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Chromosomes of European Bison, Domestic Cattle and their Hybrids

[With 1 Table & Plates VI—VII]

A description is given of somatic chromosomes in *Bison bonasus*, *Bos taurus* and hybrids between these two species (1/4 wisent, 3/4 cattle). The modal number of chromosomes in all the animals examined is the same — $2N = 60$. There are 58 acrocentric autosomes, and X chromosome is a large submetacentric element. The Y chromosome in the European bison is a small submetacentric element. A relatively high percentage of peridiploid metaphase plates was found in B_1 hybrids. The role of morphological studies of chromosomes is discussed as a means of solving the problem of sterility of male hybrids.

I. INTRODUCTION

The results of karyological studies on domestic cattle (*Bos taurus*) have been presented in numerous papers, establishing that this species possesses 60 chromosomes, *i.e.* 58 acrocentric autosomes and 2 two-armed sex chromosomes, *X* — a large submetacentric, Y — a small metacentric (cf. Wurster & Benirschke, 1968).

Basrur & Moon (1967) and Bhambhani & Kuspira (1969) described the chromosomes of *Bison bison* and established that there are also 60 chromosomes in this species, among which 58 autosomes and *X* chromosomes are identical with the chromosomes of domestic cattle, while *Y* chromosome is an acrocentric of small dimensions.

The chromosomes of the European bison (*Bison bonasus)* were investigated by Melander (1959) and Koulischer *et al.* (1967). It was found that in this species *Y* chromosome is a small submetacentric element, while the remaining chromosomes, together with *X* chromosome, are identical with those described in cattle and the American bison.

The chromosomes of hybrids of American bison and domestic cattle were described by Basrur & Moon (1967). A description is given in the present paper of the chromosomes of the European bison (or wisent), domestic cattle and their hybrids. These hybrids were obtained in the Mammals Research Institute of the Polish Academy of Sciences at Białowieża by crossing female F_1 hybrids (*Bison bonasus* × *Bos taurus*) with a bull of the Frisian cattle breed (Krasińska, 1967).

Females of the F_1 generation, and also backcrosses are fertile, whereas males in generation F_1 and also in the first generation of backcrosses (B_1) are completely sterile (Krasińska, 1967). No germinal epithelium was found in the testes of these males, or there was degeneration of the germinal cells in different stages of spermatogenesis. In the latter cases reductional division was never reached (Fedyk & Krasińska, 1971).

II. MATERIAL AND METHODS

Material for the present study consists of two individuals of *Bison bonasus*; a male of the Białowieża line, and a female of the Caucasian-Białowieża line (names and numbers in accordance with the Pedigree Book: ♂ Porter no. 1864, ♀ Pusłonka no. 941), one female *Bos taurus* and one female and one male of the backcrosses (B_1) — *i.e.* 1/4 wisent, 3/4 domestic cattle.

Blood for culture of lymphocytes was taken from the jugular vein of live individuals, sedimentation of erytrocytes being made either spontaneously or by centrifuging at 1500 rpm for 10 minutes. Lymphocyte culture was set up on TC 199 medium with addition of *l*-glutamine and Phytohemagglutinin M. Culture ended after 72 hours incubation (37° C), Colcemid Ciba 0.001 mg/ml of culture being added 2—3 hours before the end. Preparations were made by the routine technique after Mellman (1965), and stained with Giemsa solution and lacto-aceto-orceine.

III. RESULTS AND DISCUSSION

The karyotype of the cow is given in Fig. 1 [1]). It is identical with the karyotypes described earlier for different breeds of domestic cattle: 58 acrocentric autosomes and two submetacentric *X* chromosomes (Gimenez-Martin & Lopez-Saez, 1966; Koulischer *et al.*, 1967; Wurster & Benirschke, 1968; Gustavsson, 1969).

The karyotype of the female *Bison bonasus* and sex complement of a male are shown in Fig. 2. There are 60 chromosomes present in the wisent karyotype, among which 58 are acrocentric autosomes with gradually decreasing dimensions, sex chromosomes: *X* — a large-size submetacentric, analogical to *X* chromosome in *Bos taurus*, *Y* chromosome is a small-size submetacentric. This description of the wisent karyotype is in agreement with the description given earlier on (Melander, 1959; Koulischer *et al.*, 1967). The wisent karyotype differs from the karyotype of domestic cattle and American bison only in respect of the morphology of *Y* chromosome: in the wisent it is submetacentric, in the

[1]) Figs 1—3 see Plates VI—VII.

