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COMPARATIVE ANALYSIS OF KARYOTYPES OF SEVERAL BREEDS OF THREE *BOVIDAE* SPP. – *BOS TAURUS*, *OVIS ARIES* AND *BUBALUS BUBALIS BUBALIS* AN EVOLUTIONARU VIEWPOINT

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Aim. The aim of the study was to analyze chromosome sets of specific species of agricultural animals of several breeds of three *Bovidae* spp., *Bos taurus*, *Ovis aries* and *Bubalus bubalis bubalis* using the techniques of G- and Ag-banding of chromosomes and to demonstrate the role of their variability in the relatedness between the three species. **Methods**. The culture of lymphocytes and chromosome preparations were prepared by the method of Moorhead et al (1960). The Sajjad N et al (2014) and by Ag-banding method of Seabright (1971). To study the phenetic interrelations, the index of the number of active nucleolus organizer regions (NOR's) in the chromosomes during the metaphase stage was used. The chromosomal aberrations were classified according to the recommendations of the International System for Chromosome Nomenclature of Domestic Bovids (2001). The dendrogram of phylogenetic relations between mammalian species was built using «STATISTICA 6.1». **Results**. The homology of the structural organization of chromosomes and the evolutionary changes in karyotypes of *Bovidae* family were analyzed, highlighting their sex chromosomes and the chromosomes with specific localized groups of gene linkage. A considerable homology of chromosome sites was found the G-banding profiles. **Conclusions**. The comparison of differentially banded chromosomes of some breeds of *Bos taurus*, *Ovis aries* and *Bubalus bubalis* demonstrates the similarity of chromosomal sets and helps detect the differences, related to the formation of metacentric chromosomes. Species-specific morphological differences in sex chromosomes of the investigated species were found in terms of the length and presence of pericentric inversions.

Key words: evolution of karyotypes, *Bovidae, Bos taurus, Ovis aries, Bubalus bubalis bubalis,* G-banding of chromosomes, nucleolus organizers.

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INTRODUCTION

Classic population geneticists believe that the variability of the chromosomal sets cannot be the basis for evolutionary species transformations, as the chromosomal polymorphism is a phenomenon frequently found in natural populations; it occurs and is maintained but with no evolutionary consequences (Lewontin R, 1974; Dobigny G et al, 2015). However, modern scientific tendencies force the change in this point of view (Shapiro J, 2009, 2013). The breakthrough, conditioned by the development of DNA analysis methods, allowed for phylogenetic reconstructions and resulted in re-envisioning the system of the organic world (Katz LA et al, 2012; Hinchliff C et al, 2015). The introduction of the methods of GTG-banding of chromosomes (Q-, G-, C-, R-, T-, Ag-banding) and chromosomal dying, (Zoo-FISH) provided for the transition from the description of the similarity (number, size, form) to the analysis of homologies and solving complicated taxonomic problems of the level of the species (Hinchliff C et al, 2015; Graphodatsky A et al, 2011).

The scientific data demonstrate the presence of regularities in the mutation process on the level of karyotype

restructuring. A researcher, MJD White, *(*Department of Population Biology, Research School of Biological Sciences, Australian National University) introduced the term "karyotypic orthoselection" to describe the non-randomness of same-type chromosomal transformations in similar karyotypes (White MJD, 1978). It is assumed that the reason for these chromosomal transformations lies in their role in protecting co-adapted gene complexes from being disrupted by the introgression from other populations.

The evolution of karyotypes in the species of *Bovidae* family was accompanied by different transformations in the chromosomal apparatus (Chaves R et al, 2000). Nevertheless, the systematicity of *Bovidae* is still controversial, as such evolutionary events as recombination mechanisms and morphological convergence resulted in a very variable number of chromosomes in this family in the range from 30 to 60 (Chaves et al, 2005; Graphodatsky A et al, 2011).

The aim of the study was to analyze chromosome sets of three species of agricultural animals of *Bovidae* family using the techniques of G- and Ag-banding of chromosomes and to demonstrate the role of their variability in the relatedness of the three species and their different breeds studied.

MATERIALS AND METHODS

The study was conducted at the Department of Genetics and Biotechnology of Animals of the M.V. Zubets Institute of Animal Breeding and Genetics, the NAAS.

