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Description of the Muscovy Duck (*Cairina moschata*) Karyotype

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The karyotype of the Muscovy duck originating from *Cairina moschata* was characterised on the basis of R and C bands. Chromosomal preparations obtained from an *in vitro* blood lymphocyte culture were RBG- and CBG-stained. The structures of nine and fourteen pairs of chromosomes were examined based on R bands and C bands, respectively. Ideograms of banded patterns of the analysed chromosomes were drawn. The sizes of individual constitutive heterochromatin blocks wereas measured. The morphology of the analysed chromosomes was assessed.

Key words: *Cairina moschata* duck, karyotype, R bands, C bands.

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Progress in avian cytogenetics in recent years has been much slower poorer than in mammalian cytogenetics. Typical bird karyotypic traits include a large diploid number of chromosomes. A typical bird karyotype is made up of several tens of chromosomal pairs. The range fluctuates from 40 in *Burhinus oecidimus* to 126 in *Upupa epops* (CHRISTIDIS 1989, 1990). The largest chromosomes, four to eight microns long, are termed macrochromosomes and they constitute only a few pairs of chromosomes. Microchromosomes are usually smaller than two microns. In all the orders studied, the tendency exists towards a reduction of small chromosomes and an increase in the number of large chromosomes (TAGELSTRÖM & RYTTMAN 1995). In an evolutionary aspect the bird karyotype emerged approximately 250 million years ago, i.e. when the accumulation of small chromosomes took place. Macrochromosomes most probably evolved by means of a Robertsonian translocation (TAGELSTRÖM & RYTTMAN 1995) or a fusion and rearrangement of chromosomes (RODIONOV 1996; BURT 2002; GREGORY 2002). The two groups of chromosomes differ between each other not only with respect to size but also in nucleotide composition. A higher content of A-T pairs is typical of macrochromosomes, whereas microchromosomes have got more G-C pairs. Earlier replication and an increased frequency of crossing-over are consequences of the high content of G-C pairs (KAELBLING & FECHHEIMER 1983; RODIONOV 1996). The fact that females are heterogametous (ZW) and males are homogametous (ZZ) is a typical bird feature. Moreover, the avian genome is characterised by a small amount of genetic material and that constitutes about 33% of human genome size. This relatively small genome is packed into a large number of chromosomes, and is a result of an evolutionary process of microchromosome genome minimization mainly due to a reduction in the amount of repeats (FILLON *et al*. 1998; BURT 2002; GREGORY 2002).

Birds are the most numerous group of vertebrates but is their genome that is the least known. Genetically, the best known genetically bird species is the domestic chicken *Gallus domesticus*. Hen Chicken genome sequencing has shown the existence of syntenic regions between the human and bird genomes. Despite the divergence between birds and mammals, DNA sequence conservatism has been preserved (NANDA *et al*. 1999; SCHMID *et al*. 2000; ICGSC 2004; MASABANDA *et al*. 2004). The known *Gallus domesticus* genome may be used in genetic research and should become a model in comparative analyses involving other bird species (SCHMID *et al*. 2000).

Cairina moschata ducks are one of the genetically least known waterfowl species. Little progress in cytogenetic studies was a stimulus to undertake the present investigations. The aim of

this study was to describe, on the basis of RBG and CBG bands of chromosomal banding, the karyotype of the Muscovy duck descending from *Cairina moschata*.

Material and Methods

Chromosomal preparations obtained from an *in vitro* lymphocyte culture of Muscovy duck peripheral blood were subjected to analysis. The blood was sampled from ten birds. Two standard banding techniques were used in the study, RBG (PERRY & WOLFF 1974) and CBG (SUMNER 1972). The first technique mentioned included the incorporation of BrdU and Hoechst 33258 in the 65th hour of incubation, and Ethidium Bromide and colchicine in the 69th hour of incubation. Both techniques were applied to analyse ten metaphase plates. The structure of nine chromosomal pairs was examined with respect to R bands, and of 14 chromosomes with respect to C bands. Ideograms of banded patterns of the described chromosomes were drawn.

