SUMMARY
of the Final Report

On
Minor Research Project Entitled

"Molecular Methods For Avian Sex Determination"

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SUMMARY OF FINDINGS

It is very easy to distinguish between males and females in birds that are sexually dimorphic, such as the house sparrow (*Passer domesticus* L.), mallard (*Anas platyrhynchos* L.) and collared flycatcher (*Ficedula albicollis* Tem.). However, males and females of many species have very similar phenotypic traits (sexual monomorphism), so that even experienced ornithologists may have problems with unambiguous sex identification. For example, at least 60% of all passerine species are sexually monomorphic in colour (Price & Birch, 1996). In nestlings, it is even more difficult than in the adults to distinguish between the two sexes. This applies to almost all bird species, as even in those sexually dimorphic as adults and juveniles, the males and females do not differ in morphological traits shortly after hatching. Usually the distinct differences between the sexes appear at the earliest a few weeks after hatching. In passerines, for example, these results in the lack of data on sex ratio in broods, as young birds leave the nest before they can be sexed on the basis of morphological traits.

In birds, females are the heterogametic sex, carrying one copy each of the Z and W sex chromosomes. Males are homogametic (ZZ). Although there is a small pseudoautosomal region on Z and W with an obligate crossing-over at meiosis, most of the W chromosome does not recombine (this is analogous to the situation in X and Y of mammals). Critical to the question of sex determination in birds is the role of the two types of sex chromosomes. Molecular sexing (Ellegren and Sheldon1997) based on polymerase chain reaction (PCR) is an attractive option, since PCR is simple to perform, rapid and requires only a minute quantity of DNA, which could be obtained from a single feather or a drop of blood. The DNA thus obtained could be used to identify a sex-specific marker. The female bird is heterogametic (ZW), and obviously the W chromosome has to be the source of the sex-linked marker. The first W chromosome gene discovered was the chromobox – helicase – DNA - binding gene (*CHD-W*) (Griffiths and Tiwari,1995). This gene is highly conserved and it has been shown that a single pair of PCR primers (P2 and P8) can be used to sex most of the avian species (Griffiths et al., 1996; Griffiths et al., 1998). These primers anneal to conserved exonic regions and amplify across an intron that varies in size between *CHD-W* and *CHD-Z* (Griffiths et al., 1998; Ellegren,1996; Kahn et al., 1998; Fridolfsson and Ellegren, 1999).

The problems associated with reliable sex identification were overcome by introducing the molecular techniques in the 1990s. These techniques are generally based on DNA hybridization or polymerase chain reaction (PCR). (Dubiec and Zagalska-Neubauer, 2006). Pigeons constitute the bird family Columbidae and order Columbiformes that includes about 310 species. The body is spindle shaped. The size varies from 20-25 cm. The body is divisible into head, neck, trunk and tail. Most part of the body is clothed in feathers. Pigeons vary greatly in coloration, common colors include: slate blue-grey with two black bars on wings, blue-grey with black flecks on wings, reddish brown, solid black, solid white. This work aims at search for DNA markers for sex determination thereby infer the phylogeny of pigeons in order to document and establish their relationship.

Method: Genomic DNA was extracted from fully developed body feathers. The species studied included the pigeons of all the breeds that could not be differentiated morphologically.

DNA Sampling, Storage and Extraction

Sampling A total of 125 individuals were sampled for gender identification. Among a total of 256 samples, there were 184 feather samples and 72 blood samples. Samples were provided by private breeders and fanciers. Blood was collected with a sterile cotton swab after clipping a toenail; one to three thoracic feathers were sampled by plucking and quills were cut into 2-5 mm long pieces samples.

Isolation of genomic DNA from collected species

Feathers. Genomic DNA was extracted from adult feather quills using a sodium hydroxide boiling method (Zhang and Tiersch 1994).

Blood samples. DNA was extracted from blood by a standard phenol/chloroform extraction method.

Results: Gender of sampled birds (125) was successfully identified using feather samples. The results were additionally confirmed using blood samples as well. All types of samples from a single individual gave the same result. Random samples were selected for electrophoresis of

PCR products, gender was determined by visualization on agarose gel as two bands in females (Z and W) or one band in males (Z) with the difference range of 150 to 250 bp between Z- and W-bands, which made separation and visualization simple and reliable (Fig.1).Primers 2550F/2718R were successfully used for amplification of CHD gene of Pigeon.

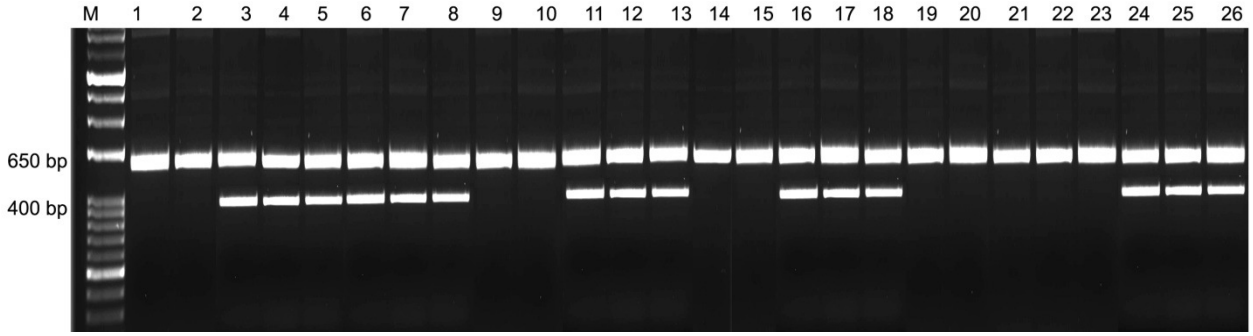

Table 1: Differentiation between male and female on the basis of gel image.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
650 400	m	m	f	f	f	f	f	f	m	m	f	f	f	m	m	f	f	f	m	m	m	m	m	f	f	f
Sample	fe	fe	fe	fe	fe	fe	bl	bl	fe	fe	fe	fe	fe	fe	bl	bl	fe	fe	fe	fe	fe	fe	bl	bl	bl	bl

(m- male, f- female; fe-feather DNA , bl-blood DNA)

On the basis of gel image out of 26 samples 11 samples were identified as males and 15 were females. In samples identified as 11 male, 9 DNA samples were extracted from feather and 2 DNA samples were from blood. In females 9 were feather and 6 were blood samples.