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Description of the Mallard Duck (*Anas platyrhynchos*) Karyotype

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The karyotype of the mallard duck, *Anas platyrhynchos,* was characterised on the basis of R and C bands. Chromosomal preparations obtained from *in vitro* blood lymphocyte cultures were RBG- and CBG-stained. The structures of nine and 14 pairs of chromosomes were analysed by the RBG and CBG chromosome banding techniques, respectively. The location of R bands, as well as the size and arrangement of constitutive heterochromatin blocks were determined. Ideograms of R and C banded patterns of the analysed chromosomes were drawn. The morphological makeup of the analysed chromosomes was assessed.

Key words: *Anas platyrhynchos* duck, karyotype, R bands, C bands.

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Domesticated forms of ducks descend from two different species, i.e. from *Cairina moschata* and *Anas platyrhynchos* (CRAWFORD 1990). The mallard, *Anas platyrhynchos,* originated in the northern hemisphere and gave rise to many duck breeds and varieties.

Cytogenetic research is carried out on few bird species due to the karyotype specificity, i.e.: a large diploid number, small size of chromosomes, the division into macro- and microchromosomes and a small amount of genetic material (CHRISTIDIS 1989; TIERSH & WACHTEL 1991). The objectives of these studies vary. They are used in research on phylogenetic relationships among individual bird species (BELTERMAN & DE BOER 1984; CHRISTIDIS 1990), and to detect chromosomal aberrations, infertility and reproduction problems (LADJALI 1994 after DUCOS *et al*. 1997; TCHELYCHEVA *et al*. 1993; LIPTOI *et al*. 2005). Knowledge on the species karyotype is used in modern projects concerning animal genome mapping (GREGORY 2002, 2004), to determine gene location and reciprocal relations between genes (BURT *et al*. 2002; MASABANDA *et al*. 2004). Cytogenetic research carried out on waterfowl is scarce and the results differ. Up to now, no standard duck karyotype has been prepared. Uniform banded patterns in turkey, duck and quail have been attempted (DODGSON *et al*. 2003). The only systematic description of bird karyotypes exists for the *Gallus* genus (LADJALI-MOHAMMEDI *et al*. 1999).

This study describes the karyotype of *Anas platyrhynchos* on the basis of RBG and CBG chromosome band staining.

Material and Methods

Chromosomal preparations were obtained from an *in vitro* lymphocyte culture of mallard duck peripheral blood sampled from 10 birds. Two standard banding techniques were used in this study, i.e. the RBG (PERRY & WOLFF 1974) and CBG (SUMNER 1972) techniques. BrdU and Hoechst 33258 were added to the cell culture in the 65th hour of incubation whereas Ethidium Bromide and colchicine were incorporated in the 69th hour of incubation. Every 10 metaphase plates were analysed. The structure of nine chromosome pairs was examined in respect to the location of R bands, and of 14 chromosome pairs with respect to the location and size of constitutive chromatin blocks. Ideograms of band patterns of the analysed chromosomes were prepared.

The description of the band pattern of the studied chromosomes, stained with the RBG technique, was done according to the conventional guidelines of ISCNDA (1989) and presented by LADJALI-MOHAMMEDI *et al*. (1999).

The CBG banding technique made it possible to determine the pattern of C bands on chromosomes

and to measure the sizes of individual heterochromatin bands as a relative value in relation to the whole length of the chromosome. The results obtained were statistically analysed. The index of arms [q/p] and centromeric index [p/(p+q)] were calculated to evaluate the morphological makeup of chromosomes.

Results

There were 80 chromosomes in the mallard duck somatic cells (Fig. 1). The sizes of individual chromosomes varied. The largest chromosomes tested were 4 to 10 μm long according to the metaphase plate. The smallest chromosomes were 0.65 to 1.55 μm long. The remaining chromosomes were visible under the microscope in the form of points. The RBG banding pattern was prepared for nine examined chromosomes (Fig. 2). The total number of R bands on the analysed chromosomes amounted to 126, including 71 dark positive bands and 55 light negative bands.

