Folia biologica (Kraków), vol. **55** (2007), No 1-2

Description of the *Anser anser* Goose Karyotype

Ewa WÓJCIK and Elżbieta SMALEC

Accepted September 20, 2006

WÓJCIK E., SMALEC E. 2007. Description of the *Anser anser* goose karyotype. Folia biol. (Kraków) **55**: 35-40.

The karyotype of the Italian goose originating from *Anser anser* was characterised on the basis of R and C bands. Chromosomal preparations obtained from an *in vitro* culture of blood lymphocytes were stained with the RBG and CBG techniques. The RBG technique enabled the analysis of the structure of nine pairs of chromosomes whereas the CBG technique – fourteen pairs of chromosomes from the total of eighty goose chromosomes. The morphology and the R and C banding patterns were described. The size and arrangement of the blocks of constitutive chromatin were determined. Ideograms of R and C banded patterns were drawn. The morphological structure of the analysed chromosomes was evaluated.

Key words: *Anser anser*, goose, karyotype, R bands, C bands.

Ewa WÓJCIK, Elżbieta SMALEC, Department of Animal Genetics and Breeding, University of Podlasie, Prusa 14, 08-110 Siedlce, Poland.
E-mail: wojcik@ap.siedlce.pl
esmalec@ap.siedlce.pl

Among ten goose species, two have been domesticated (CRAWFORD 1990). One of these is *Anser anser* which is a European species and is represented by many breeds. The Italian goose, the most common in the world (SMALEC 2001), has been recently been bred in Poland as the Koluda breed (WĘŻYK *et al*. 1993; ROSIŃSKI *et al*. 1999). Cytogenetic research was inspired by scant knowledge about waterfowl genomes (VIGNAL *et al*. 1999). Chromosome band staining techniques introduced in the 1970s enabled the precise identification of homological pairs as well as the identification and analysis of chromosomal aberrations. Chromosome band patterns were determined for man and many species of farm animals (ISCNDA 1989; ISCN 1995; ANSARI *et al*. 1999; ISCNDB 2000). The poultry band pattern standard, which includes the nine largest pairs of chromosomes, was obtained only for *Gallus domesticus* (LADJALI-MOHAMMEDI *et al*. 1999).

Birds are one of the least examined animal groups due to karyotype specificity, i.e. small chromosomes, a large diploidal chromosome number or the division of the chromosomes into macro- and microchromosomes. The largest chromosomes, ranging from four to eight microns, constitute only a few chromosome pairs. The remaining ones, described as microchromosomes, are usually smaller than two microns and in many cases are observed as points (CHRISTIDIS 1989). Compared with macrochromosomes, microchromosomes are characterised by a higher content of guanine-cytosine pairs (G-C). Half of the identifiable genes are situated on microchromosomes (FILLON *et al*. 1998; GREGORY 2002). Bird microchromosomes are characterised by a three-fold higher crossing-over frequency than the macrochromosomes, as a result the possibility of their correct segregation during the meiotic division increases (RODIONOV 1996).

Conventional banding techniques do not always enable differentiating bird chromosomes even in relation to the centromere location (BITGOOD & SHOFFNER 1990). One of the most frequently applied chromosome banding techniques is the RBG banding method. R bands undergo early replication in phase S and late condensation (HOLMQUIST 1988), they also contain many CpG islands and are rich in G-C pairs. The chromosome sites on which R bands occur in telomeres are characterised by a high frequency of chiasm creation, splitting and rearrangement (BERNARDI 1989; HOLMQUIST 1992). Another popular chromosome banding method is the CBG banding method. Constitutive chromatin, constituting about 20% of the genome, is a structural part of C bands. It is placed in centromeric, telomeric and interstitial parts of chromosomes (BURKHOLDER & DUCZEK 1982). Also sex chromosomes W, Y and animal B-chromosomes of animals can be entirely or mostly made up of

heterochromatin (ŚWITOŃSKI 1998). The region of C bands possesses a small number of CpG islands and no interspersed elements. This is due to the fact that C bands are not sensitive to DNase I, they are characterised by a late replication period, a low acetylation level of histone proteins, the presence of HMG-I proteins and a low degree of meiotic recombinations (DISNEY *et al*. 1989; SUMNER 1994).

