

# SOME CASES OF NOR INSTABILITY IN HORSE CHROMOSOMES

## ALGUNOS CASOS DE INESTABILIDAD DE LAS NOR EN CROMOSOMAS DE CABALLO

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### Additional keywords

Chromosome rearrangements. Chromosomal instability.

### Palabras clave adicionales

Reorganización cromosómica. Inestabilidad cromosómica.

### SUMMARY

By using cytogenetic and FISH techniques, we detect and analyse 1) variation in the number and size of NOR, 2) high chromosomal instability within NORs and 3) chromosome rearrangements involving NORs in domestic horse (*Equus caballus*).

### RESUMEN

Mediante técnicas citogenéticas y de hibridación *in situ* fluorescente (FISH), se detecta y analiza la variación en el número y tamaño de NOR, la elevada inestabilidad cromosómica de las NOR y la existencia de reorganizaciones cromosómicas que implican a las NOR en el caballo doméstico (*Equus caballus*).

### INTRODUCTION

The nucleolar organizer region (NOR) is one of the most variable region of the eucaryotic genome. Although the number and location of NORs in normal karyotype is as a rule species-specific

and constant, in many species NORs are very polymorphic. The phenomena such as deletion, duplication-amplification, activation-inactivation, transposition, chromatid breaks and high mutagen sensitivity are attributed to NORs (e. g. Schubert and Rieger, 1980; Nicoloff *et al.*, 1982; Mamaev and Mamaeva, 1990 and 1992; Garrido-Ramos *et al.*, 1995). Here, using cytogenetic and FISH techniques we detect and analyze 1) variation in the number and size of NORs, 2) high chromosomal instability within NORs, and 3) chromosome rearrangements involved NORs in domestic horse (*Equus caballus*).

### MATERIAL AND METHODS

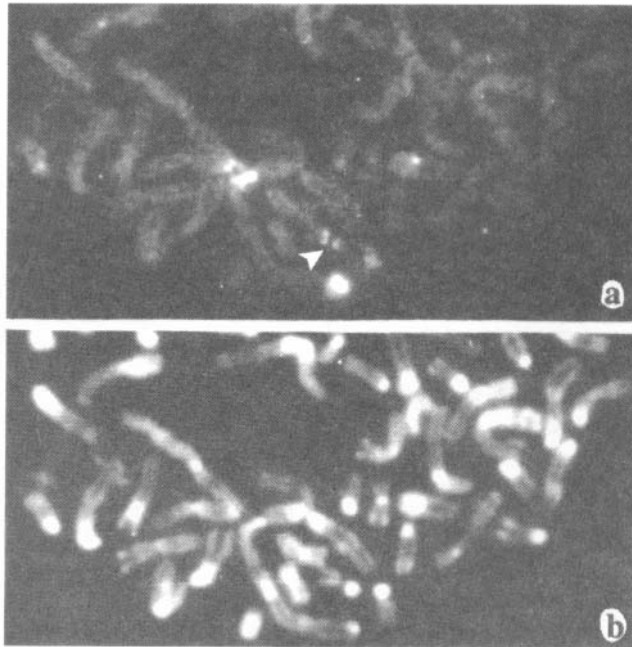
The study was carried out on chromosome spreads of 26 horses of the following breeds Arabian -6, Thoroughbred -5, Polish Primitive horse -6, Hucul -8

and Cold-blood (Lovicko-sokhatsevski horse) -1. Chromosome spreads were prepared from blood lymphocytes cultured in medium RPMI-1640 with addition of autologous serum and pokeweed mitogen by standard method. To obtain R-banding BrdU (50mg/ml) was introduced into the culture 7h prior to harvesting FISH with biotinylated rDNA probe was performed by standard procedure (Lichter and Cremer, 1992). Plasmid containing a 7.3 kb fragment of human rRNA genes (18S-5.8S-28S) was genially provided by Dr. B V Skryabin. Hybridization signals were visualized using fluorescein-conjugated avidin

DCS and the amplification of signal was done using biotinylated antiavidin D antibodies (Vector Laboratories). Direct R-banding of BrdU-substituted chromosomes was obtained for the simultaneous visualization of fluorescent signals and well-identified banded chromosomes.

## RESULTS AND DISCUSSION

At least 30-50 cells per individual were analyzed after FISH with rDNA probe for evaluating the polymorphism of rRNA gene location on horse chromosomes The fluorescent signals



**Figure 1.** a) FISH of rDNA probe on horse chromosomes. Arrow, NOR-break in chromosome 31. b) R-banding on the same metaphase performed for chromosome identification. (a) FISH de una sonda sobre cromosomas de caballo. La flecha indica una ruptura en la NOR del cromosoma 31. b) Bandedo R de la misma metafase realizada para la identificación de los cromosomas).

