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Identification of T-bands and subtelomeric areas in chromosomes of the European domestic goose (*Anser anser)*

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The aim of the research was to identify T-bands and subtelomeric areas in chromosomes of the European domestic goose (*Anser anser)*. T-bands are an exceptionally high temperature--resistant subgroup of R-bands. They are characterized by elevated C-G pair saturation. Moreover, T-bands are chromosome regions of high gene content, even in comparison with the R-bands. As a result of T-banding, bands were found to be present on all of the analysed chromosomes. On submetacentric chromosomes, telomeric bands were observed within both arms. On the acrocentric chromosome of the third pair and the Z and W sex chromosomes, they could be identified only on the metaphase chromosomes, still undivided into chromatids. On the fifth, acrocentric chromosome pair, a band was found only in the distal part of the q arm. As a result of extended incubation of the preparations in the hot PBS buffer, the subtelomeric region was found to be degraded on all of the analysed chromosomes. Degraded subtelomeric regions were identified within the distal parts of both the submetacentric chromosome arms, as well as on the arm q of the acrocentric chromosomes.

KEY WORDS: *Anser anser* geese / karyotype / T-bands / subtelomeric area

The most complete information on the karyotype of a given species is acquired by directly observing chromosomes obtained from dividing cells. The use of standard Giemsa or Leishman staining enables morphological classification of chromosomes depending on the size and position of the centromere. More precise investigations of chromosomes consist in differential/band staining of chromatids. The basic techniques of obtaining karyotype patterns are GTG [21, 22] and RBG [18] staining. A specific variety of R-bands are T-bands, first obtained by Dutrillaux in 1973 [9]. Initially, T-bands were thought to be present only in the terminal parts of chromosomes and they were thought to be telomere bands – hence their name.

T-bands are obtained through initial incubation of chromosomes in a phosphate or PBS buffer at 87°C and subsequent staining with Giemsa solution or acridine orange (OA).

T-bands are considered in the literature as a heat-resistant subgroup of R-bands [10]. As regards the mechanisms of T-band staining, crucial importance is ascribed to thermal incubation, with due account taken of the ion concentration, temperature and the duration of the initial processing. T-bands are particularly rich in C-G pairs. In the human karyotype, they constitute 15% of all the bands but they contain as many as 65% of all the genes mapped so far [9, 10, 13, 14].

The genome of vertebrates does not have uniform density. Cuny et al. [4] distinguished five DNA classes with different densities. Light isochores: L1 and L2 and heavy ones: H1, H2 i H3. The H3 isochores, which have the highest saturation of G-C pairs and the highest gene density, are present within the T-bands. Moreover, T-band regions experience active meiotic recombination and intensive chiasm formation [11, 14, 15, 19].

The physical ends of the chromosomes of mammals and other vertebrates are formed by tandemly repeated hexamers (TTAGGG)n. Towards the geometrical centre of the chromosome, the constant telomeric sequence undergoes subtle changes and forms the subtelomere area. This is a non-coding area. The sequences that are present in it can gradually become less and less similar to the basic sequence. Telomeres and the subtelomere area together form the so-called terminal restriction fragment (TRF) [1, 2, 3, 5, 25].

The use of microphotometry has made it possible to quantitatively analyse the subtelomeric region. In the vicinity of T-bands there is chromatin with the highest density. It is treated as a marker that helps detecting the changes within the chromosomes that were formed by endoreduplication [5, 6].

A density analysis of chromatin within T-bands and the subtelomere area using the Dutrillaux method [9] was performed only on chromosomes obtained from Chinese hamster ovary (CHO) cells and on human chromosomes acquired from fibroblasts [6, 7, 8].

Prolonged incubation of chromosomes in a hot phosphate buffer degrades chromatin and makes holes in the subtelomeric regions of both sister chromatids or in the pericentromeric regions of certain chromosomes. They can appear in one or both of the chromatids and differ in size. Moreover, the areas with degraded chromatin correspond to the high density areas detected with microphotometry [7, 8].