The aim of the study is to describe the karyotype of the goose, *Anser anser,* after application of the RBG and CBG techniques of chromosome staining.

Material and Methods

Samples were taken from ten White Koluda geese. Chromosomal preparations obtained from an *in vitro* culture of peripheral blood lymphocytes were stained by means of two standard techniques: RBG (PERRY & WOLFF 1974) and CBG (SUMNER 1972). The RBG staining 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. In each case ten metaphase plates were analysed. The choice of the techniques made it possible to analyse the structure of nine pairs of chromosomes in respect to the localisation of R bands, the location and the size of constitutive chromatin blocks of fourteen pairs of chromosomes, and to draw ideograms of band patterns of analysed chromosomes. The ideograms were drawn on the basis of ten metaphase plates from each individual bird.

The description of the band pattern of chromosomes stained with the RBG technique was done following the human and chicken standard: ISCNDA 1989; LADJALI-MOHAMMEDI *et al*. (1999).

The CBG staining technique enabled determining the pattern of C bands on chromosomes. Moreover, the sizes of individual heterochromatin bands were measured and expressed as relative values in relation to the whole chromosome length. The obtained results were characterised by their mean values ($\overline{X}$) and standard deviations (S). The morphological structure of the first, second, third, fourth and Z W chromosomes was evaluated by calculating the index of arms [q/p] and centromeric index [p/(p+q)].

Results

The number of chromosomes in *A. anser* somatic cells was 80. The size of individual chromosomes varied (Fig. 1). The length of the largest

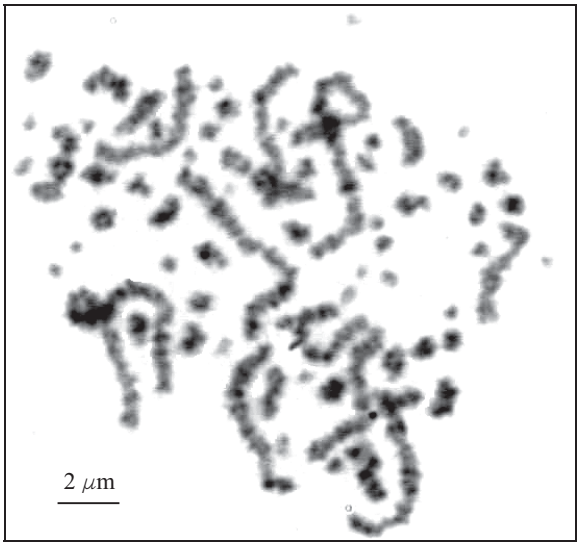

Fig. 1. Picture of the metaphase plate of the chromosomes of *Anser anser* (RBG banding).

chromosomes ranged from 4 to 11μm according to the metaphase plate, whereas for the smallest chromosomes the range was from 0.60 to 1.45μm. Ideograms of R bands of nine analysed pairs of chromosomes were made (Fig. 2). Altogether, 131 R bands were found on the analysed chromosomes, including 10 dark positive ones.

The first of the chromosomes belonging to the largest pair was submetacentric with an index of arms 1:1.697 and centromeric index 0.410. Twenty-nine R bands were recorded. Two regions and eleven R bands, six positive and five negative bands, were found on the p arm of the chromosome. On the q arm of the chromosome three regions and eighteen R bands were determined. Half of these were positive bands.

The second chromosome was submetacentric, its index of arms was 1:1.515 and centromeric index 0.429. Twenty-six R bands were recorded. On the p arm of the chromosome two regions and twelve R bands were determined, including six positive ones. On the q arm of the chromosome two regions were found and fourteen R bands were recorded. There were seven positive and seven negative bands.