The description of the RBG band pattern of the examined chromosomes was prepared according to the conventionally accepted rules of ISCNDA (1989) and LADJALI-MOHAMMEDI *et al*. (1999).

In addition to determining the C band pattern on the chromosomes, CBG banding made it possible to measure the size of individual heterochromatin bands and express them as a relative value in relation to the whole length of the chromosome. The results were described by means of basic statistical measures (mean [$\overline{X}$] and standard deviation [S]). The morphology was assessed by calculating the index of arms [q/p] and centromeric index [p/(p+q)].

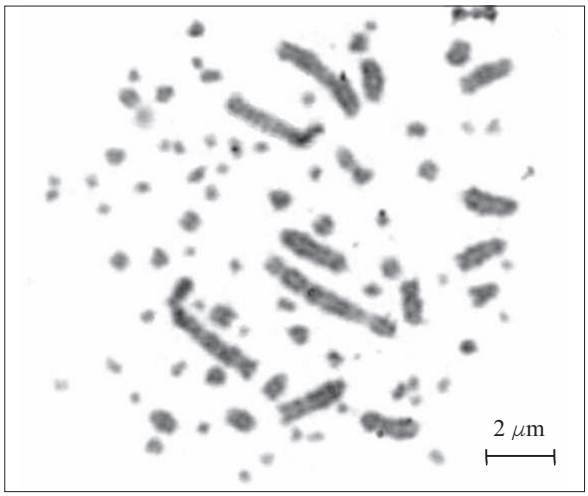

Fig. 1. Picture of the metaphase plate of the chromosomes of *Cairina moschata* duck (RBG banding).

Results

The analysis of the Muscovy duck karyotype was carried out by means of two chromosome banding techniques, RBG and CBG.

Differences in size were observed among 80 Muscovy duck chromosomes located in somatic cells (Fig. 1). The largest chromosomes were 4 to 12 μm long, whereas the smallest chromosomes analysed were 0.65 to 1.6 μm long. The remaining microchromosomes were observed under the microscope as points. An ideogram of the RBG band pattern of the nine analysed pairs of chromosomes was prepared (Fig. 2). In total, on the examined chromosomes there were found 129 R bands, in-

