

NUCLEOLAR ORGANIZER REGIONS, G-AND C-BANDS IN SOME BRAZILIAN SPECIES OF DIDELPHIDAE

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ABSTRACT

Karyotypes of four didelphid marsupial species (*Marmosa murina*, *M. cinerea*, *Caluromys philander* and *C. lanatus*) showed a similar complement of $2n = 14$, $NF = 20$. The results demonstrate an extensive homeology of the G-banding patterns but there are considerable differences in the constitutive heterochromatic patterns and nucleolar organizer regions (NORs), as shown by C-banding and silver staining techniques.

INTRODUCTION

A few more than 20 didelphid marsupial species have been so far cytogenetically described, based mainly on the studies of standard nondifferentially stained karyotypes. These data show that three distinct types of chromosomal complements characterize marsupials from this family: $2n = 14$ found in *Caluromys*, *Metachirus*, *Marmosa* and *Dromiciops* (Reig *et al.*, 1977; Rofe and Hayman, 1985); $2n = 18$ found in *Monodelphis* (Reig *et al.*, 1977; Merry *et al.*, 1983; Langguth and Lima, 1987) and $2n = 22$, found in *Didelphis*, *Chironectes*, *Lutreolina* and *Philander* (Reig *et al.*, 1977; Yonenaga-Yassuda *et al.*, 1982).

Data concerning longitudinal differentiation of G bands, distribution of constitutive heterochromatin and nucleolar organizer regions in the karyotypes of

didelphids are still scarce (Sinha and Kakati, 1976; Fernandez-Danosó *et al.*, 1979; Yonenaga-Yassuda *et al.*, 1982; Merry *et al.*, 1983; Casartelli *et al.*, 1986). Rofe and Hayman (1985) found a high level of conservation in G-banded chromosomes from cultured fibroblasts of 15 species of marsupials, but the C bands and NORs were variable.

In this report we present the G-, C- and NOR banding patterns of the didelphids *C. philander*, *C. lanatus*, *M. murina* and *M. cinerea* in an attempt to contribute to the elucidation of evolutionary relationships in this family.

MATERIAL AND METHODS

Caluromys philander (7 males and 6 females), *Marmosa cinerea* (6 males and 3 females), and *Marmosa murina* (7 males and 4 females) were collected in the State of Pernambuco, Northeastern Brazil; *Caluromys lanatus* (2 males and 1 female) were from the State of Rondonia, Northern Brazil.

Taxonomic identification of the specimens was made by Dr. A. Langguth, Federal University of Paraíba, João Pessoa, Brazil. The specimens (skins and skulls) are kept in the collection of the Biology Dept., Federal University of Pernambuco, Recife, Brazil.

Chromosomal preparations were made from bone marrow cells and G and C banding patterns were obtained according to Seabright (1971) and Sumner (1972), respectively. NORs staining followed the technique described by Howell and Black (1980).

The relative sizes of the sex chromosomes from five cells of each animal were obtained through measurements made on enlarged micrographs of mitotic metaphases. These values represent a percentage of the haploid set.

RESULTS

A diploid number of 14, $NF = 20$ and an XX/XY sexual determination mechanism was found in the four species, which had an identical autosomal karyotype, consisting of four pairs of biarmed chromosomes and two pairs of acrocentrics. The X chromosome was acrocentric in all four species but differed in size. It represented 6.0% of the haploid set in *C. philander* and *C. lanatus*; 5.0%, in *M. murina* and 8.3%, in *M. cinerea*. The Y chromosome differed both in shape and size,

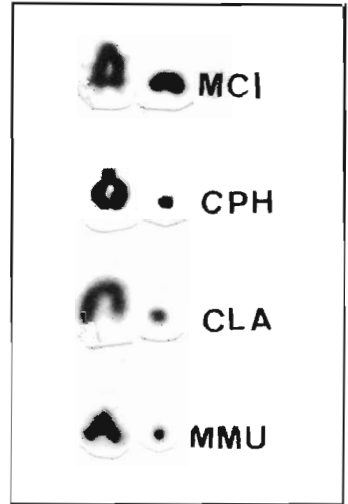


Figure 1 - Sex chromosomes of males of: *Marmosa cinerea* (MCI); *Caluromys philander* (CPH); *Caluromys lanatus* (CLA); *Marmosa murina* (MMU).

since in *C. philander*, *C. lanatus* and *M. murina* it was a minute, typical punctiform chromosome, and in *M. cinerea*, a large acrocentric that represented 4.5% of the haploid chromosomal set (Figure 1).

