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Identification of Sexes in Poultry Using DNA Based Methods

[1] Manoj Kumar Singh, [2]Jinu Manoj[*]

[1]Department of Livestock Production Management
2Department of Veterinary Public Health and Epidemiology

College of Veterinary and Animal Sciences

Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India.

Abstract: *Genetic sex of most of avian species cannot be identified by the external appearance especially for young birds. Identification of sex in avians can be performed by many techniques, but DNA based methods are more reliable and are most convenient method of sex differentiation in commercially valuable birds or endangered birds. The constitution of sex chromosomes of female birds is ZW and ZZ for males. The DNA sequence that is present only on the W chromosome, which is conserved among species, can be used as a target of PCR-based sex identification. The chromobox-helicase-DNA-binding gene or CHD gene is remarkably conserved in females as CHD-W and has an analogous sequence on Z chromosome (CHD-Z). The specific P_2-P_8 primers amplify the homologous parts of CHD-W as well as CHD-Z, could be the best way to identify the sex of many avian species. Other molecular methods like RAPD, RFLP, AFLP, micro and minisatellites are some of the efficient sex differentiation techniques in avians.*

Keywords: *CHD gene, DNA, identification, poultry, sex*

1. INTRODUCTION

Identification of the sex of birds is important for captive breeding of endangered species and also for basic research such as molecular ecology and developmental biology. Genetic sex of most of avian species cannot be identified by the external appearance especially for young birds [1]. Application of the polymerase chain reaction (PCR) for the identification of genetic sexes of birds is ideal because it requires only a small sample and minimizes trauma to birds. The feathers can be used as source of genomic DNA. DNA extraction from feathers is friendly to birds as it reduces handling stress, blood loss and chance of infection in them [2]. The constitution of sex chromosomes of birds is ZW for females and ZZ for males. Thus any DNA sequence that is present only on the W chromosome and is widely conserved among species would be a target of PCR-based sex identification [3]. Sex determination of various birds was reported by using molecular methods [4, 5, 6, 7, 8].

DNA typing in avian species is first reported [9] based on repetitive sequences, of *Eco*RI family, which was found in the DNA of the chicken W chromosome using *Xho*I family probe. The *Xho*I and *Eco*RI family repetitive sequences were highly specific to the W chromosome [9, 10] and are clearly identifiable after a relatively small number of reaction cycles in PCR [11]. The chicken W chromosome-specific *Xho*I repetitive unit can be used as a probe for identifying the sex of early embryos [12, 13, 14]. A novel model for the development and use of a one-step, SYBR green-based real-time PCR and melting curve analysis for the rapid and specific detection and verification of the gender of birds using sex-specific or P_2/P_8 primer sets was reported [15]. This allowed molecular sexing of birds in a gel-free, quick, and inexpensive as well as high-throughput manner.

RFLP

Sex identification of avian embryos at early stages of development can be achieved by restriction fragment length polymorphism technique [16]. This method is recommended as a management tool for endangered species conservation programs. The RFLP technique has been used as complementrary method in minisatellite [17] and DNA probes like DQSG10, pV47-2, pMg1 for sex identification in geese, brown skua and purple swanphen respectively [18, 19].

RAPD

Random amplified polymorphic DNA (RAPD) markers could be used in sex identification. If the selected RAPD marker is on the W chromosome, it would be amplified only in females and provides a female specific marker [20, 21, 22]. Since the length of primers determines the length of target size, when its length decreases primers could encounter a great number of target sites and increase the chance of amplifying a sex specific locus and this method is species-specific [21]. A female-specific DNA marker using RAPD fingerprinting was identified for pigeon [23], *Columbidae* birds [24] and ostrich (*Struthio camelus*) [25].

AFLP

Amplified Fragment Length Polymorphism (AFLP) method has been used in ostrich (*Struthio camels*) and shag (*Phalacrocorax aristotelis*) to sex identification [26, 27, 28].

Micro and minisatellites

Use of microsatellite or short tandem repeats (STRs) were reported for sex identification in birds [29, 30, 31]. The use of minisatellites or VNTRs (Variable number of tandem repeats) was also reported for sex differentiation. The human minisatellite probe 33.15 was used for sex identification in South American parrots [17, 32].

CHD genes in avians

CHD gene composes an improved basis for DNA sexing [1, 33, 34, 35]. CHD-W found on the chicken W chromosome [1] and a unique sequence EE0.6 (0.6 kb *Eco*RI fragment) found on the long arm of the chicken W chromosome [4] have been utilized for the purpose of sex identification. The latter sequence is widely conserved on the W chromosome, not only in *Carinatae* species but also in *Ratitae* species [5]. However, both CHD and EE0.6 sequences have their counterpart sequences on the Z chromosome, such as CHD-Z and XH0.6RSM in the oriental white stork (*Ciconia boyciana*), and the similarity of W- and Z-linked sequences is variable among species, which causes occasional ambiguous results in the sex determination [1, 4]. The EE0.6 sequence found on the long arm of the chicken W chromosome is conserved in all species of birds both in *Carinatae* and *Ratitae* [4, 5]. The sex of 36 species belonging to 16 different orders of *Carinatae* was determined by comparing the W- and Z-linked EE0.6 sequences [3].