The third largest acrocentric autosome possessed an index of arms and centromeric index equalling 1:4.979 and 0.171, respectively. Altogether twenty R bands were observed on the third chromosome. On the p arm of the chromosome one region was detected and three R bands were recorded: one wide positive band and two negative ones. On the q arm of the chromosome two regions and seventeen R bands were recorded. Nine bands were positive and eight bands were negative.

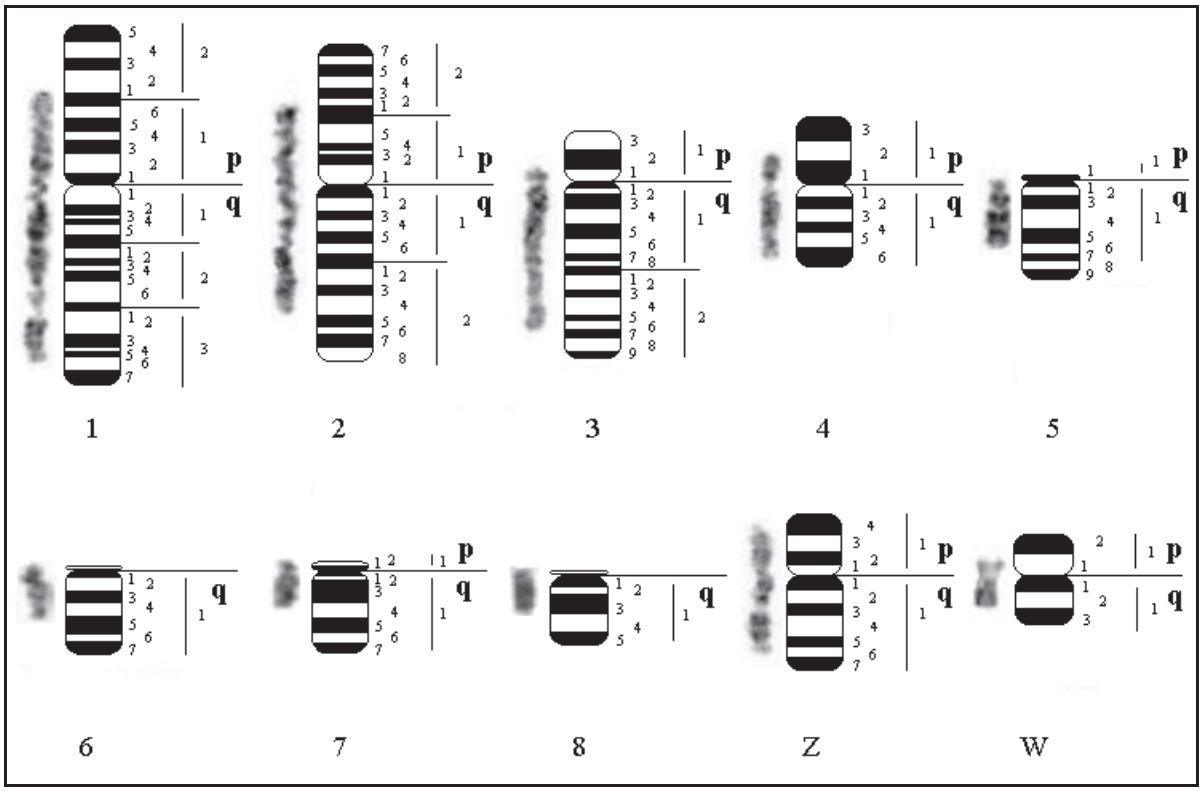

Fig. 2. Ideogram and karyogram of the chromosomes of *Anser anser* (RBG banding).