American bison acrocentric and in domestic cattle metacentric (cf. Basrur & Moon, 1967; Wurster & Benirschke, 1968).

Karyotype of male and female backcrosses of the wisent and cattle are shown in Fig. 3. The lack of structural differences between the autosomes of parental forms make it impossible to identify parental genomes in the karyotype of B_1 hybrids. In the latter all autosomes are acrocentric, identical with the autosomes of domestic cattle, wisent and F_1 hybrids. Sex chromosomes in the male's karyotype consist of the submetacentric *X* chromosome and the small metacentric *Y* chromosome.

In the karyotype of the female B_1 hybrid (Fig. 3) there are two *X* chromosomes differing slightly in dimensions. It would, however, appear that this does not form sufficient grounds for finding structural differences in *X* chromosome between the species *Bos taurus* and *Bison bonasus*. In the karyotype of the female *Bos taurus* (Fig. 1) the two *X* chromosomes also differ in the degree of spiralization of the chromatin. In addition the two *X* chromosomes of the female B_1 hybrid may originate either both from the genome of domestic cattle or from one *X* chromosome each of the genome of domestic cattle and European bison.

Tabela 1

Distribution of chromosome number (2 N) of B_1 hybrids of the European bison and domestic cattle.

Sex	<	56	57	58	59	60	61	62	63	64	Total	Peridiploid, %
♀	7	1	—	3	2	13	2	1	—	—	29	31.0
♂	5	2	1	2	4	17	1	—	2	1	35	37.1
Total	12	3	1	5	6	30	3	1	2	1	64	

The results of analysis of the metaphase plates of B_1 hybrids are given in Table 1. In both cases the modal value $2N = 60$. A relatively large number of peridiploid metaphase plates were, however, found (in female 31.0% and in the male as many as 37.1%), with a relatively wide range of variation: $2N = 60 \pm 4$. Zartman & Fechheimer (1967) in studying aneuploidy in different lines of cattle established on an average 16.6% of peridiploid cells, the range of variation being far narrower $2N = 60 \pm 2$.

From 2 — 5% of tetraploid metaphase plates were found in the individuals of *Bison bonasus* and *Bos taurus* examined, whereas in the two hybrids examined no polyploid metaphase plates were found at all. This would appear singular, since Popescu (1969) found from 4.4—10.8%

(average 6.6%) of polyploid cells in hybrids of *Bos grunniens* × *Bos taurus*, while Basrur & Moon (1967) recorded from 10.2 — 15.9% polyploid plates [1]) in backcrosses of *Bos taurus* × *Bison bison*. Zartman & Fechheimer (1967) also observed from 5.2 — 10.8% (average 7.2%) of polyploid cells in different lines of cattle.

The karyotypes of B_1 hybrids between *Bison bonasus* and *Bos taurus* described above can be compared with the karyotypes of cattalo (hybrids of *Bos taurus* × *Bison bison*). The differences between karyotypes of the initial species of both these hybrids are similar and concern only the morphology of Y chromosomes. In *Bos taurus* Y chromosome is a small metacentric, in *Bison bison* a small acrocentric (Basrur & Moon, 1967), while in *Bison bonasus* Y chromosome is a small submetacentric (Fig. 2).

Basrur & Moon (1967) found from 23.9 — 31.2% [1]) peridiploid metaphase plates in hybrids with different percentages of American bison and domestic cattle blood. These are values of the same order as those observed in backcrosses of *Bison bonasus* × *Bos taurus*, in addition the range of variation is equally wide: $2N = 60 \pm 4$.

Great hopes were placed in studies on chromosomes as a means of solving the problem of the sterility of males in F_1 and B_1 generations of hybrids. Male F_1 hybrids and males of the first generations of backcrosses originating from crossing *Bison bison* × *Bos taurus* and from crossing *Bison bonasus* × *Bos taurus* are completely sterile. Completely empty seminiferous tubules, or inhibition of spermatogenesis in the early stages, were observed in them (Basrur, 1969; Fedyk & Krasińska, 1971). Whereas in both cases we encounter chromosome sterility (Dobzhansky, 1964), the causes of sterility of males only in generation F_1 must be considered as lying in the structural differences between the Y chromosomes of the initial species. Comparison of the morphology of Y chromosomes in certain species of *Bovinae* possessing $2N = 60$, does not however confirm this assumption. We shall illustrate this by the following example: males F_1 originating from crossing *Bison bison* × *Bison bonasus* and *Bos taurus* × *Bos indicus* are completely fertile, while males F_1 originating from crossings in combinations of *Bison bonasus* × *Bos taurus* and *Bison bison* × *Bos taurus, Bison bison* × *Bos indicus* are sterile (Gray, 1954). While Y chromosome in *Bison bison* and *Bos indicus* is acrocentric, in *Bison bonasus* it is submetacentric and in *Bos taurus* metacentric (Wurster & Benirschke, 1968). Thus males F_1 from crossings of species with morphologically different Y chromo-

