The karyotype of domestic waterfowl: Ducks – RBG chromosome pattern

Ewa Wojcik and Elzbieta Smalec

Materials and methods

Samples for examination were taken from 30 birds, 10 from each species representing Pekin and Muscovy ducks. Ten Mule ducks as a cross between Pekin and Muscovy were examined for the comparison of the parental species. It was sampled from eight-week-old Pekin ducks, eleven-week-old Muscovy ducks and their crosses. In each group the proportion of males and females was 50:50%. Chromosomal preparations were obtained from an in vitro lymphocyte culture by means of the RBG technique (Perry and Wolff, 1974) which included the incorporation of BrdU and Hoechst 33258 in 65th hour of incubation, and EB and colchicine in the 69th hour of incubation. Ten metaphase plates were analysed for each bird. The first eight largest pairs of autosomes and sex ZW chromosomes were examined. The localisation of R-bands was determined on selected chromosomes in each duck species and ideograms of the banded patterns of the chromosomes were drawn based on average observations and measurements from separate metaphases. An analysis was done on separate chromosomes cut from metaphase plates. The observations of dark – light bands were connected with the absorption profile. The profiles of separate chromosomes were done so many times as to obtain the number of analysed chromosomes for a given pair as presented in the table. Finally all the schematic pictures were averaged to get one standardised chromosome ideogram. Figure 1 presents an example of the manner of obtaining the final ideogram.

Results

The applied technique of banding enabled determining the pattern of R-bands on the largest chromosomes of Pekin duck (Anas platyrhynchos) and Muscovy duck (Cairina moschata). The interspecies crosses were used in the comparative studies. A partial ideogram of eight largest pairs of autosomes and sex chromosome ZW was drawn. In order to facilitate the description of the chromosomes, regions were determined on their arms and positive R (dark) and negative (light) bands were counted. The results of observations and analyses are presented in the form of metaphase plates, karyograms and ideograms of R-bands (see Figures 2ab, 4ab and Figures 3, 5).

Pekin duck (Anas platyrhynchos)

Chromosome 1. 1-1. P arm: Two regions. 9 R-bands. The first proximal region included two positive bands (11, 13) and two negative bands (12, 14). In the second region of the p arm of the chromosome, three positive (21, 23, 25) and two negative (22, 24) bands were identified.

1-2. Q arm: Four regions. 23 R-bands. In the first, second and third region of the q arm, three dark bands (11, 13, 15; 21, 23, 25; 31, 33, 35 respectively) were followed by three light bands (12, 14, 16; 22, 24, 26; 32, 34, 36 respectively). The fourth distal region of the q arm consisted of three dark bands (41, 43 and 45) evenly distributed and separated by two light bands (42 and 44).
Chromosome 1.

1-1. P arm: Two regions. 11 R-bands. In the first region of the p arm of the chromosome, three dark bands (11, 13, 15) were followed by three light bands (12, 14, 16). The first narrow band was located in the central part of the arm. The remaining two positive R-bands were wide.

1-2. Q arm: Two regions. 11 R-bands. The first region contained two positive bands (11, 13) and two negative bands (12, 14). In the second region of the q arm, four dark bands (21, 23, 25, 27) were separated by three light bands (22, 24, 26).

Chromosome 2.

2-1. P arm: Two regions. 11 R-bands. In the first region of the p arm of the chromosome, three dark bands (11, 13, 15) were followed by three light bands (12, 14, 16). In the second region, two narrow light bands (22, 24) were separated by three dark bands (21, 23, 25). The first narrow band was located in the central part of the arm. The remaining two positive R-bands were wide.

2-2. Q arm: Two regions. 11 R-bands. The first region contained two positive bands (11, 13) and two negative bands (12, 14). In the second region of the q arm, four dark bands (21, 23, 25, 27) were separated by three light bands (22, 24, 26).

Chromosome 3.

3-1. P arm: One region. One positive band (11) was found.

3-2. Q arm: Two regions. 15 R-bands. The first region had four positive bands (11, 13, 15, 17) and four negative bands (12, 14, 16, 18). The second region of the q arm included four positive bands (21, 23, 25, 27) and three alternating negative bands (22, 24, 26).

Chromosome 4.

4-1. P arm: One region. One positive band (11) was found.

4-2. Q arm: One region. 11 R-bands. Six positive bands were observed, including two outermost ones: a centromeric (11) and a telomeric (111) band; and four central dark bands which were grouped in pairs (13, 15; 17, 19). The positive R-bands were separated by light R-bands (12, 14, 16, 18, 110). Sometimes, narrow light bands (14 and 18) were not observed, especially when the chromosomes on the metaphase plates were short.

Chromosome 5.

5-1. P arm: One region. One positive band (11) was observed.

5-2. Q arm: One region. One region with nine R-bands. Five dark bands (11, 13, 15, 17, 19) and four light bands (12, 14, 16, 18).

Chromosome 6.

6-1. Q arm: One region. 9 R-bands. Five dark bands (11, 13, 15, 17, 19) were separated by four light bands (12, 14, 16, 18).