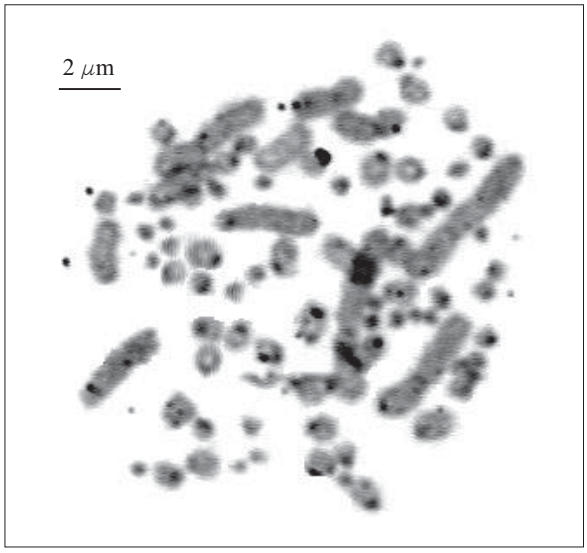

Fig. 3. Picture of the metaphase plate of the chromosomes of *Anser anser* (CBG banding).

The submetacentric chromosome of the fourth pair had an index of arms 1:1.328 and centromeric index 0.443. Nine R bands were observed. On the p arm of the chromosome one region and three R bands were determined. On the q arm of the chromosome, within one region, six R bands, three positive and three negative ones, were observed.

Ten bands were detected on the acrocentric chromosome of the fifth pair. Within one region one positive band was determined on the p arm whereas the q arm harboured, within one region, nine bands including five positive and four negative ones.

The acrocentric chromosome of the sixth pair had seven R bands including four positive bands situated within the q arm.

On the acrocentric seventh chromosome nine R bands were recorded. One positive and one negative band were found on the p arm, whereas seven R bands were recorded on the q arm : four positive and three negative ones.

The eighth acrocentric autosome had five R bands including three positive ones located within the q arm.

The index of arms and centromeric index of the submetacentric sex Z chromosome were 1.381 and 0.374, respectively. Altogether, eleven bands were determined. On the p arm of the chromosome one region including four R bands was found, whereas on the q arm of the chromosome seven R bands were recorded within one region.

The W heterochromosome was submetacentric, its index of arms was 1:1.370 and centromeric index 0.423. Five R bands were recorded. One positive R band was found on the p arm, whereas two positive bands were found on the q arm.

Darkly stained blocks of constitutive chromatin made it possible to identify fourteen pairs among