The submetacentric chromosome, showing an index of arms and centromeric index 1:1.701 and 0.373, respectively, was found to be the largest

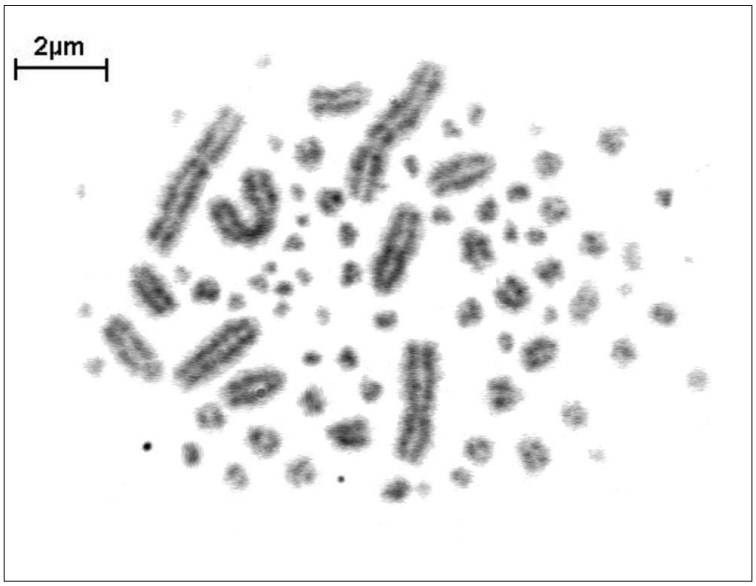

Fig. 1. Picture of a metaphase plate of the chromosomes in *Anas platyrhynchos* (RBG banding).

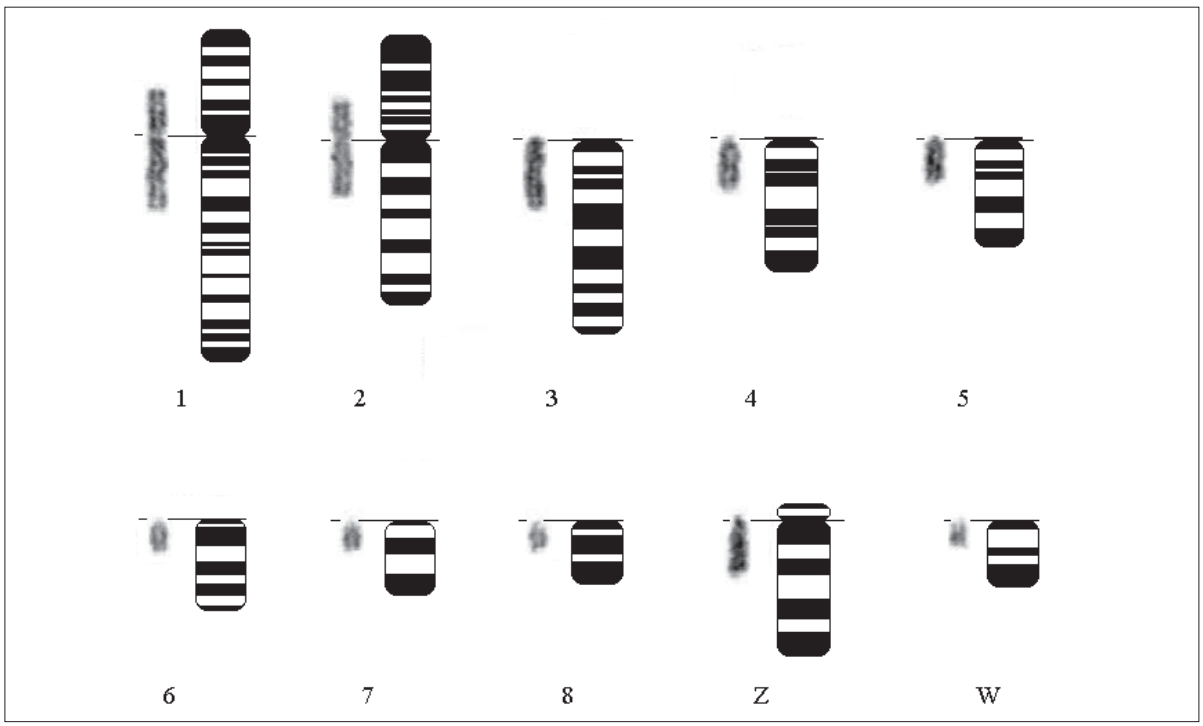

Fig. 2. Ideogram and karyogram of the chromosomes of *Anas platyrhynchos* (RBG banding).

chromosome of the mallard duck. A total of 32 R bands were found. On the p arm of the chromosome nine R bands were recorded, including five positive ones. On the q arm of the chromosome 23 R bands were noted: 12 positive and 11 negative ones.

The second submetacentric chromosome was characterised by a q/p ratio of 1:1.458 and centromeric index of 0.420. Twenty-two R bands were found. The p arm had six dark and five light bands. A total of 11 R bands were found on the q arm, six of these bands were positive and five negative.

