quail) can be kept in pairs, trios, or one male for five to six females (Thear, 1998). It is easy to determine the sex of Japanese quails because from about three weeks onwards, the reddish brown chest of the male will start to become noticeable. His markings are also more distinct than those of the female (Vali, 2008).

Before the age of three weeks, it is virtually impossible to determine the sex of the birds. The colored varieties of Coturnix do not have an apparent difference in feathering between the male and female. The only way to tell them apart is to go by size difference (the female is bigger), by sexual behavior patterns and by vent examination (Amoah and Martin, 2010; Vali, 2009). Also, identifying and distinguishing the sex of males and females of most of the Cotunix is based on an analysis of their external morphology, especially in young birds which is very difficult (Takagi et al., 1972; Ogawa et al., 1998; Jensen et al., 2003). Extensive deoxyribo nucleic acid (DNA) sampling could indicate the precise relationship. DNA should provide a versatile way to discriminate male and female birds. Unfortunately, the selection of a suitable sex-linked marker has proved difficult (Griffiths et al., 1998). Many species of animals have sex chromosomes that differ in males and females. For example, mammals have an XX (female)/XY

(male) system of male heterogametic (Baquero et al., 2009). Birds have the opposite; the sex chromosome of birds is ZW for females and ZZ for males.

Most birds have two chromo helicase DNA (CHD)[1] binding protein sex-linked genes, one W-linked (CHD-W) and one Z-linked (CHD-Z) (Doosti et al., 2009). The CHD-Z gene is found in both sexes, while the CHD-W gene is unique to females. Recent studies showed the difference in length between introns in the CHD-Z and CHD-W genes (Ellegren, 1996). Therefore they can be used for sex determination and differentiation between male and female birds (Caetano and Ramos, 2008). Application of the polymerase chain reaction (PCR) in the identification of genetic sexes of birds is ideal because it requires only a small sample, such as a drop of blood or a single plucked feather for DNA extraction, thus minimizing trauma to individual birds (Itoh et al., 2001). This technique is very smart, cheap and high sensitive for sex determination of monomorphic birds, while other techniques including avian laparoscopy and laparotomy, cloacal examination, biochemical surgical examination and cyto-genetic analysis have inconveniences, such as being time-consuming, costly, low sensibility, or sometimes even cause harm to the organism (Doosti et al., 2009). The purpose of this study was to determine CHD gene for sex identification in Japanese quail using PCR technique and to present a novel set of primers for sex determination.

MATERIALS AND METHODS

Sampling

Adult, sexually matured Japanese quail (ten males and ten females), were selected for sex typing by a molecular technique. Their sex was also identified by conventional techniques. After setting the molecular sex typing in the adult birds, 20 young Japanese quails were analyzed by this method.

Genomic DNA extraction

Genomic DNA extraction from one to three feather bulb of each Japanese quail was performed using the Qiagen DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation. The extracted DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm according to the method described by Sambrook and Russell (2001).

Gene amplification

The primers used in this project were designed according to the sequence of the CHD-W gene (Accession No: AB189143.1) of Japanese quail. The primers sequences are: Coja-F: 5'-GTACTGAACACCCCTCCAAG-3' (sense) and Coja-R: 5'- TCCCTATCCATAAGCTCATTC-3' (anti-sense). PCR was carried out in a 25 µl mixture containing 100 ng of genomic DNA, 0.2 pM of each primer, 1X PCR buffer, 1.5 mM MgCl2, 200 mM dNTPs, and 1 unit of *Taq* DNA polymerase (Fermentas, Germany). The procedure of the PCR reaction included 5 min of denaturation at 94°C; followed by 32 cycles of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C and a final extension of 72°C for 5 min. The PCR amplification products (10 µl) were subjected to electrophoresis in a 1% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with ethidium bromide, and images were obtained in UVIdoc gel documentation systems (UK).

[1] **CHD:** Chromo helicase DNA

RESULTS AND DISCUSSION

Genomic DNA was purified from feather bulb of old and young Japanese quails and analysis of PCR products for CHD-W gene on agarose gel revealed a fragment of about 320 bp for female Japanese quail, but no fragment was detected for male Japanese quail (Figure 1). The different band patterns were reproducible for all young and old Japanese quails with known sexes. According to these findings, this method will be useful for sex identification in young Japanese quails and other birds. Adults and particularly juveniles of many avian species are monomorphic, making the determination of sex difficult. Besides that, the difference between males and females for some species is only recognizable in adults or subadults.