[1]) Percentages calculated in accordance with Table 2 in Basrur & Moon (1967).

somes (*Bos taurus* × *Bos indicus* and *Bison bonasus* × *Bison bison*) are fertile, and on the other hand males F_1 from crossings of *Bison bison* × *Bos indicus* are sterile, despite the fact that both these species have morphologically identical, small acrocentric *Y* chromosomes (Basrur & Moon, 1967; Kieffer & Cartwright, 1968). Therefore structural differences between *Y* chromosomes of related species, forming proof of the existence of chromosomes aberration in the phylogenesis of the given group, are insufficient to explain the causes of sterility in male F_1 hybrids between these species. Undoubtedly studies on meiotic chromosomes can go further towards finding an explanation of the problem of sterility in F_1 males than morphological studies on the mitotic chromosomes of these animals.

Gartler & Burt (1964) found that *Y* chromosome in *Bos taurus* replicates at least 2 hour than the other chromosomes. The *Y* chromosome in *Bos indicus* also replicates late (Kieffer & Cartwright, 1968). It is possible that there are differences in the replication time of *DNA* of *Y* chromosome between some species, with may contribute to the sterility of F_1 males. On the other hand the heterochromatinization of one of the two *X* chromosomes in females may be the causes of the fertility of the homogametic sex in the first generation of hybrids.

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REFERENCES

1. Basrur P. K., 1969: Hybrid sterility. [In: »Comparative Mammalian Cytogenetics«. K. Benirschke, Ed.] Springer Verlag: 107—131. N.Y.
2. Basrur P. K. & Moon Y. S., 1967: Chromosomes of cattle, bison and their hybrid, the cattalo. Amer. J. veter. Res., *28*: 1319—1329.
3. Bhambhani R. & Kuspira J., 1969: The somatic karyotypes of American bison and domestic cattle. Canad. J. Genet. Cytol., *11*: 243—249.
4. Dobzhansky Th., 1964: Genetics and the origin of species. Columbia Paperback: 1—364. New York & London.
5. Fedyk S. & Krasińska M., 1971: Studies on the spermatogenesis of European bison and domestic cattle hybrids. Acta theriol., *16*, 29: 449—464.
6. Gartler S. M. & Burt B., 1964: Replication patterns of bovine sex chromosomes in cell culture. Cytogenetics, *3*: 135—142.
7. Gimenez-Martin G. & Lopez-Saez J. F., 1966: Cromosomas de mamiferos domesticos. Genet. iber., *18*: 13—139.
8. Gray A. P., 1954: Mammalian hybrids. Farnham Royal: 1—144. Bucks.
9. Gustavsson I., 1969: Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. Hereditas, *63*: 68—169.
10. Kieffer N. M. & Cartwright T. C., 1968: Sex chromosome polymorphism in domestic cattle. J. Hered., *59*: 35—36.

11. Koulischer L., Tijskens T. & Mortelmans J., 1967: Mammalian cytogenetics. I. The chromosomes of three species of *Bovidae: Bos taurus, Bison bonasus, Cephalophus grimmi.* Acta Zool. Pathol. Antverp., *43*: 135—141.
12. Krasińska M., 1967: Crosses of wisent and domestic cattle. Acta theriol., *12*: 67—79.
13. Melander Y., 1959: The mitotic chromosomes of some cavicorn mammals (*Bos taurus* L., *Bison bonasus* L. and *Ovis aries* L.). Hereditas, *45*: 649—664.
14. Mellman W. J., 1965: Human peripheral blood leucocyte cultures. [In: »Human chromosome methodology«. J. J. Yunis, Ed.] Academic Press: 21—49. New York & London.
15. Popescu P., 1969: Observations sur certaines cellules polyploidiques en cultures de leucocytes chez les hybrides interspecifiques *Bos grunniens* × *Bos taurus.* [In: »Cercetari de Genetica«. P. Raicu, Ed.] Edit. Didact. Pedagog.: 170—174.
16. Wurster D. H. & Benirschke K., 1968: Chromosome studies in the superfamily *Bovidea.* Chromosoma, *25*: 152—171.
17. Zartman D. L. & Fechheimer N. S., 1967: Somatic aneuploidy and polyploidy in inbred and linecross cattle. J. Anim. Sci., *26*: 678—682.