The genetic material of geese is the least known of all the animal species in economic use. This challenged us to undertake research whose purpose was to identify T-bands and subtelomeric areas in the chromosomes of the domestic goose (*Anser anser)*.

Material and methods

The experiments were conducted on mitotic chromosomes of ten animals (5 females and 5 males) representing the European domestic goose (*Anser anser)*. The chromosomes were isolated from lymphocytes of peripheral blood using the standard procedures.

The first step in the identfication of T-bands was optimisation of staining techniques. T-banding was carried out using three modifications of the basic Dutrillaux method [9]. The optimal staining conditions for each modification were selected experimentally. The modifications introduced into the basic technique principally concerned the applied media and incubation time.

Modification 1. It is the standard method for the production of T-bands in human chromosomes [12]. A mixture of 94 ml of distilled water and 3 ml of phosphate buffer with the pH of 6.7 was placed in a thermostat set at 87°C. After the mixture reached the required temperature of 87°C, it was spiked with 3 ml of a 3% Giemsa solution. In the mixture prepared in this way the preparations were incubated for 5-30 minutes. The time was selected experimentally. After staining, the preparations were rinsed several times with distilled water and dried at room temperature.

Modification 2. The technique suggested by MacGregor and Varley [17] for staining telomere bands in amphibian chromosomes. A PBS buffer with pH 5.1 was placed in the thermostat set at 87°C. The preparations were placed in the hot buffer and incubated for 20-60 minutes. The time was selected experimentally. From the PBS buffer the preparations were transferred into a 3% Giemsa solution in a phosphate buffer and incubated again at 87°C for 10-20 minutes. The excess of the stain was removed by repeated rinsing of the preparations with distilled water. The stained and rinsed preparations were dried at room temperature.

Modification 3. This procedure was taken from a website on the history of human chromosome staining (Primate Cytogenetics Network) and is complementary in relation to the two previous ones. The preparations stained with the Giemsa solution, both the ones initially incubated in the phosphate buffer and those incubated in the PBS buffer, were developed and hydrated with alcohol in a series of decreasing concentration (96%, 80%, and 70%). After the application of alcohol, the preparations were rinsed with distilled water and stained with an acridine orange (OA) solution with the concentration of 5 mg OA per 100 ml of the phosphate buffer with pH 6.7. Two staining variants were tested, depending on the initial incubation in the phosphate or PBS buffer. The incubation time with the pigment was selected experimentally as 10 to 40 minutes. The stained preparations were encapsulated in a drop of the phosphate buffer with pH 6.7 and covered with a cover glass.

The description of morphology and classification of the chromosomes was carried out on the basis of the standardised description of the *Gallus domesticus* karyotype [16]. For each bird 30 metaphase plates were analysed (10 for each staining modification). The pattern of T-bands was determined in the first five pairs of macrochromosomes and in the Z and W sex chromosomes.

Results and discussion

On the basis of the presented methodology, the chromosomes were stained in the three ways in order to optimally reveal T-banding. Each of the three modified staining procedures was carried out in different conditions. Out of 100 metaphase plates subjected to staining in accordance with the first modified procedure, only 15 revealed T-bands. The second modified procedure was applied for the preparations incubated in the PBS buffer in the thermostat at 87°C. Out of the 100 stained metaphase plates, chromosomes with prominent T-bands were observed on 89 plates. T-bands were observed in the distal parts of the p and q arms of the first four pairs of autosomes and Z and W sex chromosomes. Moreover, two bands were also found in the chromosomes of the third pair and one band on the q arm

of the fifth chromosome pair. Considering the results of the second modified procedure, it was selected as the optimal modification for the production of T-bands in goose chromosomes. Acridine orange also revealed T-bands but the percentage of metaphase plates with well-pronounced bands was not satisfactory. Consequently, thorough analysis concerned the preparations obtained as a result of the second modified procedure.

