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CHARACTERIZATION OF THE NUCLEOLUS ORGANIZING REGION IN ZEBU (*Bos taurus indicus*) AND IN ANIMALS WITH 1/29 CENTRIC FUSION

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ABSTRACT

A study of 40 metaphase spreads obtained from 6 Zebu sires (Nelore), with different number of slides from each, showed that the nucleolus organizing region (NOR) occurs in chromosomes 2, 3, 4, 5 and 28. The mean number of silver-stained NOR was 4.96 ± 0.31. The chromosomes were identified metrically. Twenty-five metaphase spreads obtained from animals heterozygous for 1/29 centric fusion were scored and the same chromosome pairs were identified with nucleolar organizers (3.88 ± 0.25 NOR-bearing chromosomes per metaphase). The translocation chromosome was never silver stained in any of the preparations. In both studies, other elements which are really not NOR-bearing chromosomes (presumptively classified as chromosome 1, 6, 9, 23 and 27) were stained by silver at a lower frequency than the first five pairs listed. The maximum number of silver-NOR stained chromosomes in cattle genome was ten.

Telomere-telomere association mainly involved the silver-stained chromosomes, more intensely so for pairs 2, 3, 4 and 28. The data confirm the results of some investigators but disagree with those of others, who detected other NOR-bearing chromosomes in cattle. The evolutionary significance of these results is discussed.

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INTRODUCTION

The nucleolar organizing region (NOR) corresponds to the secondary constriction or stalk of the satellite encountered in some chromosomes and is the site of production of 18S and 28S ribosomal RNA (Matsui and Sasaki, 1973; Goodpasture and Bloom, 1975; Miller *et al.*, 1978). The NOR is only stained with silver when the ribosomal cistrons were active during the preceding interphase (Miller *et al.*, 1976, 1978; Groce *et al.*, 1977).

NOR involvement in the origin of centric fusions (Ohno *et al.*, 1961; Capoa de *et al.*, 1973; Miller *et al.*, 1978) and of telomere-telomere (T-T) associations (Henderson and Bruère, 1977) has been the subject of studies on several domestic animals species. Diamond *et al.* (1975) were the first to describe a deeply stained region in telomeres of 4 to 6 of the largest chromosomes in cattle. The nature of the stained region was acidic protein, as later confirmed by Schwarzacher *et al.* (1978). These characteristics of NOR were reported for several *Bovidae* species (Henderson and Bruère, 1979; DiBerardino and Arrighi, 1979), whereas different NOR locations were reported for other mammalian species (Goodpasture and Bloom, 1975).

The chromosome pairs involved in nucleolus organization in cattle were identified to be 2, 3, 4, 5 and 28, by Henderson and Bruère (1979) and 2, 3, 4, 11 and 28, by DiBerardino and Arrighi (1979), Mayr and Czaker (1981) and DiBerardino *et al.* (1981), emphasizing that DiBerardino and Arrighi (1979) classified the shortest stained pair as 29, and subsequently corrected the classification to pair number 28 (DiBerardino *et al.*, 1981). This discrepancy and the fact that the NOR of *Bos taurus indicus* (Zebu) has not yet been described motivated the present research. NOR characterization in animals with 1/29 Robertsonian translocation was also done with the objective of better identifing Ag-positive chromosomes and the possible role of the fused chromosome in nucleolus organization.

MATERIALS AND METHODS

Six karyotypically normal Nelore animals (*Bos taurus indicus*) and 5 Marchigiana and Pitangueiras animals, which were carriers of 1/29 translocation were studied (Pinheiro *et al.*, 1979, 1981).

Chromosome preparations were made from lymphocyte cultures and subjected to silver staining by the method of Bloom and Goodpasture (1976). Forty complete metaphases with well spread and extended morphology from the animals in the first group, and 25 from the animals in the second were analyzed.

The preparations were photographed and the negative was cut and mounted as slides. The slides were projected onto thin copy paper from a fixed distance and the contours of the chromosomes were drawn with a pencil. The longitudinal axis of each chromatid was measured with the aid of a cutting wheel, and the points left by the wheel were used to calculate the mean length of each chromosome. The mean numbers of chromosome 1 with the long chromosome arm translocated were compared with the mean numbers of chromosome 29 with the short 1/29 chromosome arm fused by the Student t test. All T-T associations were also calculated and the chromosomes involved were classified.

