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Determining sex in pigeons (*Columba livia)*

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ABSTRACT

In many birds, especially in monomorphic species and breeds, it is difficult to distinguish between sexes due to lack of phenotypic differences. Originally, sex identification was based on behavioural observations, structure of the pelvis and certain anatomical features. Direct methods, such as endoscopy, laparoscopy and laparotomy, have been widely criticized for being highly invasive, stressful and difficult, especially when the size of birds is considered. Karyotyping fails in some species (ostrich, emu) due to similarity of their sex chromosomes and can be used only in case of the species with heteromorphic female ZW sex chromosomes. With the advances of molecular diagnostics new methods of sex identification have been developed and these include hybridization and polymerase chain reaction (PCR). An effective method of sex identification in pigeons uses CHD1 gene, which is present both in Z and W chromosomes.

Keywords: genetic sex, gonadal sex, phenotypic sex, behavioural sex, pigeon, *Columba livia*

1. INTRODUCTION

Sex is a set of structural and functional features that allow classifying organisms into male or female category. Sexual differences in birds can be observed on phenotypic, genetic and behavioural levels. Early determining of sex, especially in monomorphic species and races of birds, is of great importance for breeders as the earlier it is done, the lower costs of breeding are.

Pigeons can be classified into 9 groups and several utility types: fancy pigeons, bred mainly for aesthetic effect, homing pigeons, once used to carry messages over long distances and nowadays bred as a hobby, meat pigeons that provide tasty meat, and wild pigeons still found in their natural environment. Comparing to *Galliformes* birds, pigeons do not show visible sex attributes, such as wattles in turkeys or comb in roosters.

2. GENETIC SEX

Mechanisms that lead to origin of sex and differences between sexes are called sex determination. Epigenetic sex is determined by environmental conditions (e.g. in reptiles), whereas genetic sex depends on the number of chromosomes or the presence of sex chromosomes. The first is observed in *Hymenoptera* (bees, wasps), where males are haploid and females are diploid.

In birds and mammals sex is determined by the presence of sex chromosomes. In mammals the inheritance of sex is of the Lygeus type, i.e. females are homogametic (two homologous XX sex chromosomes) and males are heterogametic (two different sex chromosomes – X and Y). The actual sex depends on whether the egg (containing X chromosome) is fertilized with X or Y spermatozoid.

The SRY (sex-determining region Y) gene, encoding the protein determining testicle development, is located in Y chromosome of viviparous mammals [1]. Its presence is the main criterion of genetic sex in humans and it is located in the short arm of Y chromosome in locus Yp11.2 [2]. The protein product of SRY acts as a regulated transcription factor which triggers DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region). It is a nuclear receptor protein that in humans is encoded by the NR0B1 gene, which is located on the short arm of chromosome X [3]. This gene produces a protein, inhibiting processes leading to testicle development. It also activates WTN4 gene [4].

In birds the inheritance of sex follows the Abraxas pattern: males have two Z chromosomes and females one Z and one W. Females are heterogametic and produce two types of eggs, thus determining the sex of their chicks. In domestic hen it was demonstrated that it depends on the expression of two genes - DMRT1 (*Double Sex and Mab3 Related Transcription Factor)*, which is located in Z chromosome and is not present in W chromosome, and HINTW (*W-Linked Histadine Triad Nucleotide Binding Protein*) in W chromosome [5,6]. DMRT1 is present in all vertebrata, from fish to mammals, and its expression is found in males [5]. Hake [7] claims that avian FET1 and ASW genes, located in female W chromosome, have similar function to SRY in Y chromosome.

Despite the similarity of ZW and XY sex chromosomes, they are not identical and their evolution was independent. It was also found that Z chromosome of domestic hen is similar to human autosome number 9 [8]. Griffiths and Tiwari [9] were the first to map the first gene in avian W chromosome, namely CHD1 (*chromo-helicase-DNA-binding protein*). Subsequent studies by Ellegren [10] showed that it is both extremely conservative and universal. Its identification in gallinaceous birds is relatively simple with only single starter set in PCR (*polymerase chain reaction*) [9]. The gene is located in the non-recombinant part of W chromosome (CHD1W) and has its homolog in Z chromosome (CHD1Z) [11]. However, its alleles are not identical and differ in the length of one of the introns and the sequence of one of the exons; therefore it is possible to distinguish between sexes [12].

The discovery of this highly conservative gene in both sex chromosomes allows sex identification in many species of birds with PCR and/or PCR-RFLP method [9,10,13]. In pigeons CHD1 fragment is used; it is present in both W and Z chromosomes, yet differs in the length of one intron (the non-coding sequence) and the sequence of one exon (the coding sequence) [10,13,14]. P2 and P8 standards are routinely used in PCR and its product is digested with *Bsu*RI restriction enzyme [15].