The most prominent T-bands were observed in the first two pairs of macrochromosomes. In almost all of the analysed preparations it was possible to distinguish T-bands on both arms of the first and second pair of chromosomes. In the third acrocentric chromosome pair, it was possible to distinguish T-bands on the p arms only in the case of prometaphase and metaphase plates in which the chromosomes were still undivided into chromatids. In the fourth submetacentric chromosome pair, the same as in the first and second pair where it was possible to clearly distinguish the p and q arms, the bands were mostly observed within both arms. In the fifth acrocentric chromosome pair, T-bands were present only in the interstitial part of the q arm. The fifth chromosome pair also had the greatest number of blurry bands or the chromosomes themselves were blurry. Within the Z and W sex chro-

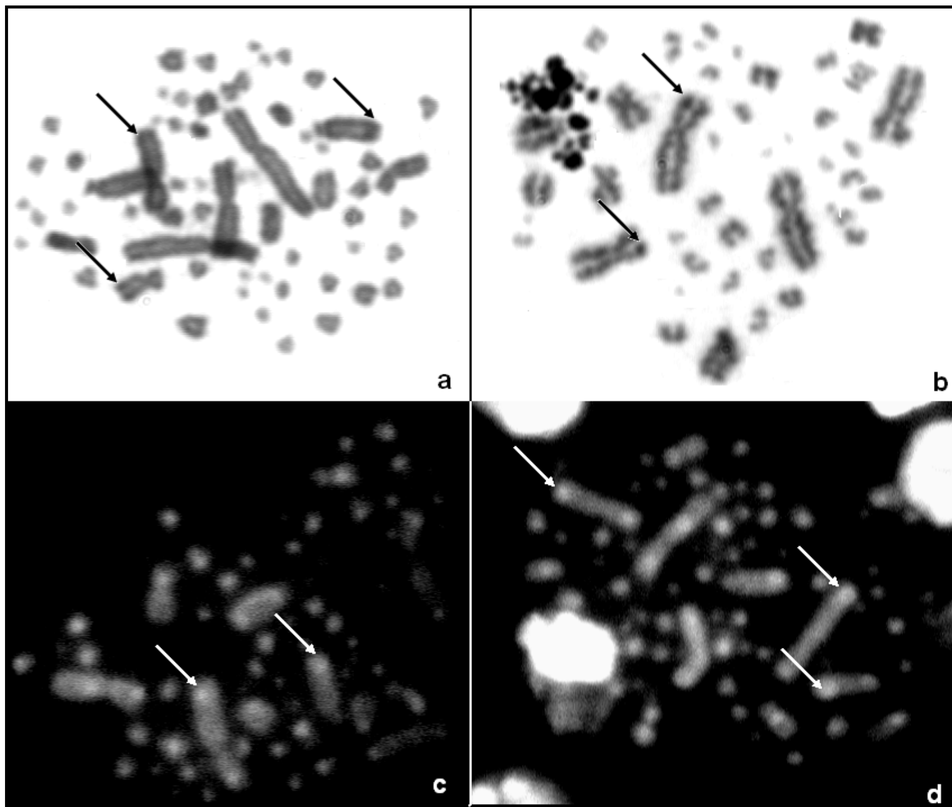

Photo. 1. Mitotic chromosomes of goose – T banding; several sample bands are marked with arrows
Fot. 1. Chromosomy mitotyczne gęsi – barwienie T; strzałkami zaznaczono przykładowe prążki

mosomes, T-bands on both arms could only be distinguished in the well dispersed metaphases, undivided into chromatids. The photograph presents the metaphase plates obtained as a result of the application of the second (Photo. 1a,b) and third procedure (Photo. 1c,d) for staining the preparations.

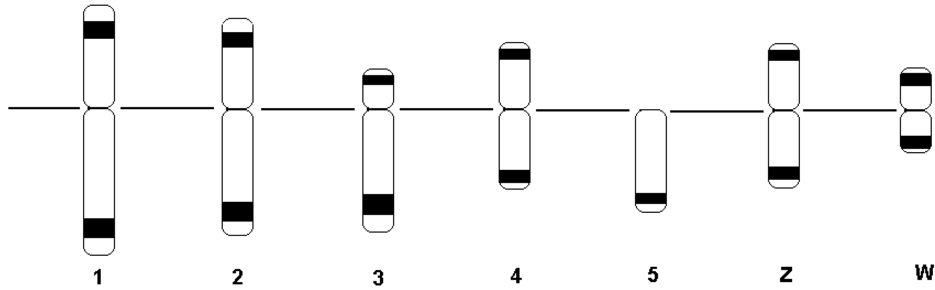

Fig. 1. Partial idiogram of geese chromosomes − T banding
Rys. 1. Cząstkowy idiogram chromosomów gęsi − barwienie T

Based on the analysis of all the preparations for which the T-banding technique was applied, a partial ideogram was created for the first five chromosomes and Z and W sex chromosomes (Fig. 1).