The material of the cytogenetic study was the peripheral blood of representatives of some species and breeds of *Bovidae* family: sheep *(Ovis arias)* of Romanivska (n = 35), Sokilska (n = 33) and Ukrainian Carpathian Mountain breeds (n = 25) **(**Tipilo K, 2020**)** – cattle *(Bos taurus)* of Ukrainian Red-and-Motley dairy (n = 74) and Ukrainian Grey breeds (n = 43) and river buffalo *(Bubalus bubalis bubalis)* of Asian origin, reared in Ukraine (n = 49).

The culture of lymphocytes and chromosome preparations were obtained following the method of Moorhead PS et al (1960). To prepare chromosomal preparations, whole venous blood was cultivated for 72 h at +37 °C in RPMI 1640 medium (Sigma, USA) with the addition of 0.1 ml/ml PHA (phytohemagglutinin, Sigma, USA), 15 % embryonic calf serum, 0.1 ml colchicine (Serva, Germany) in the concentration of 0.04 μg/ml, added two hours before the completion of the cultivation period to stop cell division. The precipitate of cells was obtained by centrifugation (1,000 rpm, 15 min) with further treatment with 5 ml of the hypotonic KCl solution (0.075 M) for 20 min. The fixation of cells was conducted in three changes of the methanol-acetic acid mixture in 3:1 ratio. The cell suspension of desired density, obtained in the last portion of the fixator, was applied in drops on a cooled and wet specimen slide.

The preparations of metaphase chromosomes were analyzed by the Giemsa-trypsin or Giemsa-trypsin or GTG-banding according to the method of (Sajjad et al, 2014) To obtain GTG-banding the preparations of chromosomes at the stage of meiosis metaphase were placed for 6–10 sec in 0.25 % trypsin solution, dissolved in 0.25 % Hanks' solution at room temperature, and stained with 2 % Romanowsky-Giemsa solution. The chromosome preparations were washed with distilled water and dried. At least 30 metaphase plates were analyzed per one animal. The chromosome preparations were analyzed using Zeiss microscope (Germany) at the magnification of 1,000.

Ag-banding was done by the method of Seabright (1971) to determine active nucleolus organizer regions (NORs) of chromosomes, 2 drops of 50 % solution of nitric-acid silver (AgNO3, Sigma-Aldrich) and 2 drops (0.1 ml) of 0.2 % formic acid were added to the specimen slide and covered with glass. Then the preparation was placed into a moistening chamber at +56 °C, protected from light for 13–14 min, until the surface of the specimen slide acquired a dark brown color. NORs were detected as dark spots on telomeres of the corresponding chromosomes.

The chromosomal aberrations were classified according to the recommendations of the International System for Chromosome Nomenclature of Domestic Bovids (Cribiu EP et al, 2001). To study the relations between three species of agricultural animals of *Bovidae family – Bos taurus*, *Ovis aries,* and *Bubalus bubalis bubalis,* we used the index of the number of active NOR in the chromosomes at the metaphase stage of mitosis. This index is specific for each species, relatively polymorphic, and minimizes the possibility of subjective assessment of relationships between species.

A phenogram of relation relations between breeds of three Bovidae spp., *Bos taurus, Ovis aries* and *Bubalus bubalis bubalis* based on the variability of active NOR was constructed, using the STATISTICA 6.1 software package (StatSoft., 2001 http://www.statsoft.ru/home/textbook/default.htm).

RESULTS

We compared the chromosomal sets of cattle *Bos taurus*, domestic sheep *Ovis aries* and river buffalo *Bubalus bubalis* using G- and Ag-banding.