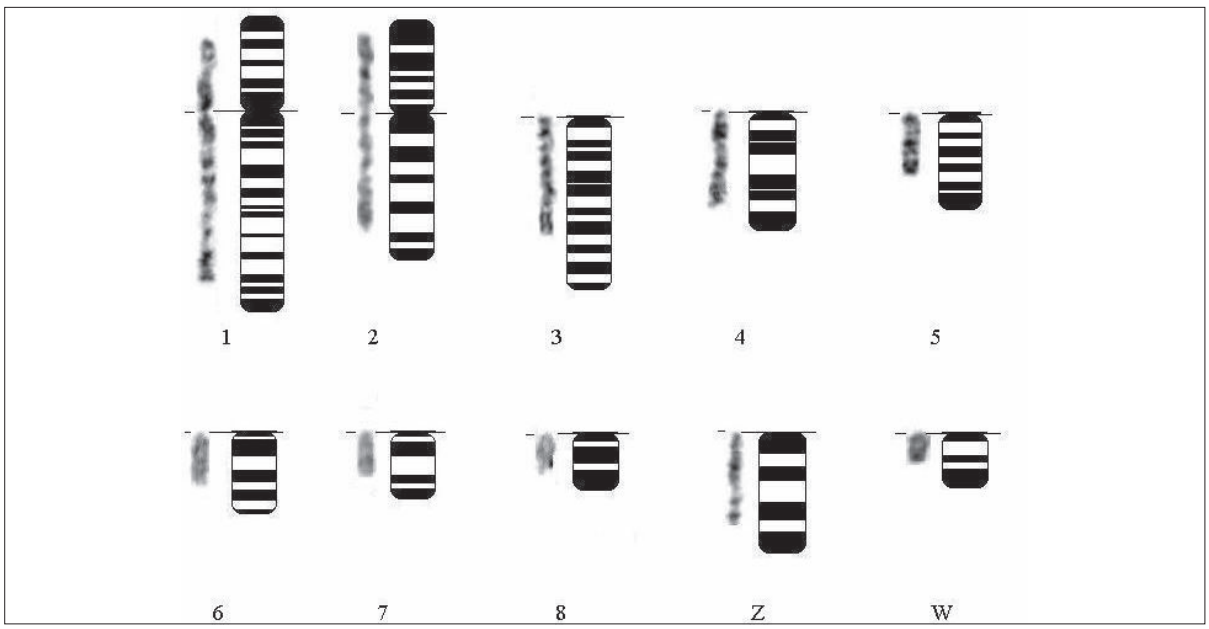

Fig. 2. Ideogram and karyogram of the chromosomes of *Cairina moschata* duck (RBG banding).

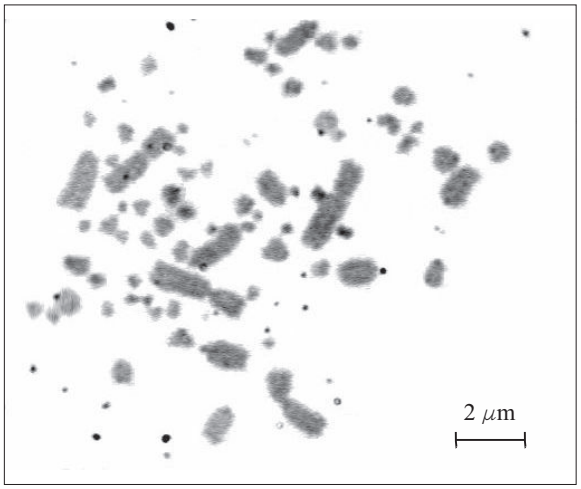

Fig. 3. Picture of the metaphase plate of the chromosomes of *Cairina moschata* duck (CBG banding).

cluding 72 dark positive ones and 57 light negative onesbands, were found on the examined chromosomes.

The first largest chromosome was submetacentric. Its centromeric index was 1:1.606 and the index of arms was 0.387. A total of 32 R bands There were observed 32 R bands. On the p arm nine R bands were identified, including five positive ones. Twenty-three R bands were counted on the q arm, 12 positive bands and 11 negative ones.

The index of arms of the second submetacentric chromosome was 1:1.507, and the centromeric index equalled 0.411. Nine R bands were located on the p arm, there were nine R bands, five dark and four light ones. The q arm had 11 R bands, including six positive ones.

The third acrocentric chromosome had one darkly stained band on a small p arm. The q arm was characterised by the presence of ten dark R positive bands.

An acrocentric chromosome of the fourth pair had one dark band on the p arm. On the q arm of the chromosome there were 11 R bands, including six positive dark bands and five negative light ones.

On the fifth acrocentric autosome one positive band on the p arm was determined. On the q arm of the chromosome, 11 R bands were observed, including six dark bands and five light ones.

The acrocentric chromosome of the sixth pair had nine R bands, including five positive onesbands, on the p arm.

Seven R bands were found, including three positive bands and two negative ones, on the seventh acrocentric chromosome.

The eighth acrocentric autosome had five R bands, including three positive and three negative ones.

The Z chromosome of the Muscovy duck was classified as belonging to the group of subtelocentric chromosomes. On the q arm of the chromosome four dark bands and three light ones were observed, amounting to seven R bands.

The W heterochromosome was characterised by the presence of five R bands, including three positive and two negative ones.