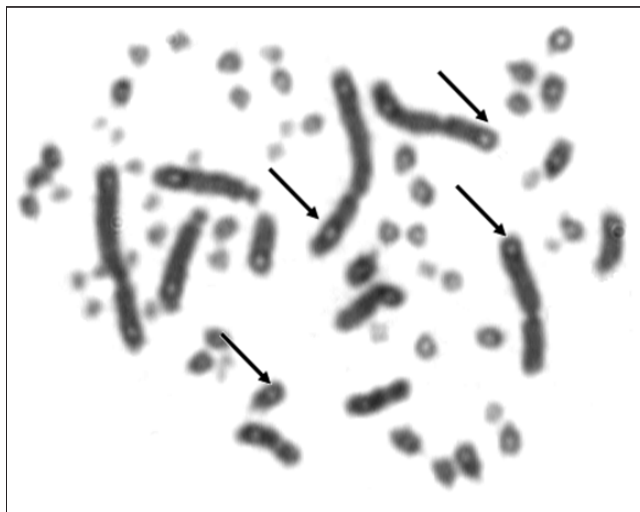

Photo. 2. Mitotic chromosomes of the geese; degraded subtelomeric areas are marked with arrows
Fot. 2. Chromosomy mitotyczne gęsi, strzałkami zaznaczono zdegradowane obszary subtelomerowe

As described above, the selection of optimal staining conditions was preceded by a number of tests associated with modifications of the incubation time in different media, depending on the assumed variant of the original method. Prolonged incubation of the

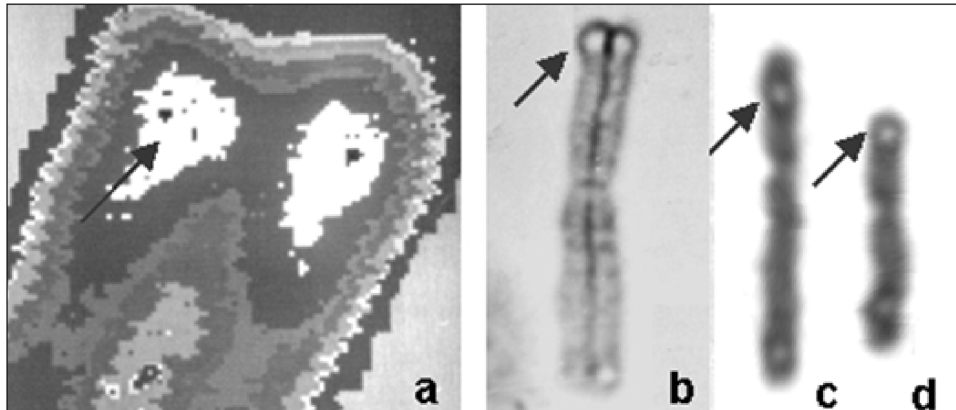

Fig. 2. Areas of high chromatin density (a) and degraded TRF regions (b) in the CHO chromosome [7] and goose chromosomes (c, d)
Rys. 2. Lokalizacja wysokiej gęstości chromatyny (a) oraz zdegradowane rejony TRF (b) w chromosomie CHO [7] i chromosomach gęsi (c, d)

preparations in the PBS buffer produced holes – damaged spots in the subtelomeric region. Degradation of this area was observed in all the chromosomes within the distal parts of the p and q arms, in the meta- and submetacentric chromosomes, as well as on the q arms of the acrocentric chromosomes (Photo. 2).