RESULTS

Table I shows the frequency of silver staining for each chromosome regardless of breed and according to mean length. The chromosomes that were frequently stained for NOR were pairs 2, 3, 5 and 28, with no. 2 having the highest staining frequency per metaphase when compared to the others. The stained chromosomes 1, 6, 7, 8, 9, 10, 22, 23, 25, 27 and 29 which are apparently not NOR-bearing chromosomes, were not all present in the same animal, nor were stained in a constant way, which may probably be an error of technique or an artifact. Chromosome 1/29 was never stained in any of the 25 preparations, and the same occurred for the sex pair.

When the Zebu animals and the animals with the translocation (*Bos taurus taurus*) were considered separately, no appreciable difference in NOR-bearing chromosomes was noted, except for presumptive number 1, which was stained 10 times in the first group vs. 2 times in the second (Table I). On the average, 4.96 ± 0.31 chromosomes per metaphase were stained in the Zebu group, and 3.88 ± 0.25 in the translocation carriers. The maximum number of silver-stained chromosomes per cell was 10.

Figure 1 shows the karyotype that best represents the NOR bands for each subspecies. Figure 2 shows the T-T associations between NOR-bearing chromosomes for the animals heterozygous for 1/29 centric fusion. The chromosome pairs involved were: 2 (twice); 3 (three times); 4 (four times); 5, 23 and 25 (once each) and 28 (twice). Chromosome 1/29 was not identified in any type of association.

Table I - Listing of NOR-bearing chromosomes classified by relative size.

Chromosomes	Frequency of silver staining (No. of times)		Total	No. of times when the chromosome pair was NOR-bearing
	Nelore animals	Animals with translocation		
1	10	2	12	–
2	28	20	48	22
3	21	17	34	5
4	25	14	39	3
5	13	11	24	5
6	5	3	8	1
7	4	3	7	1
8	5	1	6	–
9	7	1	8	–
10	6	–	6	–
11	4	–	4	–
12	4	1	5	1
13	2	–	2	–
14	3	–	3	–
15	2	–	2	–
16	2	–	2	–
17	3	–	3	–
18	2	–	2	–
19	2	1	3	–
20	2	–	2	–
21	3	–	3	–
22	6	–	6	–
23	6	1	7	1
24	3	1	4	1
25	2	4	6	1
26	2	3	5	–
27	5	2	7	–
28	18	9	27	2
29	4	3	7	–
X	–	–	–	–
Y	–	–	–	–
T	–	–	–	–