Chromosome 7.

7-1. Q arm: One region. 5 R-bands. Three positive (11, 13, 15) and two negative bands (12, 14) were noticed.

Chromosome 8.

8-1. Q arm: One region. 5 R-bands. Three positive (11, 13, 15) were followed by two negative (12, 14) bands.
Chromosome Z

Z-1. P arm: One region. 3 R-bands. Two dark bands (11, 13) were separated by a light band (12).
Z-2. Q arm: One region. 7 R-bands. Four positive R-bands (11, 13, 15, 17) were followed by three negative bands (12, 14, 16).

Chromosome W

W-1. Q arm: One region. 5 R-bands. Three dark (11, 13, 15) and two light (12, 14) bands were found.

Muscovy duck (Cairina moschata)

Chromosome 1

1-1. P arm: Two regions. 9 R-bands. The first proximal region contained two positive bands (11, 13) and two negative bands (12, 14). In the second region of the p arm of the chromosome three positive bands (21, 23, 25) and two negative bands (22, 24) were found.
1-2. Q arm: Four regions. 23 R-bands. The first, second and third region of the q arm of the chromosome had three dark bands (11, 13, 15; 21, 23, 25; 31, 33, 35, respectively) and three light bands (12, 14, 16; 22, 24, 26; 32, 34, 36). The fourth distal region of the q arm included three evenly distributed dark bands (41, 43, 45) separated by two light bands (42 and 44).

Chromosome 2

2-1. P arm: Two regions. 9 R-bands. The first region was marked by four alternating dark (11, 13) and light (12, 14) bands. In the second region, a wide central positive R-band (23) was between two bands – a narrow interstitial (21) and a wide distal (25) band. Narrow negative R-bands (22, 24) separated the positive bands.
2-2. Q arm: Two regions. 11 R-bands. In the first region, two negative bands (12, 14) followed two positive bands (11, 13) and three light bands (12, 14, 16; 22, 24, 26) separated the positive bands.

Chromosome 3

3-1. P arm: One region. One positive band (11) was observed.
3-2. Q arm: Two regions. 19 R-bands. Ten dark positive R-bands belonged to two regions. Region 1 was marked by five dark bands (11, 13, 15, 17, 19) and five light bands (12, 14, 16, 18, 110). The second region of the Q arm included five dark bands (21, 23, 25, 27, 29) and four light bands (22, 24, 26, 28).

Chromosome 4

4-1. P arm: One region. One positive band (11) was observed.
4-2. Q arm: One region. 11 R-bands. Six dark positive (11, 13, 15, 17, 19, 111) and five negative R-bands (12, 14, 16, 18, 110) were separated by five light bands (12, 14, 16, 18, 110).

Chromosome 5

5-1. P arm: One region. One positive band (11) was observed.
5-2. Q arm: One region. 11 R-bands. Six dark bands of different width (11, 13, 15, 17, 19, 111) were separated by five light bands (12, 14, 16, 18, 110).

Chromosome 6

6-1. Q arm: One region. 9 R-bands. Five dark bands (11, 13, 15, 17, 19) were separated by four light bands (12, 14, 16, 18).

Chromosome 7

7-1. Q arm: One region. 7 R-bands. Four positive (11, 13, 15, 17) and three negative bands (12, 14, 16) were noticed.

Chromosome 8

8-1. Q arm: One region. 5 R-bands. Three positive (11, 13, 15 and 17) and three negative bands (12, 14, 16) were noticed.

Chromosome Z

Z-1. Q arm: One region. One dark band (11) and one light band (12) in the proximal part of the chromosome. In the interstitial part, two wide positive bands (13, 15) were followed by a wide central negative R-band (14). The distal part included one light (16) and one dark band (17).

Chromosome W

W-1. Q arm: One region. 5 R-bands. Three dark (11, 13, 15) bands were alternately separated by two (12, 14) light bands.
Mule duck – comparison between species

In the case of the crossbreed chromosomes, letters “a” and “b” denoted a pair of homologous chromosomes. Slightly bigger chromosomes whose banded pattern resembled the chromosomes of the Pekin duck were designated with letter “a” whereas letter “b” denoted the chromosomes with the banded pattern similar to that of the Muscovy duck chromosomes. There were no differences between the banded pattern of the chromosomes of the first and fourth pair. Differences in this pattern were observed on the chromosomes of the second, third and fifth pair of autosomes. Comparison of parental sex Z chromosomes revealed the differences between the banding patterns of Z chromosome of the parents. The ideogram (Figure 6) presents only those chromosomes of Mule ducks that differed between pairs of homologues.