3. GONADAL SEX

Gonadal sex results from the normal development of gonads and the type of gonads depends on the expression of certain genes, i.e. on genetic sex. Sex identification based on karyotype analysis is an alternative to surgical methods and it was first used in pigeons by Mengden and Stock in 1976 [16]. The type of gonads is determined by sex chromosomes and fully functional gonads produce specific hormones; this, in turn, leads to the development of secondary and tertiary sexual characteristics. Gonadal sex in pigeons can only be determined with laparoscopy or vent (cloaca) endoscopy [17]. On opening the vent of a female the opening of oviduct is seen and the left side, whereas in males two conical papillae appear on both sides.

Depending on the bird size, these papillae can be 1 to 3 mms long [18]. Female pigeon reproductive system is odd and consists of the ovary and fallopian tube on the left side of the body. On the right side these organs are rudimental in result of asymmetric distribution of primordial reproductive cells on the fourth day of foetal development. Foetal testicles produce two hormones - one (T) hormone stimulates forming of mesonephric (Wolff's) ducts into male direction, and the second, produced by Sertolli's cells, is known as Müllerian-inhibiting factor (MIF). MIF inhibits the development of paramesonephric (Muller's) ducts on the tenth day. As a result, these do not develop into female direction and the right fallopian tube regresses [19]. The left fallopian tube consists of infundibulum, secreting part (magnum), and isthmus [20]. Male reproductive system is even and consists of testicles, epidydymis, spermatic (deferent) ducts and a single phallus.

4. PHENOTYPIC SEX

Phenotypic sex is a set of external features typical of each sex. In pigeons gonads cannot be seen without specialized inspection while phenotypic sex can be recognized by differences in shape and appearance, albeit with rather low certainty. According to Nowicki [21] young pigeons differ in the shape of the vent edges – in males the shape resembles that of a horseshoe as the upper edge covers the lower edge, whereas in females the shape is upside down (Fig. 1).

Some methods, especially in poultry, are based on the speed of wings moulting. Some differences in the growth of primary and secondary remiges (flight feather) can be seen in the first days after hatching and females tend to moult faster than males. Both rows of feathers of a male wing are of even length, while in females the upper row does not cover the bottom one. However, this method has not been tried yet in pigeons.

Some pigeon and hen races can be autosexed. The sex of a chick can be determined by the colour of its down. Also in pigeons some sex-linked genes that also determine the down colour have been identified, together with a few that determine adult colours. The best known example is the Texaner – in this race females are white with red collar around the neck and stripes on the wings, whereas males are uniformly cream white. This difference results from different expression of the dominant "fading" gene ($St^{F)}$ in females and males. Some other sex-linked genes can be used in autosexing, e.g. the Brown (recessive b), dominant Red (B^{A}), Extreme Dilute (d^{ex}), recessive Pale (d^{p}) and dominant Almond (St) [22,23].

Figure 1. Vents of a male (right) and female (left) Texaner pigeons (Photo: Maciej Miąsko).

Adult pigeons differ only slightly. Males are larger than females; their bodies, bills and legs are stronger, heads bigger and bills longer, while their necks are generally thinner and longer. Some breeders are able to distinguish between sexes by the space between pelvic bones – the distance between their ends measured just below the vent is bigger in females than in males.

5. BEHAVIOURAL SEX

Behavioural sex is shaped by development of hormonal system and production of the relevant male and female hormones. In this case sex can be determined by careful observations of behaviours, especially during mating seasons. Males choose their females and begin tooting with cooing and driving the females away from other males. The male outstretches his tail and rubs it on the surface. This enables recognition as in males their rectrices (tail flying feathers) are always ragged. The next sequence of sexual behaviour is reciprocal feeding and copulation which takes a short while – the female crouches, lifts her tail up and beats her wings slightly while he jumps onto her back, presses his vent to hers and beats wings vigorously to keep balance. Eventually the pair moves to the nest. This behaviour can be observed repeatedly.

Once copulation acts have been completed, eggs are laid and sit on, the latter by both parents – by males at daytime, by females at nights [24]. Hatching takes several days and the new-borns display different behaviours in reaction to human presence. Males beat their wings and stretch in the nest, while females remain calm [21].

6. SUMMARY

The article describes main differences between male and female pigeons. All current methods of sex identification can be classified as non-invasive and invasive ones. The first are based on observations of shape, colour, feathers and behaviour but these are all far from effective. Others (karyotyping, laparoscopy, and endoscopy) are far more effective, yet quite expensive. Developing a method based on DNA testing can replace current invasive methods.

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