DNA analysis is a more reliable method for identifying the sex of birds which are monomorphic in their plumage (Baquero et al., 2009). In birds, females are heterogametic, carrying one copy of each of the Z and W sex chromosomes while males are homogametic (ZZ) (Cerit and Avanus, 2007). The sex of most bird species can be determined by DNA analysis using a test based on two conserved CHD genes located on the avian sex chromosomes (Griffiths et al., 1998). Their lengths differ between CHD-W and CHD-Z genes, making sex identification possible. In this study, DNA was isolated from bird feathers bulb, because feathers bulb sample collection gives less pain to the bird than blood sample collection. Additionally, its low cost and reduced risk of contamination, and prevention of breakage of DNA with requirement of less tube transfer make this method preferable. The method in this study was based on avian CHD genes (CHDW and CHDZ). After amplifying the varied intronic regions of CHD genes by PCR primers (Coja-F and Coja-R), PCR products were screened by agarose gel, and in males no fragment was detected while females clearly showed a specific fragment. The electrophoresis results of this study were similar to the results reported by Griffiths et al. (1998).

These results show that different band patterns exist between CHD-W and CHD-Z in young and adult Japanese quails, and it is useful for sex determination of these birds. Saitoh et al. (1991) were the first to report DNA typing in an avian species. In the method described by Griffiths et al. (1998), homologous copies of the CHD (chromohelicase-DNA-binding) gene, located on Z and W avian sex chromosomes, were amplified by PCR using a single pair of primers P2 and P8. Their findings showed that P2/P8 sexing test is an effective way to distinguish a male from a female bird (Griffiths et al., 1995, 1998). Dcosta and Petitte (1998) developed multiplex polymerase chain reaction for identifying the sex of turkeys based upon the *Pst*I repeat. Their findings show that this procedure is rapid and permits the determination of many embryos' sex in a short time (Dcosta and Petitte, 1998). Suzuki et al. (2001) identified the sex of a wide range of carinatae birds by PCR. Their findings showed that EE0.6-related sequences on the Z and W chromosomes of the ostrich and emu could not be distinguished either by Southern blotting (Itoh et al., 2001). 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 at determining the sex of some endangered bird species (Birkhead et al., 2001). Cerit and Avanus (2007) determined the sex of parrot species *Nymphicus hollandicus* by P2 - P8 primer pairs without causing it any harm and obtained accurate results by DNA analysis. In the study, they identified double (ZW) and single (ZZ) bands in females and males, respectively (Cerit and Avanus, 2007). Shizuka and Lyon (2008) improved the reliability of molecular sex determination of birds, comprising CHDZ alleles of coots and common moorhens (*Gallinula chloropus*) using a W-specific marker, and then revealed that CHD-Z polymorphism evolved separately in these two closely related species. Doosti et al. (2009) presened the sex identification system for the first time in canaries. They established a fast, safe, accurate and inexpensive procedure for sex typing of canary using DNA extracted from feathers.In conclusion, the sex identification based on Coja-F and Coja-R primers is a reliable and age independent method that needs a small amount of DNA and its applicable in

both adults and young Japanese quails. Therefore CHD gene amplification is a convenient, safe and simple technique for sex typing of Japanese quails. Moreover, the procedure of this study would also be useful for sex identification of these birds and other avian species in the wild.

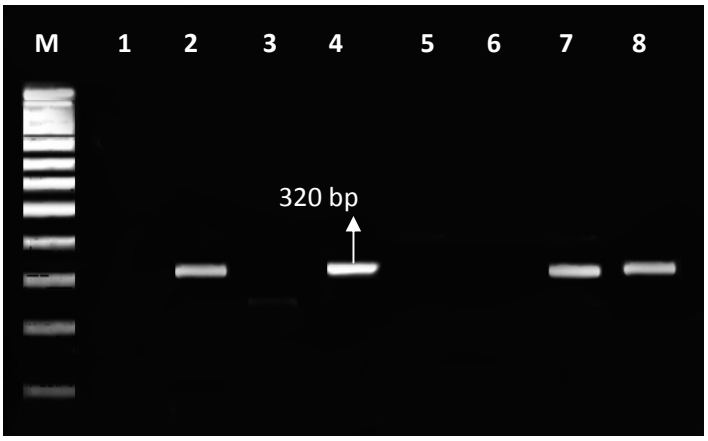

Figure 1. Sex identification of Japanese quails using Coja-F and Coja-R primers showing two different banding patterns for female and male. Lane M, 100 bp DNA ladder (Fermentas, Germany); lane 1, negative control (without DNA), lanes 2 and 3, adult female and male, respectively (defined samples); lanes 4 to 8, young birds; 4, 7, and 8, female; 5, 6, two male.