Observations of the Muscovy duck chromosomes stained by the CBG technique made it possible to localize darkly stained constitutive heterochromatin regions in the background of the remaining grey parts of chromosomes (Fig. 3). In total, 22 blocks of constitutive heterochromatin were observed (Fig. 4). Dark blocks were observed

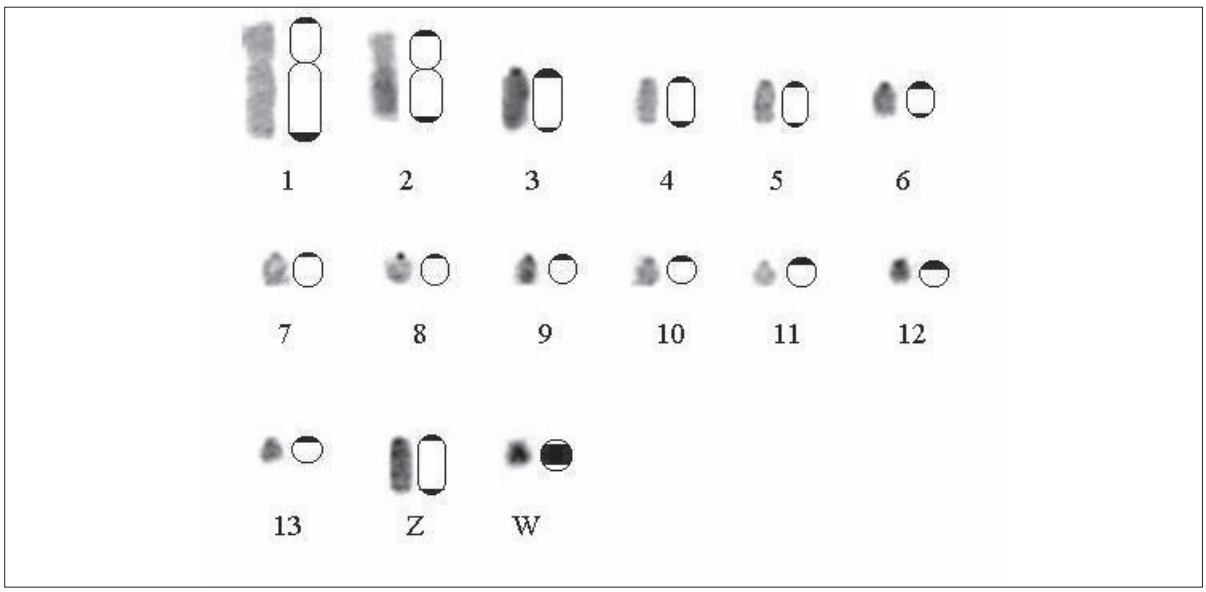

Fig. 4. Ideogram and karyogram of the chromosomes of *Cairina moschata* duck.

Table 1

Size of constitutive heterochromatin blocks of fourteen chromosomes of the *Cairina moschata*

Chromosome	Chromosome region – Statistical description				
	Proximal	distal		interstitial	
	$\overline{X} \pm S$	p arm	q arm	p arm	q arm
		$\overline{X} \pm S$		$\overline{X} \pm S$	
1	–	4.476±0.557	3.788±0.662	–	–
2	–	4.972±0.828	4.672±0.660	–	–
3	9.623±1.408	–	5.992±0.748	–	–
4	12.540±1.670	–	7.940±1.224	–	–
5	14.173±1.741	–	8.429±1.282	–	–
6	16.972±1.911	–	9.865±3.807	–	–
7	19.280±2.426	–	–	–	–
8	20.914±2.695	–	–	–	–
9	22.415±2.677	–	–	–	–
10	21.971±2.805	–	–	–	–
11	22.680±2.740	–	–	–	–
12	23.099±2.713	–	–	–	–
13	23.658±2.820	–	–	–	–
Z	9.333±1.385	–	8.822±1.114	–	–
W	–	–	–	64.069±7.674	

on both distal parts of the q arm of chromosomal pairs one to six on chromosome Z. On the autosomes of pair six to 13, heterochromatic regions were found only on one of the chromosome ends, and they were classified as proximal bands. Sex chromosome W had a darkly stained heterochromatin block in its interstitial part.

The largest constitutive heterochromatin block was found on the W chromosome (64% the whole chromosome). In relation to the whole entire karyotype, the smallest heterochromatin heterochromatic regions were detected on chromosome one and two (8-10%). In the remaining chromosomes, heterochromatin blocks constituted about 20% of chromosome their size (from 16 to 26%) (Table 1).