Differences in the arrangement of bands on the chromosomes of the second pair referred to the number of bands in the first region of arm p. The chromosome designated with letter “a” was characterised by the arrangement of bands described previously for the Pekin duck. The chromosome denoted with letter “b” had the arrangement of bands typical of the sequence described previously for the Muscovy duck. The third pair of chromosomes differed in the number of bands. On the chromosome labelled with letter “a”, there were eight bands whose arrangement was the same as for the Pekin duck. The homologous chromosome denoted with letter “b” had ten bands distributed similarly to that on the Muscovy duck chromosome. Acrocentric chromosomes of the fifth pair differed in the number of bands. The chromosome designated with letter “a” had one band less than the corresponding homologous chromosome denoted with letter “b”. The banded pattern of the “a” chromosome of the crossbreed was the same as in the Pekin duck and the pattern of the “b” chromosome closely resembled the pattern of the Muscovy duck. Homologues of the seventh pair differ in the number of bands (7 vs 5).

Sex Z chromosomes differed both in the morphological make up and the number of bands. Pekin duck Z chromosome was acrocentric and had ten R-bands whereas telometric Z chromosome of Muscovy duck included seven R-bands.

Discussion and conclusion

Organization of most avian karyotypes comprises a few macrochromosome pairs and many microchromosomes. The standard for chicken – eight macrochromosomes plus the Z and W sex chromosomes – has been described by Ladjali-Mohammedi et al. (1999). The other chromosomes for most of poultry species are identified unambiguously and classified by size.

Molecular tags allowed identifying 22 of all chicken microchromosomes (Fillon et al., 1998; Schmid et al., 2000; Masabanda et al., 2004). Huang et al. (2006) assigned 20 FISH markers to the largest 10 duck chromosomes and 4 markers to microchromosomes. In spite of the strong homology between macrochromosomes in different bird species (Schmid et al., 2000) 4 orthologous loci that were localized on the chicken chromosomes were uncertain in the duck genome or located on different chromosomes (Huang et al., 2006).

The ability to karyotype an individual or a species is fundamental for any genome-mapping attempt as both genetic and physical maps are made with reference to the chromosome position (Masabanda et al., 2004). It was recommended by Ladjali-Mohammedi et al. (1999) and Schmid et al. (2000) to apply general guidelines developed for chicken to other avian species.

Analysis of the duck karyotype was done in a limited number of papers. Two of them presented GTG banding pattern for 5 (Apitz et al., 1995) and 12 chromosomes (Denjean et al., 1997) of two duck species (A. platyrhynchos and C. moschata). Both teams described the Z and W heterochromosomes. There were some divergences in the banding pattern of duck chromosomes proposed (No. 3 and 2) that could be attributed to a different contraction during the cell cycle. The differences between duck species the authors restricted to the 2nd and Z chromosomes (Apitz et al., 1995) or to the 3rd, 5th, 7th and Z chromosomes (Denjean et al., 1997). The ideogram of eight GTG banded macrochromosomes and Z chromosome from the paper by Denjean et al. (1997) cited in the First Report on Chicken Genes and Chromosomes 2000 differ from the ideogram presented in the original papers in regard to the number of G positive bands (68 in the original paper vs 62 in the paper of Schmid et al. (2000)).

The RBG banding structure is not exactly the inverse of that described with GTG pattern (Ladjali et al., 1995). The

Figure 4. Image of the metaphase plate (a) and the partial karyogram of the chromosomes of the Muscovy duck (b) Bild der Metaphasenplatte (a) und des partiellen Karyogramms der Chromosomen der Moschusente (b)
standard of the chicken karyotype allows counting 70 R-positive bands, 73 G-positive and 78 G-negative bands.

Our investigation shows that the total number of R-positive bands of analogous chromosomes of Mullard duck reached 71, while that of Muscovy was 72. The karyotype comparison between species reflects differences of the 2nd, 3rd, 5th, 7th and Z chromosomes. APITZ et al. (1995), HAILU et al. (1995) and DUCOS et al. (1997) observed chromosome size differences between duck species.

In conclusion, while for the species Gallus the karyotype standard of R banding has already been described, there is lack of comparable studies on R banding chromosomes in ducks.

Summary

The objective of the study was to describe the karyotype of two duck species: the Pekin (Anas platyrhynchos) and the Muscovy (Cairina moschata) utilising the RBG banding technique of chromosome staining. The comparison between the species was done on Mule crosses. Chromosomal preparations obtained from an in vitro culture of blood lymphocytes were stained with the RBG technique.

The first eight largest pairs of autosomes and ZW sex chromosomes were analysed. The localisation of R-bands was determined and the ideograms of the banded patterns of the analysed chromosomes were drawn. No differences
were found in the banded pattern of the chromosomes in the first, fourth, sixth pair of autosomes and W sex chromosome. Differences in the arrangement of bands were observed on the second, third, fifth and seventh pair of autosomes and the sex Z chromosome.

Key words

Duck, Anas platyrhynchos, Cairina moschata, karyotype, mitotic chromosomes, R-bands

Zusammenfassung

Der Karyotyp des heimischen Wassergeflügels: Enten – RBG Muster der Chromosomen


Schlüsselwörter

Ente, Anas platyrhynchos, Cairina moschata, mitotische Chromosomen, Karyotyp, R-Bänder

References