ACKNOWLEDGEMENT

The authors would like to thank all members of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in Iran for their useful suggestions and sincere support.

REFERENCES

Amoah JK, Martin EA (2010). Quail (*Coturnix coturnix japonica*) layer diets based on rice bran and total or digestible amino acids. J. Appl. Biosci. 26: 1647-1652.

Baquero A, Puerta A, Gutierrez G (2009). Magnitude Effects of Sexual Reinforcement in Japanese quail (*Coturnix japonica*). Int. J. Comp. Psychol. 22: 113-126.

Birkhead TR, Hatchwell BJ, Lindner R (2001). Extra-pair paternity in the common murre. The Cooper Ornithol. Soc. 103: 158-162.

Caetano LC, Ramos ES (2008). MHM assay: Molecular sexing based on the sex-specific methylation pattern of the MHM region in chicken. Conserv. Genet. 9: 985-987.

Cerit H, Avanus K (2007). Sex determination by CHDW and CHDZ genes of avian sex chromosomes in *Nymphicus hollandicus*. Turk. J. Vet. Anim. Sci. 31(6): 371-374.

Dcosta S, Petitte JN (1998). Sex identification of Turkey Embryos using a multiplex polymerase chain reaction. Poultry Sci. 77: 718-721.

Doosti A, Fathpour H, Moshkelani S (2009). Sex identification in the Canary using DNA typing methods. BJVM, 12(3): 207-211.

Ellegren H (1996). First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. Proc. R. Soc. Lond. 263: 1635-1641.

Griffiths R, Double MC, Orr K, Dawson RJG (1998). A DNA test to sex most birds. Mol. Ecol. 7: 1071-1075.

Griffiths R, Tiwari B (1995). Identification of the sex of the last wild Spix's Macaw. Nature, 375: 454.

Itoh Y, Suzuki M, Ogawa A, Munechika I, Murata K, Mizuno S (2001). Identification of the sex of a wide range of carinatae birds by PCR using primer sets selected from chicken EE0.6 and its related sequences. Am. Genet. Assoc. 92: 315-321.

Jensen T, Pernasetti FM, Durrant B (2003). Conditions for rapid sex determination in 47 avian species by PCR of genomic DNA from blood, shell membrane blood vessels and feathers. Zool. Biol. 22: 561-567.

Ogawa A, Murata K, Mizuno S (1998). The location of Z- and W-linked marker genes and sequence on the homomorphic sex chromosomes of the ostrich and the emu. PNAS, 95: 4415-4418.

Saitoh Y, Saitoh H, Ohtomo K, Mizuno S (1991). Occupancy of the majority of DNA in the chicken W-chromosome by bent-repetitive sequences. Chromosoma, 101: 32-40.

Sambrook J, Russell DW (2001). Molecular Cloning: A laboratory manual. 3rd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shizuka D, Lyon BE (2008). Improving the reliability of molecular sexing of birds using a W-specific marker. Mol. Ecol. Res. 8: 1249-1253.

Takagi N, Itoh M, Sasaki M (1972). Chromosome studies in four species of ratitae (Aves). Chromosoma, 36: 281-291.

Thear K (1998). Keeping quail (Aguide to domestic and commercial management) third edition, published by Broad Leys Publishing Company Buriton House, station Road, Newport, Saffron Waldan, Essex CB11 3PL, UK.

Vali N (2008). The Japanese quail: A Review. Int. J. Poult. Sci. 7(9): 925-931.

Vali N (2009). Growth, Feed Consumption and Carcass Composition of *Coturnix japonica*, Coturnix ypsilophorus and their Reciprocal Crosses. Asian J. Poult. Sci. 3(4): 132-137.