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Molecular Marker Based Sex Identification in Ratite and Non-Ratite type of Birds: A Comparative Study*

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Abstract

The aim of this research was to test the *CHD* (*Chromo Helicase DNA-binding gene*) gene as molecular marker for sexing dimorphic and monomorphic birds because of its high degree of conservation and different lengths in Z and W chromosomes due to different intron sizes. Sex determination was attempted in 74 samples of 10 ratite and one non ratite species. Genomic DNA was isolated from blood samples of dimorphic birds *and* feather samples of monomorphic birds. The amplification of the *CHD* gene was performed with 2550F/2718R sets of universal primers and it was successful in non-ratite species. w1 and k7 primers were suitable for ratite type of birds.

Keywords: Molecular sexing, *CHD* gene, Ratite, Non-ratite birds.

Identification of sex in birds is useful to eliminate the unwanted male birds at an early age for economic management. In monomorphic birds, especially in young birds, sex is difficult to identify based on their external morphology. Sex-specific genetic markers such as sexing methods are capable of sexing a wide range of birds. Collectively, these methods are capable of genetically sexing nearly all species of birds, with the notable exception of the ratites.

Materials and Methods

A total of 74 samples were collected from non-ratite and ratite birds of 11 different species (Table I). Genomic DNA was extracted from the blood samples of large dimorphic birds (Miller *et al.,* 1988) and feather samples were used in small endangered monomorphic birds (Bello *et al.,* 2001). Single universal primers 2550 F (5'- GTT ACT GAT TCG TCT ACG AGA- 3') and 2718 R (5'- ATT GAA ATG ATC CAG TGC TTG 3') were used for the amplification of the *CHD1* gene in non-ratite birds (Fridolfsson and Ellergen, 1999). Another set of primers w1 (5'- CCT TTA AAC AAG CTG TTA AAG CA – 3') and k7 (5'-TCT CTT TTG TTC TAG ACA CCC T – 3') were used to amplify sex specific DNA fragments in the ratite birds (Huynen *et al*, 2002). The following three PCR amplifying methods were used for the sexing monomorphic and dimorphic birds. All PCR amplification was performed in 20 µL reaction mixers containing: 2 x reaction buffer (Gene Technologies, Chennai) and 5 p. moles of each primer. In the first reaction, dimorphic birds were sexed using 2550 F/ 2718 R primes with the annealing temperature of 53ºC for 30 sec. Where as in second protocol, monomorphic birds were sexed with same primer at a annealing temperature 55ºC. w1 and k7 primers were used to sex the emu birds with the annealing temperature of 53ºC for 45 sec.

Results and Discussion

All non- ratite birds viz., Domestic fowl, Guinea fowl, Cockateil, Macaw, White crested cockatoo, Night heron, Painted Stork, Turkey, Pigeon are sexed using PCR amplification optimized at 55º C annealing temperature where as in Japanese quail annealing temperature was optimized at 53º C. All male birds were represented by a single band fragment (*CHD-Z*) at approximately 600 bp, whereas females were represented by two amplified bands, sized around 450 and 600 bp (*CHD-Z* and *CHD-W*) are shown in Fig. 1 and 2. PCR amplification of the *CHD* gene produces a single Z-band in male and two bands (Z and W) in female, because of varying

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Table I. List of species and type of materials collected for isolation of DNA

Order	Species	Common name	No.of samples
Non- ratite bird			
Galliformes	*Gallus gallus domesticus**	Domestic fowl	8 (blood)
	*Numeadia maleagridis**	Guinea fowl	8 (blood)
	*Coturnix japonica**	Japanese quail	8 (blood)
	*Maleagridis gallapova**	Turkey	8 (blood)
Psittaciformes	*Ara chloroptera*#	Macaw	6 (feather)
	Cacatua alba#	White crested cockatoo	6 (feather)
	Nymphicus hollandicus#	Cockatiel	6 feather)
Ciconiiformes	*Nycticorax nycticorax*#	Night heron	6 (feather)
	Mycteria leucocephala#	Painted Stork	6 (feather)
Columbiformes	*Columbia livia*#	Pigeon	6 (feather)
Ratite bird			
Casuariformes	Dromaius novae hollandiae#	Australian Emu	6 (feather)
			Total – 74

* Dimorphic bird # Monomorphic birds

intron length in male and female indiviuduls (Fig. 1 and 2). *CHD* gene was the first gene discovered on the avian W chromosome (*CHDW*) (Griffiths and Tiwari, 1995). *CHD*-Z is located on the Z chromosome (Griffiths and Korn, 1997) and is present in both sexes (male ZZ; female WZ). There are very few differences between *CHD1-Z* and *CHD1-W* proteins (Fridolfsson and Ellegren, *loc. cit*). Garcia-Moreno and Mindell (2000) showed that phylogenetic analyses can be useful in rooting the related genes on opposite chromosomes (gametologs). In most avian species, the length of the *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 for example some terns, pukeko, most owls and hawks intron size of *CHDW* and *CHDZ* genes show very similar sizes (Griffiths *et al., loc. cit*) and he failed to amplify the sex specific regions of 28 bird non-ratite species because of absence of intronic length difference between Z and W chromosomes. The 2550F / 2718R primers are not suitable in ratite (Australian emu) birds because of absence of intronic length difference between Z and W chromosomes. Hence, sexing of Australian emu *i.e.* ratite type of birds was performed with, another set of primers w1 and k7.