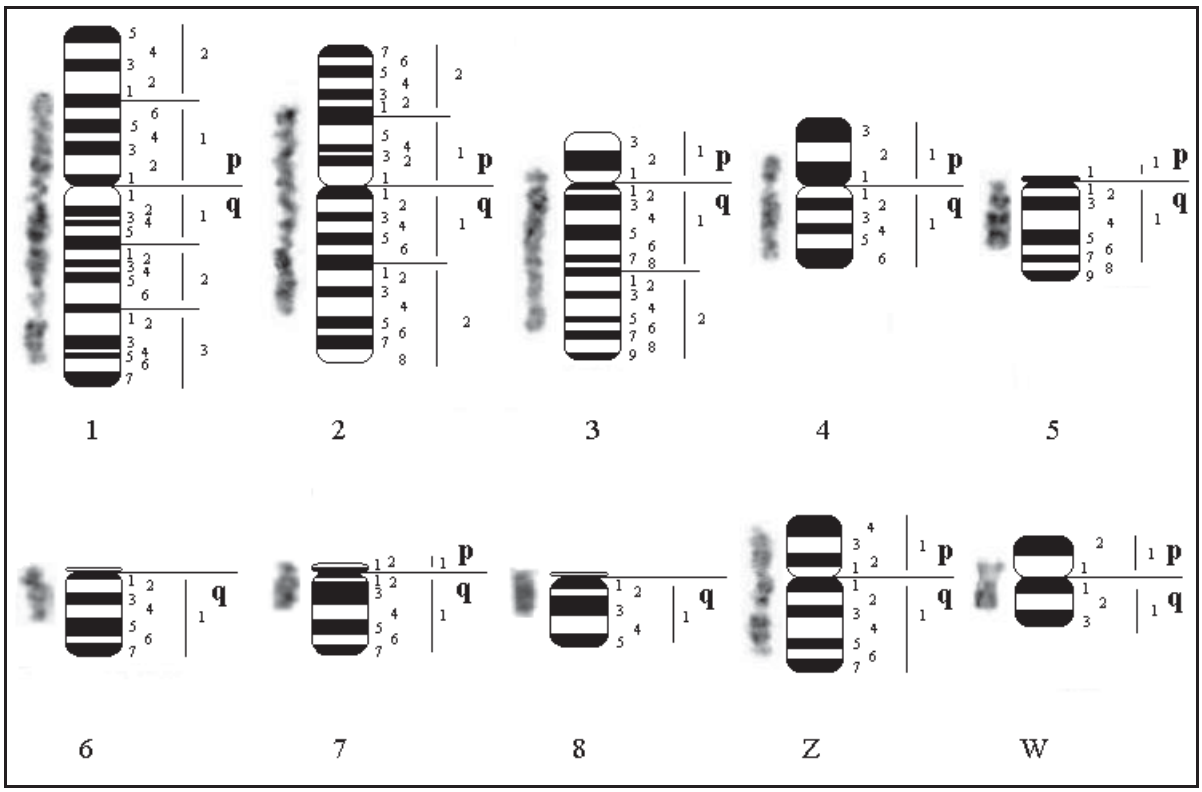

Fig. 4. Ideogram of the chromosomes of *Anser anser* (CBG banding).

Table 1

Size of constitutive heterochromatin blocks of thirteen autosomes and sex chromosomes in *Anser anser*

Chromo - some	Chromosome region – Statistical description				
	Proximal	distal		interstitial	
	$\bar{X}$±S	p arm	q arm	p arm	q arm
		$\bar{X}$±S		$\bar{X}$±S	
1	7.70±0.49	4.60±0.45	5.51±0.50	–	–
2	8.25±0.50	4.19±0.78	4.69±0.43	–	–
3	12.69±0.64	–	3.78±0.56	–	–
4	–	–	–	–	–
5	14.47±0.93	–	6.84±0.76	–	–
6	19.62±2.39	–	–	–	–
7	20.81±2.64	–	–	–	–
8	21.73±3.81	–	–	–	–
9	22.36±4.38	–	–	–	–
10	23.19±3.08	–	–	–	–
11	24.31±4.82	–	–	–	–
12	24.34±4.53	–	–	–	–
13	25.96±3.23	–	–	–	–
Z	15.15±1.42	–	7.94±1.00	–	17.86±1.96
W	–	–	–	74.86±2.99	

the total of forty pairs of chromosomes, including thirteen pairs of autosomes and a pair of sex chromosomes (Figs 3 & 4). Altogether, twenty-two heterochromatin blocks were observed on the analysed chromosomes. The regions of constitutive chromatin in the proximal part of the p and q arms of chromosomes were observed in respect to all the investigated chromosomes, excluding the fourth pair of autosomes. On the Z chromosome the centromeric band was detected only on the short arm. Heterochromatin blocks on the interstitial parts of chromosomes were observed on the Z W chromosomes. The remaining blocks of constitutive heterochromatin were identified in the distal parts of the analysed chromosomes, excluding the chromosome of the fourth pair where no heterochromatin bands were detected.

The size of heterochromatin blocks on individual autosomes ranged from 17% (the third autosome) to 26% (the thirteenth autosome) of the total length of a chromosome, whereas on the sex chromosomes it was more than two times as large. On the Z chromosome it amounted to 41% and on the W chromosome – 75% (Table 1).