In most of the studies connected with the identification of T-bands in chromosomes of other vertebrates, the medium selected for incubation was the phosphate buffer, whereas the PBS buffer was used in few methodologies [17]. On the basis of our experiments on the goose chromosomes, it was concluded that the PBS buffer produced much better results than the phosphate buffer. Furthermore, the incubation time in all of the media was longer in the case of birds than the time suggested for experiments with mammals.

Prolonged incubation of human and CHO chromosomes in a hot phosphate buffer degraded chromatin and caused small holes to appear in the subtelomeric regions of both sister chromatids or in the pericentromeric regions of certain chromosomes. The holes can appear in one or both of the chromatids and differ in size. Moreover, the areas with degraded chromatin correspond to the areas of high density detected with microphotometry. In the goose chromosomes, degraded subtelomeric areas were observed in each arm of the analysed macrochromosomes and sex chromosomes (Fig. 2).

Chromosome replication and independent segregation of chromatids is associated with maintaining a constant structure of the karyotype. The process can be spontaneously or experimentally modified, producing cells with different modifications of their ploidy. High-density chromatin is considered as a marker of changes within the chromosomes produced as a result of endoreduplication [5, 7, 8]. Endoreduplication and endomitosis are the mechanisms of mitotic modifications. They were observed in normally developing cells and in neoplastic cells [20]. Endoreduplication is associated with the production of gigantic nuclei in neoplastic cells. Presumably, it is the chief process in the polyploidization

of these cells [23, 24]. The use of microphotometry and computer graphic analysis makes it possible to observe the distribution of chromatin with different density in subtelomeric areas of chromosomes and within T-bands.

T-band regions and subtelomeric areas of metaphase chromosomes are extremely complex structures and their exploration at the level of molecular structure is still at an experimental stage.

REFERENCES

1. BAIRD D.M., 2005 − New developments in telomere length analysis. ***Experimental Gerontology*** 40, 363-368.
2. BLACKBURN E.H., 2001 − Switching and signaling at the telomere. ***Cell*** 106, 661-673.
3. BOLZAN A.D., BIANCHI M.S., 2006 − Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations. ***Mutation Research*** 612, 189-241.
4. CUNY G., SORIANO P., MACAYA G., BERNARDI G., 1981 − The major componentes of the mouse and human genomes. I. Preparation, basic properties and compositional heterogeneity. ***European Journal of Biochemistry*** 115, 227-233.
5. DRETS M.E., 2000 − Insights into structure of the subtelomeric chromosome segments. ***Genetics and Molecular Biology*** 23 (4), 1087-1093.
6. DRETS M.E., 2004 − Cytological indications of the complex subtelomeric structure. ***Cytogenet. Genome Research*** *104, 137-141.*
7. DRETS M.E., MENDIZÁBAL M., 1998 − Microphotometrical image analysis of the subtelomeric region of T-banded endoreduplicated chromosomes of Chinese hamster ovary cells. ***Genetics and Molecular Biology*** 21 (2), 219-225.
8. DRETS M.E., MENDIZÁBAL M., 1998 − The underlying structure of the subtelomeric region detected by microphotometrical scanning and chromosome graphic image analysis. ***Mutation Research*** 404, 13-16.
9. DUTRILLAUX B., 1973 − Nouveau systeme de marquage chromosomique: Les bands T. ***Chromosoma*** 41, 395-402.
10. DUTRILLAUX B., 1977 − New chromosome techniques. In: Yunis J.J (ed): Molecular structure of human chromosomes. Academic Press, New York, 233-265.
11. GRADINER K., HORISBERGER M., KRAUS M., TANTRAVAHI U., KORENBERG J., RAO V., REDDY S., 1990 − Analysis of human chromosome. 21: Correlation of physical and cytogenetic maps: gene and CpG Island distributions. ***The EMBO Journal*** 9, 25-34.
12. GUSTASHAW K.M., 1991 − Chromosome Stains. In: Barch M.J. (ed): The ACT Cytogenetics Laboratory Manual, Second Edition. The Association of Cytogenetic Technologists, Raven Press Ltd., New York.
13. GUSTAVSSON I., 1980 − Banding techniques in chromosome analysis of domestic animals. ***Advances in Veterinary Science and Comparative Medicine*** 24, 245-289.
14. HOLMQUIST G.P., 1992 − Chromosome bands, their chromatin flavors, and their functional features. ***American Journal of Human Genetics*** 51, 17-37.
15. IKEMURA T., WADA K.N., 1991 − Evident diversity of codon usage patterns of human genes with respect to chromosome banding patterns and chromosomes: relation between nucleotide sequence data and cytogenetic data. ***Nucleic Acids Research*** 19, 4333-4339.