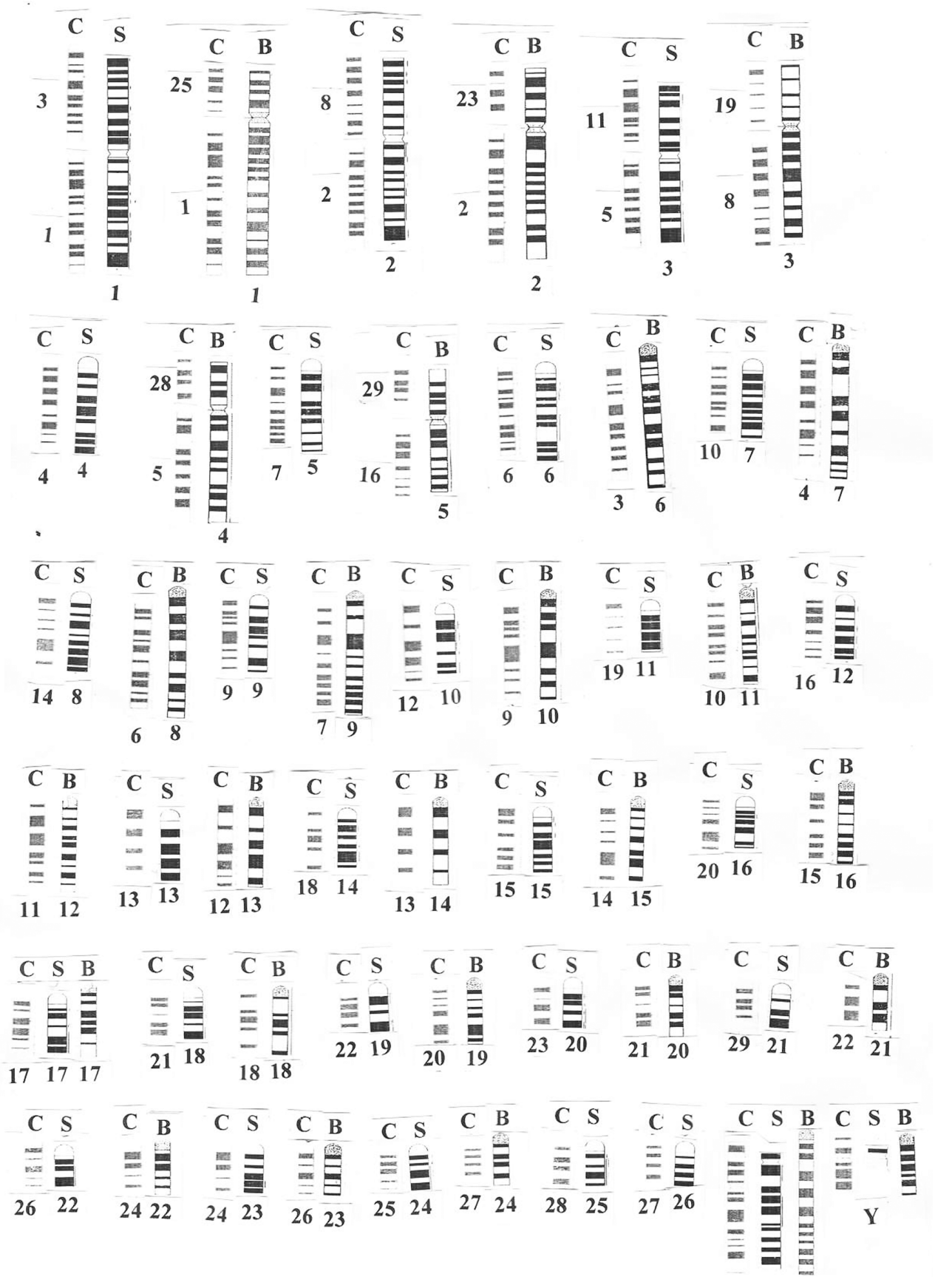

Fig. 1. A schematic presentation of karyotypes based on GTG-banding of cattle (C), sheep (S), and river buffalo (B)