Sixteen R bands were found in the third acrocentric chromosome. A small surface of the p arm was occupied by a darkly stained R band. The q arm had eight positive and seven negative bands.

The fourth acrocentric chromosome had 12 R bands. The p arm included one positive R band. On the q arm there were six positive bands and five negative bands out of the total number of 11 R bands.

The acrocentric chromosome of the fifth pair had ten R bands. There was one dark band on the p arm, whereas on the q arm five dark and four light bands were observed.

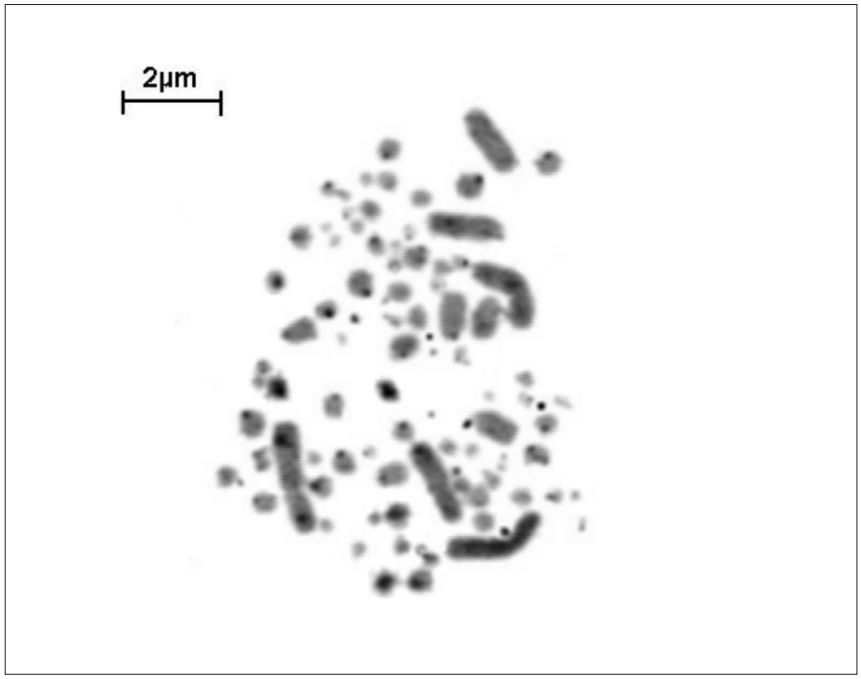

Fig. 3. Picture of the metaphase plate of the chromosomes in *Anas platyrhynchos* (CBG banding).