In ratite birds, sex determination was achieved by using sex specific w1 and k7 primers. These primers are well amplified in flightless emu (*Dromaius novaehollandiae*) and are given three different distinguishing banding patterns of Z and W sex chromosome regions. The banding pattern showed presence of a 350 bp band and another approximately 300 bp band in both male and female samples which is sex specific region of Z chromosomes (Fig 3). An additional 150 bp band apart from these two bands was observed in case of female samples which is sex specific region of W chromosome. The same finding was also observed by Jeyakumar *et al.* (2013) and the same was reviewed by Dash *et*

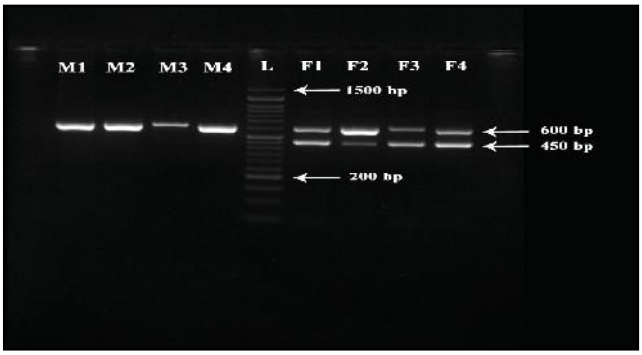

Fig.1 EtBr stained banding patterns in dimorphic birds with 2550F and 2718R set of primers. M1 – Domestic fowl (♂), M2 – Guinea fowl (♂), M3 – Japanese quail (♂), M4 – Turkey (♂) L – Ladder, F1 – Domestic fowl (♀), F2– Guinea fowl (♀), F3 – Japanese quail (♀), F4 – Turkey (♀).

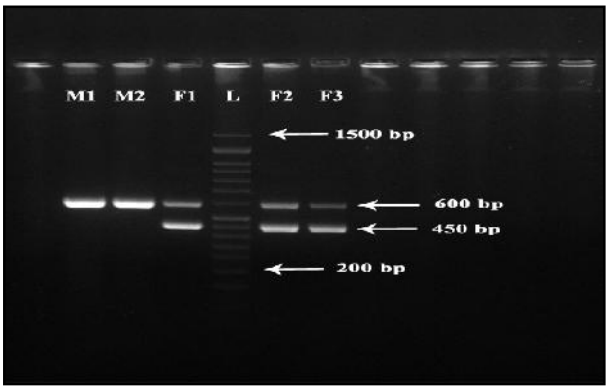

Fig.2 EtBr stained banding patterns in monomorphic birds with 2550F and 2718R set of primers. M1– Night heron (♂), M2 – Cockateil (♂), F1 – White crested cockatoo (♀), L – Ladder, F2 – Macaw (♀), F3 – Painted Stork (♀)

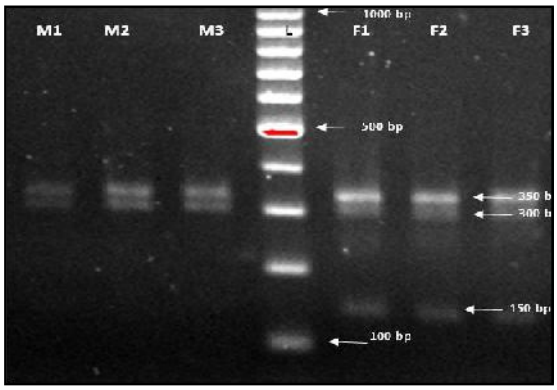

Fig. 3 EtBr stained banding patterns in Dromaius novaehollandiae with w1 and k7 set of primers. M1, M2, and M3: Emu (♂), F1, F2 and F3: Emu (♀)

al. (2014). Huynen *et al.* (*loc. cit*) designed w1 and k7 primers based on the sequence of the sex specific marker kW1. The primers were used to sex all related ratite species. Fraire and Mortella (2006) carried out sex determination in lesser rhea *(Rhea pennata pennata)* using w1 and k7 primers. Costantini *et al.* (2008) was also observed a single sex-specific band of 150 bp only in females. Gábor *et al.,* (2014) observed the use of feathers for isolation of genomic DNA was an optimal one for determining sex in young birds.

Summary

Genomic DNA was isolated from both blood and feather samples of birds depending on the size and endangered conditions of birds. In non-ratite birds, all male birds being represented by a single band fragment (*CHD-Z*) visualised at approximately 600 bp, whereas females are represented by two amplified bands, sized around 450 and 600 bp (*CHD-Z* and *CHD-W*). Whereas in ratite type of birds, the sex specific primers: w1 and k7 will be better option for sex determination. The banding pattern showed presence of a 350 bp band and another approximately 300 bp band in both male and female samples which is sex specific region of Z chromosomes. An additional 150 bp band apart from these two bands was observed in case of female samples which is sex specific region of W chromosome.

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