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CHROMOSOMY ŻUBRA, BYDŁA I ICH MIESZAŃCÓW

Streszczenie

Opisano somatyczne chromosomy *Bos taurus* (Fig. 1), *Bison bonasus* (Fig. 2) i hybrydów B_1 pomiędzy bydłem a żubrem (Fig. 3). U wszystkich tych zwierząt stwierdzono modalną liczbę chromosomów $2N = 60$, u hybrydów zaś stosunkowo wysoki procent peridiploidalnych płytek metafazalnych (Tabela 1). W dyskusji zwrócono uwagę na niewystarczalność morfologicznych badań chromosomów gatunków wyjściowych i hybrydów w wyjaśnieniu problemu sterylności samców mieszańców pokolenia F_1.

EXPLANATION OF PLATES

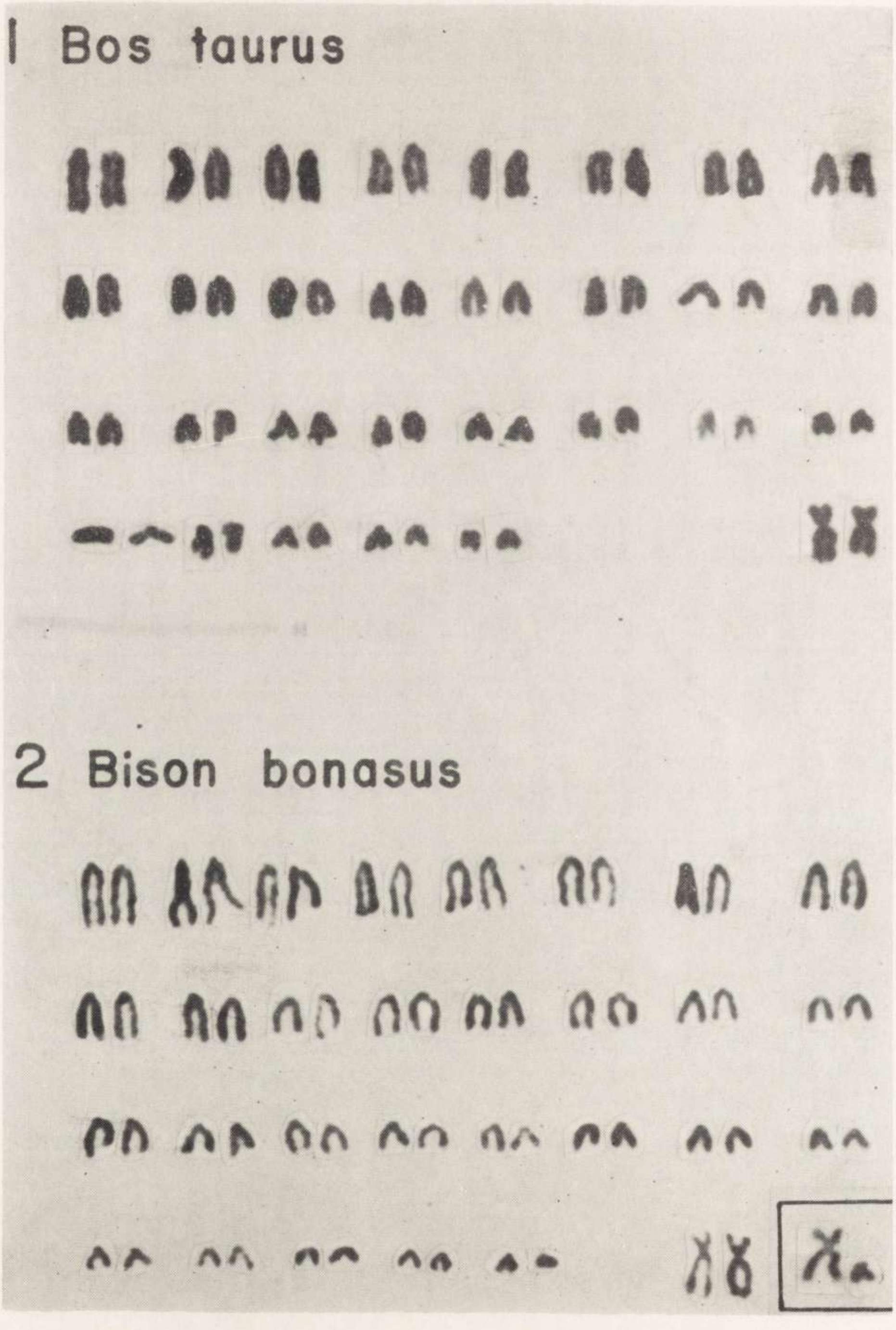
Plate VI

Fig. 1. Female karyotype of a domestic cattle (*Bos taurus*).