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**Table 1.** *Distribution of chromatid aberration involving NORs (p. cent).* (Distribución de las aberraciones cromátidas que implican a las NOR, p. cien).

Case number and sex	Breed	Frequency of the cells with NOR breaking chromosomes	Frequency of chromatid breaks in NOR of the NOR bearing chromosomes			
			1	27	28	31
1m	Polish Primitive horse	5.0	0.0	0.0	0.0	2.5
2m		56.5	6.5	0.0	0.0	34.8
3m	Hucul	17.2	5.2	0.0	0.0	6.9
4m		4.5	2.3	0.0	0.0	0.0
5f		4.0	4.0	0.0	0.0	0.0
6f		25.9	0.0	0.0	14.8	5.6
7f		4.0	0.0	0.0	0.0	2.0
8f	Arabian	8.6	0.0	0.0	2.9	1.4
9f		10.0	0.0	0.0	0.0	5.0
10f*		15.7	0.0	0.0	0.0	7.8
10f*		12.5	0.0	0.0	0.0	6.2
11m	Cold-blood	0.0	0.0	0.0	0.0	0.0

\*two parallel cultures of lymphocytes of one individual  
m = male; f = female

were most frequently observed on three chromosome pairs 1, 28 and 31. However cells of one Arabian horse and one Hucul horse had only 5 fluorescent signals. One of the chromosomes 28 did not have rDNA hybridization sites. Three Polish Primitive horses, two Hucul horses, two Thoroughbred horses and Cold-blood horse had additional fluorescent signals near the centromeric region of the one or both homologues of chromosome 27. So there are animals with 5, 6, 7 and 8 NOR-bearing chromosomes. The similar phenomena of intraspecies variation in location of rRNA genes have been described in mouse (Suzuki *et al.*, 1992), fish (Garrido-Ramos *et al.*, 1995)

amphibian species (Schmid *et al.*, 1995) and plants (Garrido *et al.*, 1994; Fukui *et al.*, 1994). Apparently such polymorphism is a general property of NORs.

The distribution and intensity of fluorescent signals were individual-specific and constant. However 15.6 p. cent cells of one Thoroughbred male had unusual large intensity of fluorescent signal on chromosome 28 which differed from other cells of the same individual and was never found in the analyzed horses. It's hardly likely that this variability is a result of the culture phenomenon. Rather amplification of rRNA genes on chromosome 28 or chromosomal rearrangement involved

NORs occurred *in vivo* and the population of lymphocytes was heterogeneous before culturing.

On metaphase spreads of 10 horses chromosome and chromatid breaks were found inside NORs but not any other regions. A pattern of NOR breaks was individual-specific trait and the frequency of cells with the breaks varied from 4 to 68 p. cent. Also we observed chromatid translocations between NOR-bearing chromosome and heterologous chromosome without NOR. We suggest that these findings can provide further explanations for the interpretation of chromosomal evolution in Equidae.

There are investigation showing that the number of rRNA genes in the NORs of chromosomes of tumour and cell line may be either greatly increased or decreased (Henderson and Megraw-Ripley, 1982; Mamaev and Mamaeva 1990 and 1992) We found the same in the normal individual lymphocytes

An interesting finding which emerged in our study is a presence of chromosome and chromotid breaks observed inside NORs (but not any other regions) on metaphase spreads of 10 horses FIHS with rDNA probe showed the rRNA genes localized on both ends of breakes

(figure 1). Patterns and frequencies of NOR-breakes were individual specific (table I). Chromosome fragility in NORs has not been described in horse yet (Ronne,1992). It seems the lot of medium we used for cultures of lymphocytes from 11 horses had an admixture of chemical which caused the chromatid breaks within NORs or there was deficiency of any substances in the medium affected cell metabolism. It is known that mutagene sensitivity of plant chromosome NORs is higher than expected (e.g. Schubert and Rieger, 1980). NORs are aberration hot spots involved in chromatid translocations (Nicoloff *et al.*, 1982). We didn't analysed homologous chromatid translocations which are usual in NORs (Schubert and Rieger, 1980) but we detected chromatid translocations between NOR-bearing chromosome and heterologous chromosome without NOR. NOR-bearing chromosome in involved in rearrangement was the chromosome in which NOR-breaks may occur. Apparently NOR-breaks with following heterologous chromatid translocation is one of the way of NOR-transposition in karyotype evolution and this process is likely not so rare.

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## SOME CASES OF NOR INSTABILITY IN HORSE CHROMOSOMES

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