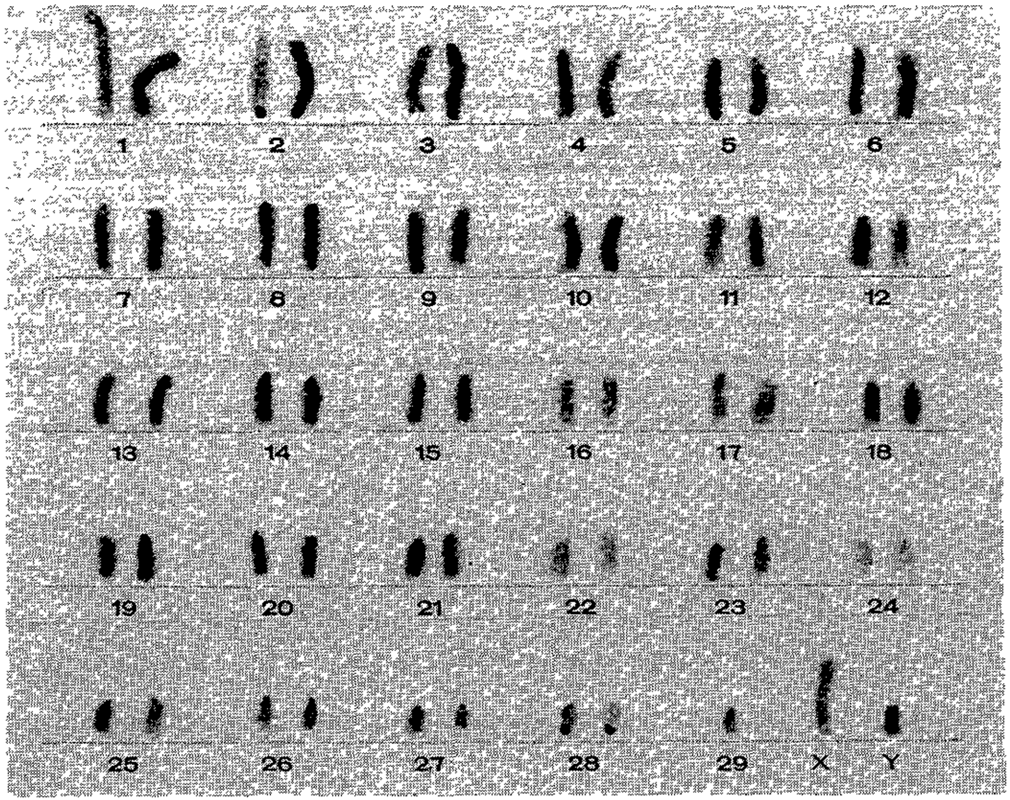

Figure 1 - NOR-banding pattern obtained for animals that were carriers of 1/29 centric fusion.

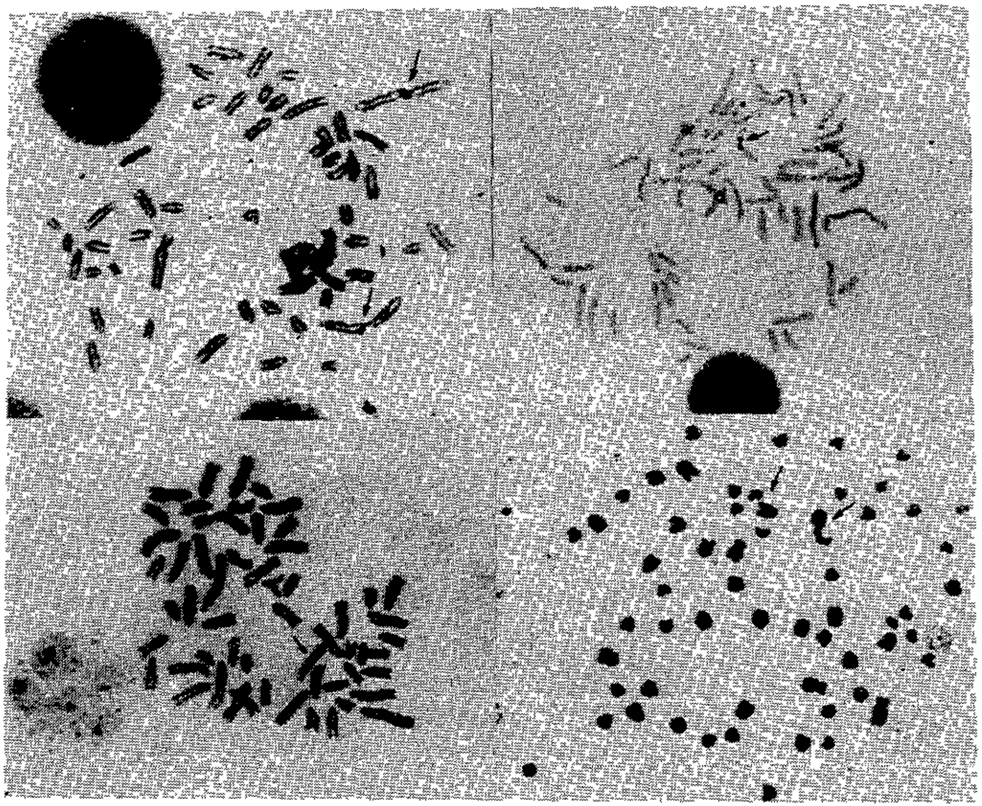

Figure 2 - Telomere-telomere association involving NOR-bearing chromosomes (indicated by arrows).

