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SEX IDENTIFICATION IN BIRDS BASED ON POLYMERASE CHAIN REACTION (PCR) AND POLYACRYLAMIDE GEL ELECTROPHERESIS (PAGE)

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Abstract. PCR and PAGE techniques could be used to identify sexes in birds, where females are heterogametic (ZW) and males are homogametic (ZZ). The sex system depends upon a female specific W-chromosome linked gene (CHD-W) and a male specific Z-chromosome linked gene (CHD-Z). The two genes could be amplified by PCR, and give different banding pattern on Polyacrylamide gel in a wide variety of birds. We examined 180 birds of six different species, identified their sex using this approach. This technique could be universally used to identify sexes of most avian species.

Key words: biochemistry, chromosome, gene, PCR, PAGE, sex.

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Определение пола у птиц на основе реакции полимеразной цепочки и электрофореза полиакриламидного геля. - Г. Муйтаба, М.С. Надим. - Беркут. 15 (1-2). 2006. - Методы могут использоваться для определения пола у птиц, поскольку у них самки являются гетерогаметными, а самцы – гомогаметными. Половая система зависит от генов, сцепленных с W-хромосомой самки (CHD-W) и Z-хромосомой самца (CHD-Z). Эти два гена могут быть амплифицированы при помощи реакции полимеразной цепочки и имеют разный характер сегментирования в полиакриламидном геле. Исследования проводились на 180 птицах 6 видов. Этот метод может использоваться для определения пола большинства видов птиц.

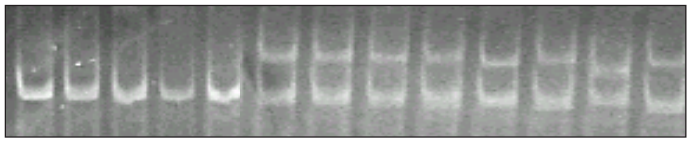
Introduction

Our knowledge about the molecular process behind sex determination has improved significantly in recent years, notably through the insights gained into the mechanisms that control sexual development in mammals, *Drosophila melanogaster* and *Caenorhabditis elegans* (see e.g. Meyer, 2000; Schutt, Nothiger, 2000; Vaiman, Pailhoux, 2000). In contrast to these advances, the molecular determinants behind sexual development in birds have largely remained a mystery (Clinton, 1998; Clinton, Haines, 1999; Ellegren, 2000). We know that the process is different from that in mammals, as it has not been possible to identify an *SRY* (a gene that confers maleness in mammals) homolog in avian genomes (Griffiths, 1991). Moreover, the failure to identify an avian *SRY* probably is a reflection of what appears to be a general, but perhaps surprising, phenomenon. Despite the fact that the occurrence of two sexes is a nearly universal feature throughout the animal kingdom, the genes involved in directing the process of sexual development seem virtually unrelated among metazoan phyla. The differences obviously raise obstacles for comparative or candidate gene approaches in studies of sexual development. Nevertheless, the first hints toward the genetic mechanism that underlies sex determination in birds recently have come from studies of the expression pattern and chromosomal localization of two genes potentially associated with avian sexual differentiation.

There is a greater need for identifying sexes in birds, especially where there is no discrimination between the two sexes. In some of the birds like Stone Curlews (*Burhinus oedicnemus*), the two sexes remain virtually indistinguishable throughout their lives, with males having a slightly more prominent black bar above the whitish one on the wing-coverts (Jonsson, 1992).

One possible approach to identify sexes in birds is their DNA analysis. In birds, females are heterogametic (ZW), while males are homogametic (ZZ). This principle has been pre-

viously used to produce sexing techniques for several avian species like European Starling (*Sturnus vulgaris*) (Griffiths et al., 1992; Braudbury et al., 1997), Purple Gallinule, (*Porphyrio melanotus*) (Millar et al., 1996), Lesser Black-backed Gull (*Larus fuscus*) (Bradbury, Griffiths, 1997), Norfolk Island Boobook Owl (*Ninox novaeseelandiae undulata*) (Double, Olsen, 1997) and the Black Stilt (*Himantopus novaezelandiae*) (Millar et al., 1997). This method involves the isolation of a W-linked marker, which could be done by RAPD (Rapid Amplified Polymorphic DNA). The problem associated with this method is that RAPD tends to be species specific (Lessels, Mateman, 1998).