Discussion

The morphological structure of the chromosomes of *A. anser* has been described in many papers. The

morphological description of the chromosomes of *A. anser* presented by SILVERSIDES *et al*. (1988) and APITZ *et al*. (1995) has been confirmed by the results in this study. While investigating the goose karyotype BELTERMAN and DEBOER (1984) observed a metacentric morphological form of the fourth autosome. SILVERSIDES *et al*. (1988), HIDAS (1993, 1999) and AELTERMAN *et al*. (1995) classified the fourth pair of chromosomes as submetacentric. In the chromosome atlas presented by BELTERMAN and DEBOER (1984) the W sex chromosome is considered acrocentric, in contrast to the present study, which classified the W chromosome as representing the group of submetacentric chromosomes. This conclusion agrees with the results of SILVERSIDES *et al*. (1988) as well as APITZ *et al*. (1995).

Differentiating banding on Italian geese chromosomes was carried out by SILVERSIDES *et al.* (1988) and APITZ *et al.* (1995). Both authors analysed the first five pairs of autosomes and sex chromosomes, but used a different banding technique (GTG). In the ideogram presented, APITZ *et al.* (1995) recorded 32 G-positive bands and 45 G-negative bands. LADJALI *et al.* (1995) conducted comparisons of R and G bands on hen chromosomes. The authors found that R bands do not reflect the generally accepted standard of G band reverse stereotype. SCHMID *et al.* (2000) refer to the currently established standard of banded patterns prepared for the *Gallus* genus by LADJALI-MOHAMMEDI *et al.* (1999) which is a reference point in the investigations on other bird species. In the present study 70 R-positive bands were found, the number of the bands was the same for *Gallus gallus*.

There are no papers so far mentioning the RBG chromosome staining of the goose. This technique distinguished the banding pattern of the largest goose chromosomes. R bands can be detected by the methods of luminosity. The use of DNA-binding base pair of chromomycin A_3 allowed MAYR *et al.* (1990) to illuminate R banding pattern on *A. anser* goose macro- and microchromosomes.

Heterochromatin blocks were observed by APITZ *et al*. (1995) on all the analysed chromosomes excluding the fourth pair of autosomes. The presented results are concordant, apart from the distal parts of both arms of the analysed chromosomes. The cited authors do not mention the localisation of heterochromatin in the form of telomeric bands. Lack of C bands in the distal parts of chromosomes may be due to short digestion times of the chromosomes with barium hydroxide. MAYR *et al*. (1990) applied the opposite techniques of chromatin staining using fluorochromes (chromomycin, A-2/distamycin, A/DAPI, and then DAPI/actinomycin) in order to characterise the arrangement of heterochromatin on macro- and microchromosomes. They found heterochromatin blocks in the distal parts of chromosomes of the *A. anser* first and second pair. Moreover, they proved the presence of guanine-cytosine regions (CMA-2 positive) in the telomeres of both arms of the first and second pair of chromosomes, on the long arm of the autosome of the third and fifth pair and the Z chromosome. Earlier studies (HAMMAR *et al*. 1966) mentioned the occurrence of C bands at the same sites.

SŁOTA (1998) and ŚWITOŃSKI (1998) attempted to estimate the size of heterochromatin blocks using subjective estimates (the size of the heterochromatin region was described by means of "+" signs). The subjective studies were continued by KOZUBSKA-SOBOCIŃSKA *et al*. (1999) who aimed at characterising the karyotypes of various animal species. In the present study the measurements of the size of heterochromatin blocks were carried out and presented as relative values in relation to the chromosome length. The measurement of the size of heterochromatin blocks enabled the general determination of the variation of chromosome structure.

References

APITZ M., WAGNER K. U., SAAR W. 1995. Karyotype characteristics in domestic ducks and geese. Proc. 10th Europ. Symp. on Waterfowl, Halle, Germany: 465-472.