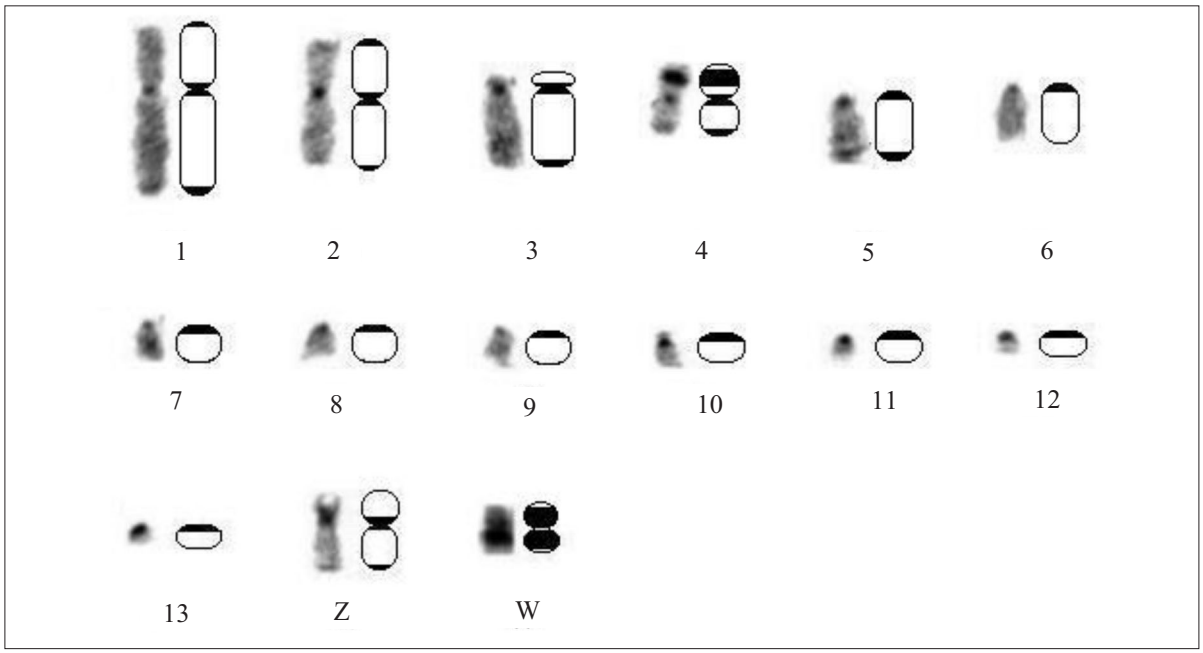

Fig. 4. Ideogram of the chromosomes of *Anas platyrhynchos* (CBG banding).

Table 1

Size of constitutive heterochromatin blocks of thirteen autosomes and sex chromosomes in *Anas platyrhynchos*

Chromosome	Chromosome region – Statistical description				
	Proximal	distal		interstitial	
	$\bar{X}$±S	p arm	q arm	p arm	q arm
		X±S		X±S	
1	–	4.919±0.802	4.462±0.855	–	–
2	–	6.053±1.131	5.392±1.016	–	–
3	9.816±1.836	–	7.134±1.200	–	–
4	13.211±1.995	–	9.069±1.675	–	–
5	15.340±2.151	–	10.209±1.676	–	–
6	19.498±2.930	–	11.938±4.615	–	–
7	20.244±3.469	–	–	–	–
8	21.863±3.004	–	–	–	–
9	22.168±3.099	–	–	–	–
10	–	–	–	71.045±7.969	
11	23.093±4.385	–	–	–	–
12	23.990±4.548	–	–	–	–
13	23.783±4.301	–	–	–	–
Z	–	–	7.504±1.049	–	–
W	–	–	–	68.613±7.499	

There were nine R bands on the acrocentric chromosome of the sixth pair, including five positive and four negative ones.

The number of R bands on an acrocentric chromosome of the seventh pair was five, three bands were positive and two were negative.

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

The Z chromosome was acrocentric; its ratio of q and p was 1:5.726, whereas its centromeric index value was 0.162. Ten R bands were observed. On the p arm of the chromosome, two positive bands separated by one light negative band were found. Out of the seven bands on the q arm of the chromosome four bands were positive and three bands were negative.

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

The CBG banding of mallard duck chromosomes revealed a total of 21 blocks of constitutive heterochromatin on 13 pairs of autosomes and sex chromosomes (Figs 3 & 4). No heterochromatin bands of the proximal region of the first and second pair of autosomes and Z chromosome were observed. In proximal regions of successive chromosomes (from the 3rd to the 5th), 10 to 15% of the chromosome surface was darkly coloured. Successively in respect to size (6th to 13th, excluding 10th), the chromosomes were characterised by proportionally larger areas occupied by heterochromatin (about 20%). Dark heterochromatin blocks in the distal regions of the p and q arms of the large chromosomes of the first and second pairs constituted approximately 5% of the whole chromosome. Interstitially occurring heterochromatin constituted 69 and 71% of the surface of the W chromosome and the tenth autosome (Table 1), respectively.

Discussion

In this study a constant number of chromosomes, equalling 80, was observed in the mallard duck. Different opinions exist as to the number of chromosomes in mallard duck somatic cells. Either 40 (BELTERMAN & DE BOER 1984; RUIXIAN *et al*. 1988; DENJEAN *et al*. 1997 and DUCOS *et al*. 1997) or 39 pairs of chromosomes (GUANCHAO & LICHENG 1982 after DUCOS *et al*. 1997) have been observed. DUCOS *et al*. (1997) recorded 80 chromosomes in 90% of ducks of both species. The remaining 10% of birds had from 76 to 82 chromosomes.

Mallard duck chromosome morphology was presented by MURAVSKA and BAUMGARTNER (1983), RUIXIAN *et al*. (1988), MAYR *et al*. (1989), APITZ *et al*. (1995), DENJEAN *et al*. (1997), and DUCOS *et al*. (1997).