Fig. 2. Female karyotype of a wisent (*Bison bonasus*), and sex complement of a male.

Plate VII

Fig. 3. Male and female karyotypes of B_1 hybrids ($^1/_4$ wisent, $^3/_4$ domestic cattle).



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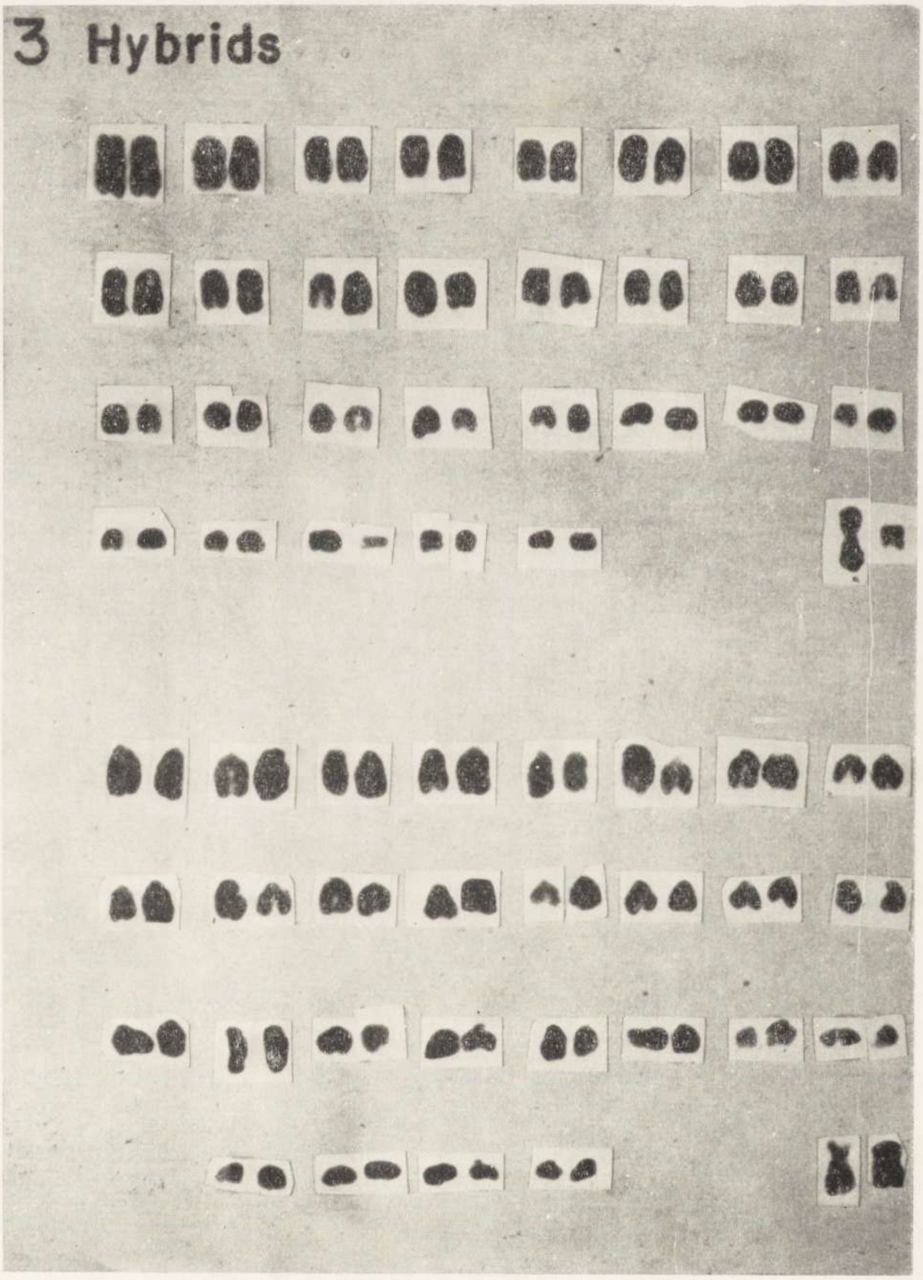

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