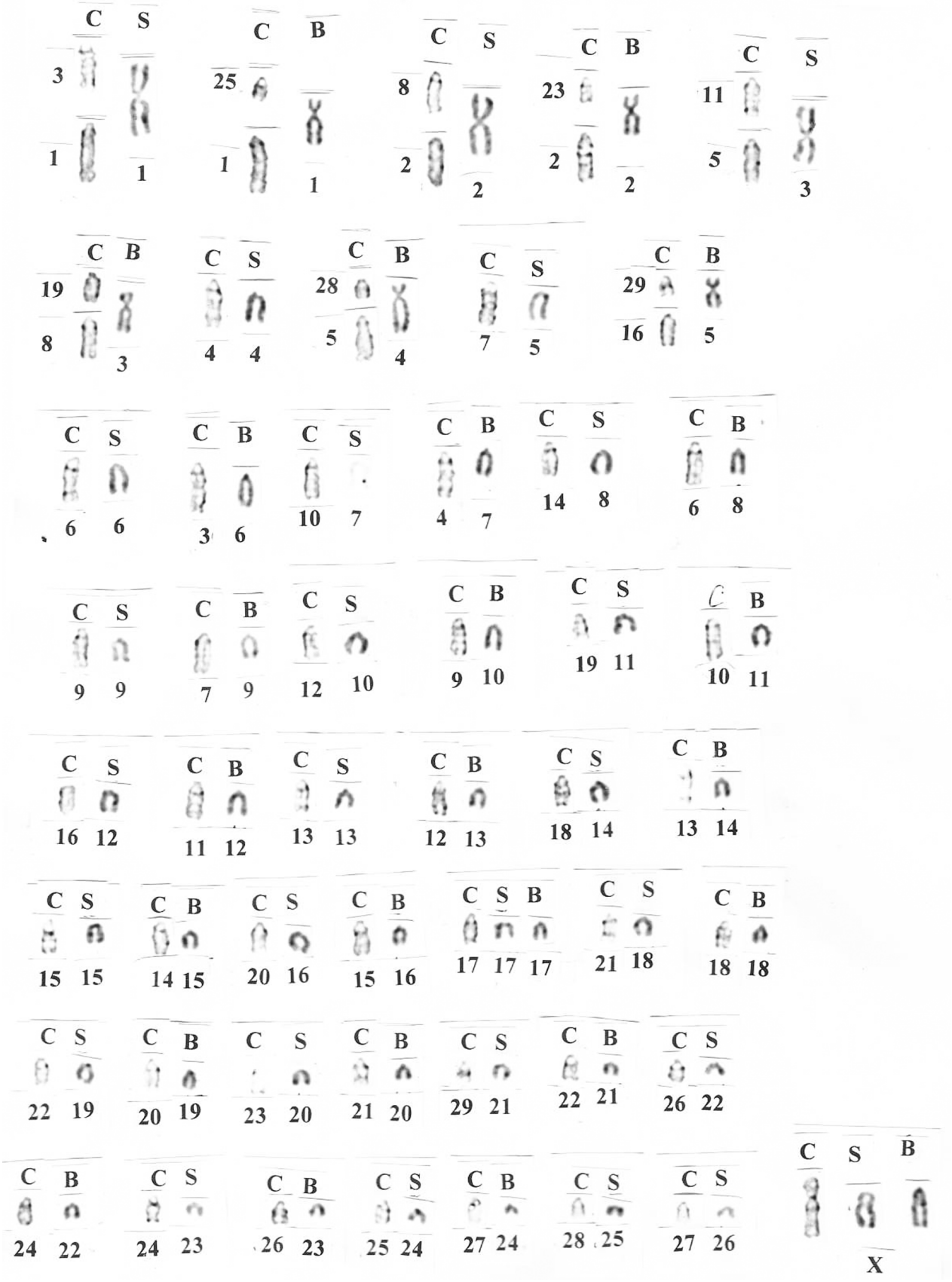

Fig. 2. The karyotypes of the representatives of cattle (C), sheep (S), and river buffalo (B) according to GTG-banding, magnification 10×100 light microscope using oil immersion)