Out of 80 chromosomes examined in the present study, the submetacentric makeup of the first two pairs of autosomes and the acrocentric makeup of the Z sex chromosome made it possible to measure the arm length of these chromosomes. According to LADJALI-MOHAMMEDI *et al*. (1999), beginning from the ninth chromosome in the hen, it is difficult to determine the centromeric and telomeric ends of chromosomes that have very short p arms. There is a discrepancy in assigning the position of the W sex chromosome in the karyogram. In the present work the tenth position was assigned to the W heterochromosome, which is in agreement with the report by APITZ *et al*. (1995). DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) found the W chromosome in the place of 11^{th}-12^{th} pair. MURAVSKA and BAUMGARTNER (1983) classified the W chromosome as occurring between the 9^{th} and 12^{th} pair. The Z chromosome, which had an acrocentric makeup and a short p arm, was classified as the fourth in respect to size. The results are in agreement with those reported by APITZ *et al*. (1995). DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) classified the Z chromosome as representative of subtelocentric chromosomes.

In the available literature there is no description of the karyotype of mallard duck chromosomes stained by means of the RBG technique. DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) mention that they applied RBG staining on mallard duck chromosomes. However, they did not include a description of the chromosomes analysed in their published work. Their papers do include the characterisation of mallard duck chromosomes stained with GTG bands. The authors suggest that GTG bands do not reflect the reversity of RBG bands. The phenomenon was observed by LADJALI *et al*. (1995) in their study of hen chromosomes. While analysing the mallard duck karyotype in respect to G bands, DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) observed a total of 165 G bands, including 75 positive bands, on 12 chromosomes analysed and the Z sex chromosome. The ideogram of mallard duck chromosomes included in the work by Denjean and co-authors published in 1997 in the 'First Report on Chicken Genes and Chromosomes 2000' (SCHMID *et al*. 2000) is abridged to eight autosomes and the Z sex chromosome, the band arrangement on the chromosomes presented being different from the original work of the authors. In this study 126 R bands were found altogether on eight autosomes and sex chromosomes ZW, including 71 positive bands. Positive dark R bands were observed in the centromeric and telomeric parts of all chromosomes tested. Additionally, positive R bands were found in the interstitial part of the chromosomes. In the ideograms stained by the method of GTG bands presented by DENJEAN *et al*. (1997) and DUCOS *et al*. (1997), no positive G bands were detected in the centromeric and telomeric parts of the analysed chromosomes. The remaining bands were observed in the interstitial parts of the chromosomes examined by the aforementioned researchers. Altogether on eight autosomes and the sex chromosome Z the authors counted 147 G bands, including 68 dark positive bands. The researchers did not analyse sex chromosome W.

According to SCHMID *et al*. (2000) the karyotype standard prepared by LADJALI-MOHAMMEDI *et al*. (1999) for *Gallus domesticus* should be a model in research on other bird species. In the standard R band pattern model for domestic hen chromosomes (LADJALI-MOHAMMEDI *et al*. 1999), 70 R positive bands were recorded. In the present study a very similar number of R positive bands was found, amounting to 71.

Using the hypothesis formulated by SCHMID *et al*. (2000), HUANG *et al*. (2005, 2006) showed a strong homology between the chromosomes of *Anas platyrhynchos* and *Gallus domesticus* as well as other bird species.

MAYR *et al*. (1989) applied fluorochrome chromatin staining techniques with DA/DAPI to identify heterochromatin on macro- and microchromosomes.

A block of constitutive heterochromatin on mallard duck chromosomes was observed in the proximal, distal and interstitial parts of chromosomes. While examining mallard duck chromosomes, APITZ *et al*. (1995), DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) found that the first and second pairs as well as chromosome Z completely lack C bands. In the present study no heterochromatic bands were observed in the proximal parts of the aforementioned chromosomes. C bands were identified in both distal parts of the first and second autosome and on the q arm of the Z chromosome. Similar observations were reported by RUIXIAN *et al*. (1988). They found that on the two-armed chromosomes of the first and second pair, a small spot of telomeric heterochromatin occurs. In the present work the presence of constitutive heterochromatin blocks was also observed in the distal parts of the q arm of the third to sixth chromosome pairs. Thin heterochromatic bands observed in this work were obtained by longer digestion of chromosomes with barium hydroxide. Constitutive heterochromatin blocks in the interstitial parts were found on the tenth autosome and W heterochromo-

some. The chromosomes were almost completely filled with heterochromatin, apart from their proximal and distal parts. APITZ *et al*. (1995), DENJEAN *et al*. (1997) and DUCOS *et al*. (1997) described the W chromosome as completely heterochromatic. RUIXIAN *et al*. (1988) mentioned the occurrence of one or two completely heterochromatic microchromosomes in the mallard duck. The measurements of heterochromatin blocks collected in the present study made it possible to determine the size of individual heterochromatic blocks as relative values in relation to the whole length of the chromosome. The average amount of heterochromatin in autosome 10 amounted to about 71% whereas in the W heterochromosome it was about 69%.

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