16. LADJALI-MOHAMMEDI K., BOTGOOD J.J., TIXIER-BOICHARD M., PONCE DE LEON F.A., 1999 − International System for Standardized Avian Karyotypes (ISSAK): standardized banded karyotypes of the domestic fowl (*Gallus domesticus*). ***Cytogenetics and Cell Genetics*** 86 (3-4), 271-276.
17. MACGREGOR H.C, VARLEY J., 1988 − Working with Animal Chromosomes. 2nd edition. John Wiley & Sons, London, New York, Brisbane, Toronto, Singapore.
18. PERRY P., WOLFF S., 1994 − New Giemsa method for differential staining of sister chromatids. ***Nature*** 261, 156-158.
19. RODIONOV A.V., CHECHIK M., KANASHINA O., MYAKOSHINA Y., PANASENKO S., VISHNYAKOVA N., GAGINSKAYA E.R., 1996 − Crossing-over frequency and linkage study in poultry: a cytogenetic approach. Abstr. XXV International Conference on Animal Genetics ISAG, Tours, France, 108.
20. SARTO G.E., STUBBLEFIELD P.A., THERMAN E., 1982 − Endomitosis in human trophoblast. ***Human Genetics*** 62, 228-232.
21. SEABRIGHT M., 1971 − A rapid banding technique for human chromosomes. ***Lancet*** 2, 971-972.
22. SEABRIGHT M., 1973 − Improvement of trypsin method for banding chromosomes. ***Lancet*** 1, 1249-1250.
23. THERMAN E., KUHN E.M., 1989 − Mitotic Modifications and Aberrations in Cancer. ***CRC Critical Reviews in Biochemistry*** 1, 293-305.
24. THERMAN E., SARTO G.E., KUHN E.M., 1989 − The course of endomitosis in human cell. ***Cancer Genetics and Cytogenetics*** 19, 301-310.
25. ZAKIAN V.A., 1989 − Structure and function of telomeres. ***Annual Review of Genetics*** 23, 579-604.

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Identyfikacja prążków T i obszarów subtelomerowych w chromosomach europejskiej gęsi domowej *(Anser anser)*

Streszczenie

Celem badań była identyfikacja prążków T oraz obszarów subtelomerowych w chromosomach gęsi domowej *(Anser anser)*. Prążki T stanowią podzespół prążków R, wyjątkowo odporny na wysoką temperaturę. W ich obrębie występuje duże nasycenie par C-G. Ponadto prążki T są rejonami chromosomów o wysokiej zawartości genów, nawet w porównaniu z prążkami R. W wyniku zastosowania barwienia T stwierdzono obecność prążków na wszystkich analizowanych chromosomach. Na chromosomach submetacentrycznych prążki telomerowe obserwowano w obrębie obu ramion. Na akrocentrycznym chromosomie trzeciej pary, chromosomach płci Z i W można je było wyróżnić tylko na chromosomach metafazowych jeszcze nie podzielonych na chromatydy. Na akrocentrycznej piątej parze chromosomów stwierdzono obecność prążka tylko w dystalnej części ramienia q. Na skutek wydłużonej inkubacji preparatów w gorącym buforze PBS stwierdzono degradację rejonu subtelomerowego na wszystkich analizowanych chromosomach. Zdegradowany rejon subtelomerowy obserwowano w obrębie dystalnych części obu ramion chromosomów submetacentrycznych oraz na ramieniu q chromosomów akrocentrycznych.