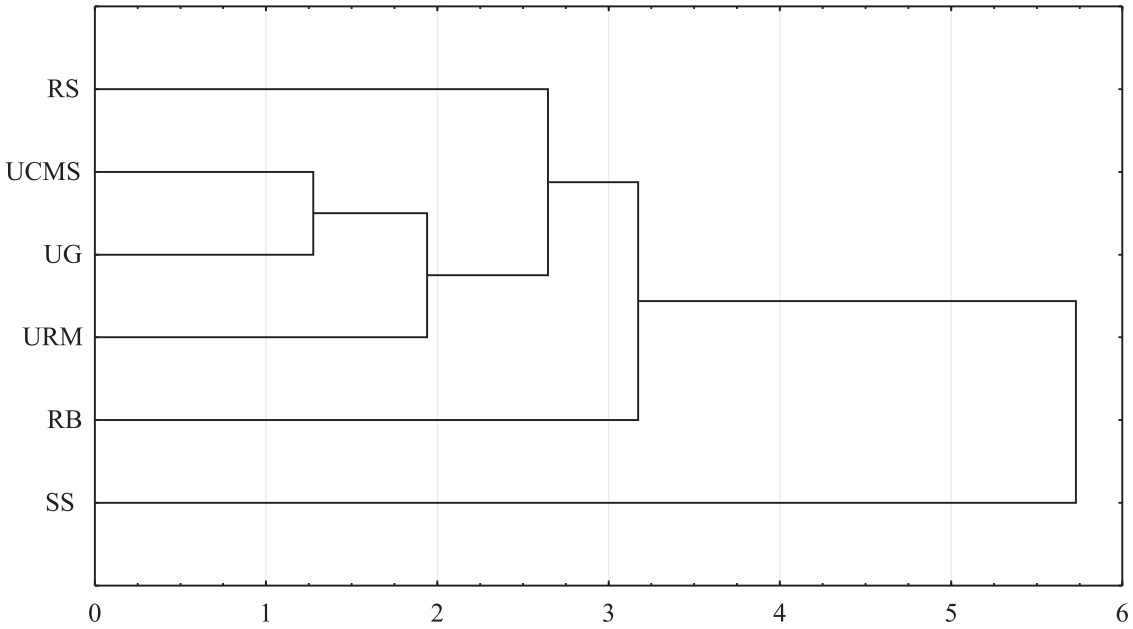

Fig. 3. The dendrogram of phenetic interrelations between breeds of three *Bovidae* spp., *Bos taurus*, *Ovis aries* and *Bubalus bubalis bubalis* based on the variability of active NOR. UG – Ukrainian Grey cattle breed; URM – Ukrainian Red-and-Motley dairy cattle breed; RS – Romanivska breed of sheep; SS – Sokilska breed of sheep; UCMS – Ukrainian Carpathian Mountain breed of sheep; RB – river buffalo

A homology of chromosome sites was found in the representatives of the investigated species by the GTG-banding profiles (Fig. 1 and 2). The karyotypes of *Bos taurus*, *Ovis aries* and *Bubalus bubalis bubalis* differ in the number of chromosomes and their morphology.

There was an observed loss of the pericentromeric G-positive band on the arms of chromosomes 1p, 2q, 4p and 5q. The pericentromeric G-positive band was found to be preserved in the third chromosome of river buffalo. Almost similar results were obtained while studying the karyotype of sheep, where the loss of the pericentromeric G-positive band was also found in the arms of chromosomes 1p and 2q, and the pericentromeric G-positive band was preserved in the third chromosome. In addition, the pericentromeric G-positive band in chromosome 3p of river buffalo and 3q in sheep was found to be smaller than in the corresponding homologous chromosomes of cattle (19 and 11, respectively). The remaining autosomes of river buffalo and sheep were found to be similar in the G-banding model to the homologues of cattle. Sex chromosomes X and Y of cattle were smaller than those of river buffalo and sheep (Fig. 1). The structures of G-bands in two sex acrocentric chromosomes of cattle and river buffalo under comparison are very similar, contrary to those of sex chromosomes of sheep. The only differences lie in size, as stated above, and in the positive distal band of Y-chromosome of cattle which was found to be larger than that in Y-chromosome of river buffalo.

The analysis of the chromosome morphology of *Bos taurus* and *Bubalus bubalis bubalis* demonstrated the differences in the length of sex chromosomes of the animals of these species. Different sizes of distal G-negative bands in Y-chromosome of river buffalo than those in cattle.

For known conservative sites, we found associations of 1/3, 2/8, 5/11 in sheep and 1/25, 2/23, 8/19, 5/28, 16/29 in buffaloes.

A phenogram was constructed using the variability of active NOR's of the three species studied (Fig. 3), where the river buffalo in our hands remarkably formed a separate branch that was more distant than the one of the Romanivska sheep breed to other studied cattle breeds. Sokilska sheep appeared most distant, but more related to river buffalo than to the other Ovis breed (Romanivska).

As for Romanivska sheep and cattle of the Ukrainian red-and-motley dairy breed, they form a more tight cluster, inside which there is also a tight cluster of Ukrainian-Carpathian mountain breed sheep and Ukrainian gray breed cattle. Ukrainian-Carpathian mountain sheep appear to be more related to Ukrainian grey breed than to Romanivska sheep.

