MOLECULAR SEX IDENTIFICATION IN BIRDS

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Molecular sex identification belongs to the most reliable methods of sexing. It is independent of phase of birds development, can be conducted in monomorphic birds, and even when the size difference between Z and W chromosomes is low, for example like in ostriches (Malago et. all, 2002). These techniques are split on two methods: based on DNA hybridization, and based on polymerase chain reaction (PCR). The main topic of this research was the most frequently used method belonging to the second group - identification based on amplification of CHD1 gene fragment. This molecular analysis is based on the presence of CHD1 gene in Z and W chromosomes. Obtaining of PCR product is possible with one of the three pairs of primers: 2550F/2718R (Fridolfsson and Ellegren, 1999), P2/P8 (Griffiths et al., 1998) and 1237L/1272H (Kahn et. al., 1998). Analysis of obtained PCR product can be performed by analyzing the difference in intron size between CHD1 gene fragments (CHD1-Z and CHD1-W) or in sequence of exon in CHD1 gene (CHD1-Z and CHD1-W) in sex chromosomes (Z and W) (Cerit and Avanus, 2007).
CHD1 gene contains at least two introns which are distinct in length, depends on sex chromosome in which they are present. After amplification the sequence and electrophoretic separation in agarose gel, one stripe for male and two stripes for female are obtained (Gabor et al., 2014). In case of choosing analyze of difference in exon in CHD1 gene, restriction enzymes (BsuRI, HaeIII, MaeII, BshN) are used. Digesting by restriction enzyme enables obtaining specific number of sequence fragments for Z and W chromosomes. Electrophoretic separation will difference the sexes
For some bird species, the PCR product may give 1 band for both sexes. This is due to the amplification of a short copy of the gene in the W chromosome, resulting in an undetectable Z chromosome product in females, while males still show one band in the gel (Fridolfsson and Ellegren, 1999). This arrangement is visible in the birds of the Anatidae, Gruidae, Scolopacidae, Falconidae and Accipiteridae families (Fridolfsson and Ellegren, 1999). In turn, using the 1237L / 1272H pair (Kahn et al., 1998), it is possible to create more non-specific fragments and less pronounced bands in the gel than using the P2 / P8 pair, which may be due to greater genetic variation between species in the primer pair attachment region 1237L / 1272H (Jensen et al., 2003). Restrictive enzymes are used to obtain different results for both sexes. In the case of Z and W chromosome fragments that do not differ in size, but are heterogeneous in terms of nucleotide sequence, the use of a suitable restriction enzyme will result in obtaining female and male specific sequence fragments. The gel separation following digestion with an appropriate restriction enzyme differentiates both sexes. For example, when using the BsuRI enzyme, 3 bands are obtained in the gel for the female (ZW), and 2 for the male (ZZ) due to the different number of restriction fragments depending on the chromosome.
Sex identification will allow the development of strategies for the conservation of genetic resources in different bird species and will allow a full understanding of the state and dynamics of population development of different bird species.

Keywords - Molecular sex identification, CHD1 gene in birds, CHD-Z gene, CHD-W gene

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