ANSARI H. A., BOSMA A. A., BROAD T. E., BUNCH T. D., LONG S. E., MAHER D. W., PEARCE P. D., POPESCU C. P. 1999. Standard G-, Q-, and R-banded ideograms of the domestic sheep (*Ovis aries*): homology with cattle (*Bos taurus*). Report of the committee for the standardization of the sheep karyotype. Cytogenet. Cell Genet. **87**: 134-142.

BELTERMAN R. H. R., DEBOER L. E. M. 1984. A karyological study of 55 species of birds, including karyotypes of 39 species new to cytology. Genetica **65**: 39-82.

BERNARDI G. 1989. The isochore organisation of the human genome. Annu. Rev. Genet. **23**: 637-661.

BITGOOD J., SHOFFNER R. N. 1990. Cytology and cytogenetics. (In: Poultry Breeding and Genetics. B. D. Crowford ed. Amsterdam-Oxford-New York-Tokyo, Elsevier): 401-427.

BURKHOLDER G. D., DUCZEK L. L. 1982. The effect of chromosome banding techniques on the histone and nonhistone proteins of isolated chromatin. Can. J. Biochem. **60**: 328-337.

CHRSTIDIS L. 1989. Karyotypic analyses in birds. (In: Cytogenetics of Animals, C. Halmaun ed.): 125-132.

CRAWFORD R. D. 1990. Origin and history of poultry species. (In: Poultry Breeding and Genetics. Elsevier Amsterdam-Oxford-New York-Tokyo ed.): 1-41.

DISNEY J. E., JOHNSON K. R., MAGNUSON N. S., SYLVESTER S. R., REEVES R. 1989. High-mobility group protein HMG-I localizes to G/Q and C-bands of human and mouse chromosomes. J. Cell Biol. **109**: 1975-1982.

FILLON V., MORISSON M., ZOOROB R., AUFFRAY C., DOUAIRE M., GELLIN J., VIGNAL A. 1998. Identification of sixteen chicken microchromosomes by molecular markers using two colour fluorescent in situ hybridization (FISH). Chrom. Res. **6**: 307-313.

GREGORY T. R. 2002. A bird's-eye view of the C-value enigma: genome size, cell size, and metabolic rate in the class *aves*. Int. J. Org. Evol. **56**: 121-130.

HAMMAR B. 1966. The karyotypes of nine birds. Hereditas **55**: 367-585.

HIDAS A. 1993. Cytogenetic studies on a species hybrid goose breed. Proc. 8th North American Colloquium on Domestic Animal Cytogenetic and Gene Mapping, Guelph, Canada: 153-155.

HIDAS A. 1999. Molecular cytogenetic studies in domestic goose. Proc. 13th European Colloquium on Cytogenetics of Domestic Animals, Budapest, Hungary. Állattenyésztés és Takarmányozás **48**: 78-80.

HOLMQUIST G. P. 1988. DNA-sequences in G-bands and R-bands. (In: Chromosome and Chromatin Structure. K. W. Adolph ed. Boca Raton, Florida, CRC Press): 76-121.

HOLMQUIST G. P. 1992. Review article: Chromosome bands, their chromatin flavors, and their functional features. Am. J. Hum. Genet. **51**: 17-37.

ISCN 1995. An international system for human cytogenetic nomenclature. Mitelman F. ed. S. Karger, Basel.

ISCNDA 1989. International System for Cytogenetic Nomenclature of Domestic Animals (1989). The Second Intern. Conf. on Standardization of Domestic Animal Karyotypes. Di Berardino D., Hayes H., Fries R., Long S. 1990. Cytogenet. Cell Genet. **53**: 65-79.

ISCNDB 2000. International System for Chromosome Nomenclature of Domestic Bovids (2000). Di Berardino D., Di Meo G.P., Gallagher D. S., Hayes H., Iannuzzi L. 2001. Cytogenet. Cell Genet. **92**: 283-299.