Multiple primers have been designed to screen the intron size difference in CHD genes. The most commonly used primer pairs for sex identification in avian species were P_2 – P_8 [1] and 1237L – 1237H [33]. In most avian species the length of CHD gene is slightly longer in the W chromosome as compared to the Z due to the presence of additional DNA bases in intron region. However in some species like pukeko, most owls and hawks intron size of CHDW and CHDZ genes show very similar sizes [1, 33]. In *Ratitae*, SS and OSM_5 primers were used in sex identification of ostrich which belongs to *Ratitae* family [36, 37]. PCR-based methods to sex-type ostriches were reported [28, 36, 38]. Identification of sex of the endangered old world vultures by PCR based method, facilitated the breeding of these birds in captivity [39].

DNA sexing techniques which target the conserved CHD genes in owls [40] and in pin-tailed Manakin (*Ilicura militaris*) [41] using the CHD genes were also reported. Sex identification in Black-faced Spoonbill amplified CHD-W and CHD-Z genes, with products of 658 and 464 base pair respectively [42], while sex determination in Cockatoo (parrot) species (*Nymphicus hollandicus*) from feather also possible [2].

The highly conserved CHD-W gene can be used as a universal tag for avian sexing based on single-strand conformation polymorphism (SSCP) protocol [43]. The sex determination with primers USP_1 and USP_3 is applicable for Galliformes and some Falconiformes and the primers P_2 and P_3 for Galliformes. The molecular evolution of the coding sequence of the CHD1Z and CHD1W genes revealed that these two genes evolve independently but are highly conserved at nucleotide as well as amino acid levels [34].

Similar reports were also found in neognath and paleognath bird CHD sequences [44]. Palaeognathous birds have been morphologically conserved karyotypes and less differentiated ZW sex chromosomes. The molecular basis of chromosome orthologies and sex chromosomal differentiation by comparative chromosome painting, revealed that the karyotypes and sex

chromosomes of palaeognathous birds are highly conserved not only morphologically, but also at the molecular level [7]. The comparative cytogenetic maps of the Z and W chromosomes of palaeognathous birds, *Eudromia elegans* and *Struthio camelus* revealed that there are partial deletions in the proximal regions of the W chromosomes in the two species and the W chromosome is more differentiated in *E. elegans* than in *S. camelus* [8].

Gender identification in Zebra Finches (*Taeniopygia guttata)* by a W-chromosome linked marker is reported [45]. The structure and brain expression of the sex chromosome genes CHD1Z and CHD1W in Zebra Finches revealed that the two genes maintained a high degree of similarity especially within the C, H and D domains, but outside of these regions larger differences were observed [6]. *CHD1Z* mRNA was expressed at a higher level in the male brain than in the female at various post-hatch ages and the proteins have been also diverged in their function. Two genes, ATP5A1 and CHD1, which was assigned to the female-specific W chromosome of birds, were present on the Z chromosome also [46]. This indicated a common ancestry of the two sex chromosomes, with the evolution from a pair of autosomes.

Other genes

The *DMRT1* gene which is Z linked is expressed early in male development [47] and the *PKCIW* gene which is W linked which appear early in female gonads [48]. The Z-linked gene, *DMRT1*, supports the Z-dosage model of avian sex determination and two W-linked genes, ASW and FET1, represent candidate female determinants [49, 50].

The W-linked gene, HINTW, is expressed in the gonads of female birds just before sexual differentiation and is distinctly different from its homolog on the Z chromosome (HINTZ), thus ,makes a candidate gene in avian sex determination [48]. HINTW showed evidence of adaptive molecular evolution related to female development [51]. The phylogenetic analysis within galliform birds (chicken, turkey, quail, and pheasant) showed that individual HINTW copies within each species are more similar to each other than to gene copies of related species [52].

The presence of WPKCI gene on the W chromosome with its locus in the nonheterochromatic end region was found to be express significantly greater during the early stages of development of female embryos [48]. WPKCI gene is involved in triggering the differentiation of ovary by interfering with the function of protein, PKCI and it is expressed in early female embryos, which can be used for determination of sex in avians. The female specific WPKCI gene was conserved among all the *Carinatae* species of birds examined by them.

The sex of quail and duck embryos can be identified based on *Sox9* gene expression [53]. *Sox9* is a member of the *Sry-type HMG-box* (*Sox*) gene family. It encodes a transcription factor and is important for sexual differentiation in chicken.

2. CONCLUSION

Identification of sex in avians can be performed by many techniques, but DNA based methods are more reliable. It is most convenient method of sex differentiation in commercially valuable birds or endangered birds. The feathers can be used as source of genomic DNA. DNA extraction from feathers is friendly to birds as it reduces handling stress, blood loss and chance of infection in them. Molecular methods including RAPD, AFLP, RFLP, micro and minisatellites, amplification of CHD genes are some of the efficient sex differentiation techniques in avians. The P_2 -P_8 primer pairs for the conserved dimorphic genes of CHD sequence could be the best way to identify the sex of many avian species.

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