DISCUSSION

Much information has been accumulated within the last 40 years regarding the comparative analysis of GTG- banded chromosomes and DNA satellites of different species of animals which allowed to determine the main tendencies in the karyotype evolution within many taxa, including the Bovidae (Di Berardino et al, 1979; Mayr et al, 1987; Chaves et al, 2005; Nguyen et al, 2008; Robinson TJ et al, 2011; Amancio AP et al, 2019).

Iannuzzi A et al (Iannuzzi A et al, 2021) compared the karyotypes of cattle and buffalo and assumed that two pericentric inversions may cause differences between sex chromosomes in these species of animals. For another comparison of Bovidae and an inversion noticed in roe deer see Kozubska-Sobocińska et al, 2007.

The information, obtained in our study and in the study of Di Meo GPD et al (DI Meo GPD et al, 2005) about the differences in the form and size of Y-chromosome in the species of *Bovidae family* (in *Bos taurus* and *Ovis aries* this sex chromosome has a metacentric form,in *Bubalus bubalis bubalis* – the acrocentric form; its size in buffaloes is larger than in bulls) supplements current cytogenetic maps.

Some scientists assume that the initial karyotype of cattle (2n = 60) is the ancestral model for evolutionary comparison with other Bovidae species due to the prevalence of acrocentric chromosomes and a karyotypic formula, observed in different species (Wurster DH, 1968).

The analysis of GTG- banded chromosomes of domestic sheep, (2n = 54), demonstrated that three pairs of metacentric chromosomes of the karyotype were formed due to the centric fusion of homologous chromosomes of cattle and goats: one and three, two and eight, and five and eleven respectively (Iannuzzi L et al, 1995; Cribiu EP et al, 2001; Di Meo GP et al, 2007; Goldammer T et al, 2009).

Ovis species is the best example of the evolution of the karyotype of Bovidae family, involving Robertsonian translocations on the autosomal level. Starting with the closest species, a goat (*Capra hircus*, 2n = = 60), the autosomal karyotype of Ovis develops via a progressing decrease in the diploid number of chromosomes to 58 in urial (*Ovis vignei*), 56 in arkhar or argali (*Ovis ammon*), 54 – in domestic sheep (*Ovis aries*) and other species, in 52 – in Siberian sheep (*Ovis nivicola*) due to Robertsonian translocations and centric fusions (Iannuzzi A et al, 2013).

It is believed that all sheep with karyotype 2n = 54 originate from one common ancestor due to the formation of Robertsonian translocations of chromosomes after the fusion of acrocentric chromosomes in karyotypes 2n = 58 and 2n = 56 (Nadler CF et al, 1971; Nadler CF et al, 1973). Probably, the evolution of sheep followed the pattern of 2n = 58 → 2n = 54 in terms of monophyletic origin (Bunch T et al, 1977).

The cytogenetic analysis using the fine morphology of chromosomes is also acceptable for the comparison of karyotypes of cattle, sheep, and buffaloes. The scientific data demonstrate that all the species of *Bovidae* family demonstrate a high degree of homology among autosomal chromosomes; they are closely related and have a similar genetic order (Iannuzzi L et al, 2009; Amaral ME et al, 2008; Iannuzzi A et al, 2021). The constitutive losses of heterochromatin were found during chromosomal aberrations which led to the formation of five pairs of biarmed chromosomes in river buffalo and three pairs – in sheep (Iannuzzi L et al, 2009).

We chose the index of the number of active nucleolus organizers per cell for our study. This feature is as species-specific as any other quantitative (instance.g., the number of gene alleles) or qualitative feature (e.g., nucleotide composition of the gene). In most animal species, the nucleolus organizers are known to occupy certain sites on specific chromosomes (Britton-Davidian J et al, 2012). Specific study methods using RNA polymerase I main transcription factors, e.g. upstream binding factor (UBF) and immunofluorescence demonstrate that not all NORs are actively transcribed, and it depends on the proliferative status of the cell and the stage of the cellular cycle Smirnov et al (2006). Some authors (Chi J et al, 2005; Cernohorska H et al, 2011; Ropiquet A et al, 2010) state that the evolutionary transformation refers mainly not to the organization of the chromosome but to the organization of the karyotype. The results of comparative cytogenetic studies from different countries (Buckland RA, 1978; Robinson et al, 2011) demonstrated that agricultural animals can be conventionally divided into two groups by the directions and tempo of the karyotypic evolution. The representatives of *Bos taurus* preserved the karyotype structure, close to the karyotype structure of the mammalian ancestor. The karyotypes of *Ovis aries* and *Bubalus bubalis* were subjected to “catastrophic evolution” which resulted in numerous breaks and fusions of ancestral chromosomes.