Discussion

The number of chromosomes in the Muscovy duck originating from *Cairina moschata* amounted to eighty. Views on the number of chromosomes in duck somatic cells differ and are quite often divergent. This is due to difficulties in identifying pairs of homologous chromosomes. Conventional staining methods do not always make it possible to distinguish chromosomes because of a the large number of microchromosomes that are often visible as points of different size. Techniques of chromosome banding enable precise identification of homologous pairs. BELTERMAN and DE BOER (1984) and RUIXIAN *et al*. (1988) observed 40 pairs of chromosomes in the Muscovy duck. DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) recorded 80 chromosomes in 90% of examined ducks, whereas for the remaining 10% the number of chromosomes ranged from 76 to 82. GUANCHAO and LICHENG (1982 after DUCOS *et al*. 1997) described 39 pairs of chromosomes.

There is disagreement between different researchers as to the sex chromosome classification. In the present work sex chromosomes Z were assigned to the fourth position, in relation to the chromosome size, and they were classified as subtelocentric, which agrees with the classification of MIGLIORE *et al*. (1986), APITZ *et al*. (1995), DENJEAN *et al*. (1997). CHANG *et al*. (1977, after DUCOS *et al*. 1997) classified the Z chromosome to belong to the group of acrocentric chromosomes, whereas in the opinion of HUANG and SUNG (1988) the chromosome was of a telocentric kind. In the present work sex chromosome W was placed in the eighth position. Also APITZ *et al*. (1995) classified heterochromosome W to base the eighth largest chromosome. DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) found the acrocentric W chromosome in the place of the 11th – 12th pair.

There is no research pertaining to the chromosome RBG banding in the Muscovy duck. In their studies, DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) encountered marked substantial difficulties in growing chromosomes for RBG banding. Cell

culture ended in rapid degradation and the cells obtained were fragile and breakable. The researchers' explanation of this phenomenon was an assumption that the cells lacked resistance to synchronization or BrdU incorporation. The authors described the karyotype of Pekin and Muscovy ducks, exposing differences in the banded pattern of chromosomes stained by the GTG banding method. APITZ *et al*. (1995) also carried out differentiating GTG banding on the Muscovy duck chromosomes. In the studies on chicken chromosomes, LADJALI *et al*. (1995) found that the Muscovy duck chromosomes did not reflect the RBG reverse banding. SCHMID *et al*. (2000) suggest that the *Gallus domesticus* banded pattern standard prepared by LADJALI-MOHAMMEDI *et al*. (1999) should be a reference for other experiments that deal with avian cytogenetics. LADJALI-MOHAMMEDI *et al*. (1999) detected 130 R bands, including 70 positive ones. In the present study a comparable number of R bands was obtained. It amounted to 129, including 72 dark positive bands.

In their studies on the Muscovy duck chromosomes, APITZ *et al*. (1995), DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) found that the first and second chromosome pairs chromosomes and the Z chromosome are completely deprived of C bands. The aforementioned authors do not mention the occurrence of telomeric C bands in the remaining chromosomes. Thin heterochromatin stripes found in the discussed study discussed in both distal parts of the first and second autosome and on the q arm of the Z chromosome are a result of a prolonged time of heterochromatin block digestion with barium hydroxide. The presence of constitutive heterochromatin blocks in the distal parts of the q arm of the third-to-sixth chromosome pairs was also confirmed.

APITZ *et al*. (1995), DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) did not detect bands on the Z chromosome. By contrast, MIGLIORE *et al*. (1986) showed chromosome Z to possess a band of constitutive heterochromatin in its proximal part. The observation was confirmed by the results of the present study.

A constitutive heterochromatin block was detected in the interstitial part of the W heterochromosome. The chromosome was in 64% made up of heterochromatin. APITZ *et al*. (1995) suggested that chromosome W was totally completely heterochromatic. A similar opinion was presented by DENJEAN *et al*. (1997) and DUCOS *et al*. (1997).

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