DISCUSSION

Although the technique used to classify chromosomes is subject to error, mainly due to variation in chromosome extension and size and to chromosome position on the metaphase plate, we may conclude that in the subspecies *Bos taurus indicus* the NOR-bearing chromosome pairs are 2, 3, 4, 5 and 28. These data agree with those obtained by Henderson and Bruère (1979), but differ from those obtained by DiBerardino and Arrighi (1979) in that we found no evidence for the participation of chromosomes 11 and 29. With respect to the latter, a small NOR-bearing pair (28) side by side with chromosomes 29 and 1/29 can be seen in Figure 1, a fact that removes the possibility of chromosome 29 being the nucleolus organizer. DiBerardino *et al.* (1981) corrected the previous findings (DiBerardino and Arrighi, 1979), classifying, with the help of R-bands, the shortest stained chromosome pair as 28. As to chromosome number 11, we could postulate chromosome heteromorphism or a difference in chromosome size that may lead to erroneous classification. Concerning other stained chromosomes found in this study, besides the mentioned possibilities of classification error, one could think in terms of artifacts or of structures similar to those found by Mair and Czaker (1981).

The telomeric associations involving nucleolus organizer chromosomes have been studied by Henderson and Bruère (1977) in sheep. These investigators demonstrated that there are individual peculiarities in terms of number of nucleolus organizer chromosomes, but that T-T associations are not preferential. In the present study, despite the small number of metaphases analyzed, the association between NOR-positive chromosomes seems to occur at random. Some researchers have associated this complex with the origin of many chromosome rearrangements, including centric fusion (Ohno *et al.*, 1961; Capoa de *et al.*, 1973; Miller *et al.*, 1978; Brasch and Smith, 1979; Mattei *et al.*, 1980). As to the family *Bovidae*, no chromosome aberrations that might have arisen from breaks in the distal q region of NOR-positive chromosomes have been reported (Henderson and Bruère, 1979) and the consequences of the T-T associations encountered in these animals remain unknown.

Speciation in Bovidae seems to occur mainly by centric fusion, with a large fraction of chromosome structures remaining unchanged. Thus, marked conservatism not only in terms of fundamental number of chromosome arms, but also in terms of G- and NOR-banding patterns persists side by side with different karyotypes (Wurster and Benirschke, 1968; Evans *et al.*, 1973; Schnedl and Çzaker, 1974; Buckland and Evans, 1978; Henderson and Bruère, 1979). From this point of view, considering mainly NOR-banding patterns, the frequency of other types of chromosome rearrangements, such as inversion or tandem fusion, can be considered low in bovines, a fact further supported by the small number of abnormalities of these types described thus far. On the other hand, centric fusions alone could not produce the dimorphism existing between species in the family Bovidae, including the reproductive barriers. This clearly shows the presence of specific alterations at the gene level, perhaps caused by chromosome rearrangements that normally go undetected. More recently, DiBerardino and Iannuzzi (1981), in a study of NOR-banding patterns in buffaloes, observed that, in addition to the lack of conservatism noted when these patterns were compared to those of bovines, there was a loss of a NOR-bearing chromosome when the tandem fusion that reduced the chromosome constitution of swamp buffaloes from 50 to 48 chromosomes occurred. Further studies are needed to clarify the questions raised by the present study with respect to the conservatism encountered by Henderson and Bruère (1979).

Finally, the possibility of using NOR-banding patterns in their quantitative aspects as a genetic marker for possible correlation with productivity and/or fertility is quite stimulating. If such a correlation does exist, young animals could be selected with more certainty.

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RESUMO

O estudo de 40 metáfases obtidas de 6 reprodutores zebuínos (Nelore) mostrou que a região organizadora do nucléolo (NOR) se apresenta, com maior frequência, nos cromossomos 2, 3, 4, 5 e 28. A média de cromossomos marcados por metáfases foi de 4,96 ± 0,31. Os cromossomos foram identificados metricamente. Com o emprego desta mesma técnica fòram analisadas 25 metáfases obtidas de animais heterozigotos para a fusão cêntrica *1/29*, tendo sido *identificados os mesmos pares cromossômicos como* organizadores nucleolares (Média de 3,88 ± 0,25 cromossòmos NOR positivos por

metáfase). Em nenhuma das metáfases o cromossomo translocado se apresentou marcado. Em ambos os estudos, outros elementos, que na realidade não são cromossomos portadores de NOR, se apresentaram corados pela prata, (pares 1, 6, 9, 23 e 27), numa freqüência menor do que a encontrada para os cinco primeiros pares descritos.

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