Based on the hypothesis of Robertson W-M (Robertson W-M, Rees B, 2005), the loss of a centromere

of one chromosome and its preservation in the other occurs during centralized translocations of monocentric type. The same phenomenon explains the losses in biarmed chromosomes of river buffalo and sheep (Iannuzzi A et al, 2021). Our study shows that in four out of five pairs of biarmed chromosomes of river buffalo, and in two out of three sheep a G-positive band, close to centromeric sites, was lost along with the loss of the block of an acrocentric chromosome, involved in the central linkage. It is known that intercalary heterochromatin (G-range) and constitutive heterochromatin (C-range) do not contain structural genes. If this is true, then the loss of a G-positive band should not be of special relevance. Practically, similar to other chromosomes, R-positive structures (euchromatin) in the pericentromeric region of biarmed pairs were preserved in the species under investigation (Ianuzzi L et al, 1990).

However, the function of heterochromatin in the evolution of the karyotype of species is yet to be determined. Nevertheless, there are scientific communications on special relevance of heterochromatin sites, including: (a) stabilizing the specialized regions of chromosomes, such as centromeres and telomeres (Baird DM, Farr CJ, 2006) facilitating the transformations of chromosomes; (c) determining the fertility barrier; (d) preserving euchromatin (Saksouk N et al, 2015); (e) decreasing recombination in the neighboring euchromatin (Morrison O, Thakur J., 2021).

The sequencing of genomes of many organisms and their comparative analysis demonstrated high conservative nature of DNA sequences – large areas, sometimes with the length of an entire arm of the chromosome, are homologous in different taxa. According to current notions (Graphodatsky AS et al, 2011), the evolution of karyotypes occurred not via the appearance of new nucleotide sequences but rather via the "reshuffling" of the existing ones. Probably, it is due to this reshuffling that considerable diversity of karyotypes is observed in different organisms: even close species often have different numbers of chromosomes with different morphology (McEachern S et al, 2009; Sacerdot C et al, 2018).

Thus, in the compared species of Bovidae, the morphological structure of chromosomes is characterized by a high degree of similarity. However, classical cytogenetic analysis does not allow to reveal all the subtleties of the organization of gene orders in chromosomes. Therefore, even to compare related species of animals, it is necessary to study their genomes at the level of gene maps, in particular, using molecular genetic markers (Chokan T et al, 2016).

CONCLUSIONS

The comparison of differentially banded chromosomes of some breeds of *Bos taurus*, *Ovis aries* and *Bubalus bubalis* demonstrates the similarity of chromosomal sets and helps detect the differences, related to the formation of metacentric chromosomes in sheep. Species-specific morphological differences in sex chromosomes of the investigated species were found in terms of the length and presence of pericentric inversions. The analysis of phenetic relations between the three species of Bovidae based on the variability of the number of active NOR's demonstrated their degree of genetic similarity.

Adherence to ethical principles. All the procedures performed in the studies involving animal participants were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg,1986.
Conflict of interests. The authors declare the absence of any conflicts of interests.
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Порівняльний аналіз каріотипів трьох порід *Bovidae* spp. – *Bos taurus*, *Ovis aries* та *Bubalus bubalis bubalis* spp. – точка зору

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Мета. Метою роботи є аналіз хромосомних наборів окремих видів сільськогосподарських тварин родини *Bovidae* з використанням технік диференційного фарбування хромосом G- і Ag-banding і демонстрація ролі їх мінливості в еволюції. **Методи.** Підготовку культури лімфоцитів і приготування препаратів хромосом проводили за методом Moorhead et al (1960). Препарати метафазних хромосом аналізували із використанням методик G-banding за Sajjad N et al (2014) та Ag-banding за Seabright (1971). Класифікацію хромосомних аберацій проводили відповідно рекомендацій International System for Chromosome Nomenclature of Domestic Bovids

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Ключові слова: еволюція каріотипів, *Bovidae, Bos taurus, Ovis aries, Bubalus bubalis bubalis, диференційне фарбування хромосом, ядерцеві організатори.*

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