KOZUBSKA-SOBOCIŃSKA A., SŁOTA E., BUGNO M., DANIELAK-CZECH B., REJDUCH B. 1999. Application of the MultiScan™ system to assess chromosome polymorphism. Rocz. Nauk. Zoot. **26**: 9-19. (In Polish).

LADJALI K., TIXIER-BOICHARD M., CRIBIU E. P. 1995. High resolution chromosome preparation for G- and R- banding in *Gallus domesticus*. J. Hered. **86**: 136-139.

LADJALI-MOHAMMEDI K., BITGOOD J. J., TIXIER-BOICHARD M., PONCE DE LEON F. A. 1999. Intern. System for Standarized Avian Karyotypes (ISSAK): standardized banded karyotypes of the domestic fowl (*Gallus domesticus*). Cytogenet. Cell Genet. **86**: 271-276.

MAYR B., LAMBROU M., KALAT M., SCHLEGER W., BIGELBACH A. 1990. Characterization of heterochromatin by sequential counterstain-enhanced fluorescence in three domestic bird species. Hereditas **8**: 468-475.

PERRY P., WOLFF S. 1974. New Giemsa method for differential staining of sister chromatids. Nature **261**: 156-158.

RODIONOV A. V. 1996. Micro versus macro: a review of structure and functions of avian micro- and macrochromosomes. Genetika **32**: 597-608.

ROSIŃSKI A., BIELIŃSKA H., BADOWSKI J., MIASTKOWSKA K. 1999. Effect of feeding system on body weight and carcass composition in the White Koluda Goose. Proc. 1st World Waterfowl Conf. Taichung, Taiwan: 519-523.

SCHMID M., NANDA I., GUTTENBACH M., STEINLEIN C., HOEHN M., SCHARTL M., HAAF T., WEIGEND S., FRIES R., BUERSTEDDE J. M., WIMMERS K., BURT D. W., SMITH J., A'HARA S., LAW A., GRIFFIN D. K., BUMSTEAD N., KAUFMAN J., THOMSON P. A., BURKE T., GROENEN M. A., CROOIJMANS R. P., VIGNAL A., FILLON V., MORISSON M., PITEL F., TIXIER-BOICHARD M., LADJALI-MOHAMMEDI K., HILLEL J., MÄKI-TANILA A., CHENG H. H., DELANY M. E., BURNSIDE J., MIZUNO S. 2000. First report on chicken genes and chromosomes. Cytogenet. Cell. Genet. **90**: 169-218.

SILVERSIDES F. G., CRAWFORD R. D., WANG H. C. 1988. The cytogenetics of domestic geese. J. Hered. **79**: 6-8.

SŁOTA E. 1998. Chromosome polymorphism in swine. Rocz. Nauk. Zoot., Ph.D. Theies, IZ, Kraków **7**: 1-58. (In Polish).

SMALEC E. 2001. Italian White goose. Animal Health and Production Compedium CAB International UK Wallinford--Oxford, (www.cabi.org).

SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304-306.

SUMNER A. T. 1994. Functional aspects of the longitudinal differentiation of chromosomes. Eur. J. Histochem. **38**: 91-109.

ŚWITOŃSKI M. 1998. B chromosomes in the common fox (*Vulpes vulpes*), their structure, distribution, inheritance and significance. Rocz. AR Poznań. Ph.D. Thesis **174**: 1-65. (In Polish).

VIGNAL A., FILLON V., VIGNOLES M., MORISSON M., CROOIJOMANS R. P. M. A., GROENEN M. A. M. 1999. The development of comparative molecular genome analysis in duck. Proc. 1st World Waterfowl Conf., Taichung, Taiwan: 603-606.

WĘŻYK S., ROUVIER R., ROSIŃSKI A., ROUSSELOT-PAILLEY D. 1993. With the Polish goose to Europe. Przeg. Hod. **5**: 26-28. (In Polish).