Showing banding pattern obtained with different sexes of Houbara Bustard on PAGE.

Различия в сегментировании у особей разного пола у джека.

In contrast to RAPD, CHD-W is present in a wide range of birds and has a highly conserved DNA sequence (Griffiths et al., 1996). CHD-W and CHD-Z have been previously used to identify sex in Houbara and other land birds (Aloia, Griffiths, 1999), but it has a severe limitation in selection of Restricion enzymes, which is practically not applicable. This paper demonstrates that how we can correctly identify sexes in most of birds without involving the use of Restriction enzymes, by directly running the amplified products on Polyacrylamide gel.

Materials and Methods

This study was initiated with the prior approval of the institutional Review Board of Hazara University Mansehra, Pakistan. Blood samples were taken in vacutainers from Common Buzzard (*Buteo buteo*), Lagger Falcon (*Falco jugger*), Gray Partridge (*Perdix perdix*), Little Brown Dove (*Streptopelia senegalensis*), Common Pheasant (*Phasianus colchicus*) and Houbara Bustard (*Chlamydotis undulata*). DNA was extracted by standard Proteinase K and Phenol-Chloroform technique (Sambrook et al., 1989).

Two PCR primers P2 (5′-TCTGCATCGCTAAATCCTTT) and P3 (5′-AGATATTCCGGATCTGATAGTGA) were designed to amplify CHD-W and CHD-Z respectively.

PCR reaction volume of 25 ul include 2.5 units Taq DNA polymerase, PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl) with final conc of 1X, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 uM of each primer and approximately 200 ng of genomic DNA.

PCR conditions consisted of an initial denaturation at 94 °C for 3 min followed by 35 cycles of PCR amplification at:

94 °C for 45 sec: Denaturation;

55 °C for 30 sec: Annealing;

72 °C for 1.5 min: Extension.

The last extension step was extended to 10 min followed by incubation at 4°C. The amplimers were directly run on 8 % non-denaturing Polyacryamide gel (containing Polyacrylamide, N,N Methylene-bis-acyrylamide, TBE, Ammonium Persulphate and TEMED). Amplified products were mixed with loading dye containing 0.25 % Bromophenol blue prepared in 40 % Sucrose solution and loaded into the wells. Electrophoresis was carried out at 100 volts for 90 minutes and the gel was stained with Ethidium Bromide (10 mg/ml) solution for visualization on UV Transilluminator.

Results

Results obtained by running the amplified product of DNA samples obtained from Buzzard, Lagger Falcon, Gray Partridge, Little Brown Dove, Pheasant and Houbara on Polyacryamide gel (PAGE) showed the particular

banding pattern of the two sexes. Female birds gave two bands on the PAGE, while male birds gave only one band (Fig.). This is an easy approach that could be used to identify sexes not only in the above mentioned birds but also in almost other bird species.

Discussion

PCR and PAGE are the powerful techniques that could be used for sex identification in a wide range of birds i.e. Buzzard, Lagger Falcon, Gray Partridge, Little Brown Dove, Pheasant and Houbara. Molecular based sexing system described here is based on two genes, CHD-W and CHD-Z. P2 and P3 are the primers used for the amplification of two genes. Females produce two amplified products of different size and sequence while males produce two amplified products of equal size and almost similar sequence. Although the band separation is difficult on Agarose gel but it can be easily performed on Polyacrylamide gel (PAGE). So, females produce two bands on PAGE while males produce only one band when observed under UV-light.

The same approach has been previously used for sex identification in houbara bustard and other land birds making use of the same two genes (Aloia, Griffiths, 1999). That technique suffers from a serious problem of selection of Restriction enzymes, which is a difficult job for a new species. The molecular genetic techniques described in this paper are easy to use and applicable to almost all avian species.

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