The autosomes of trypsin G-banded karyotypes of the four species showed a high degree of chromosomal conservation (Figure 2), but striking differences in C bands and NORs were detected. Autosomal pericentromeric C bands were observed in all the species even though there were interspecific differences related to the amount of this heterochromatin. The largest C bands were those found in *M. cinerea*, followed by the ones of *M. murina*. In *C. philander* and *C. lanatus*, the amount of pericentromeric heterochromatin was so small that it was difficult to visualize C-bands in some metaphases (Figure 3).

The heterochromatic pattern of the X chromosomes was represented by large distal and proximal C bands in *M. cinerea* and *C. philander*. *M. murina* showed only the proximal band and *C. lanatus* had this heterochromatic block plus an interstitial band. The Y chromosome of these species was apparently completely heterochromatic. G-bands of the sex chromosomes were not sufficiently clear to allow any comparative analysis (Figures 2 and 3).

Nucleolar organizer regions (NORs) were present in the pericentromeric region of acrocentric pair number 5 in the silver-stained karyotype of the four species. In *M. cinerea* and *M. murina* NORs were also present in the telomeric region of the long arms of the acrocentric pair number 6. *M. murina*, in addition, had a NOR in the telomere of the short arm of submetacentric pair number 3 (Figure 4).

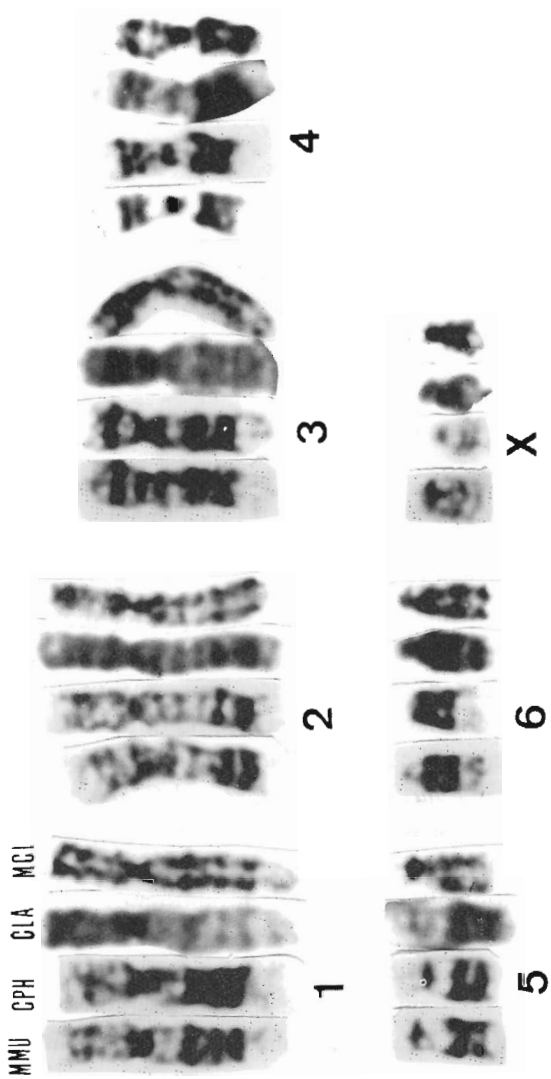


Figure 2 - Comparison of the G-banded chromosomes in a haploid set of *Marmosa murina* (MMU); *Caluromys philander* (CPH); *Caluromys lanatus* (CLA) and *Marmosa cinerea* (MCI).

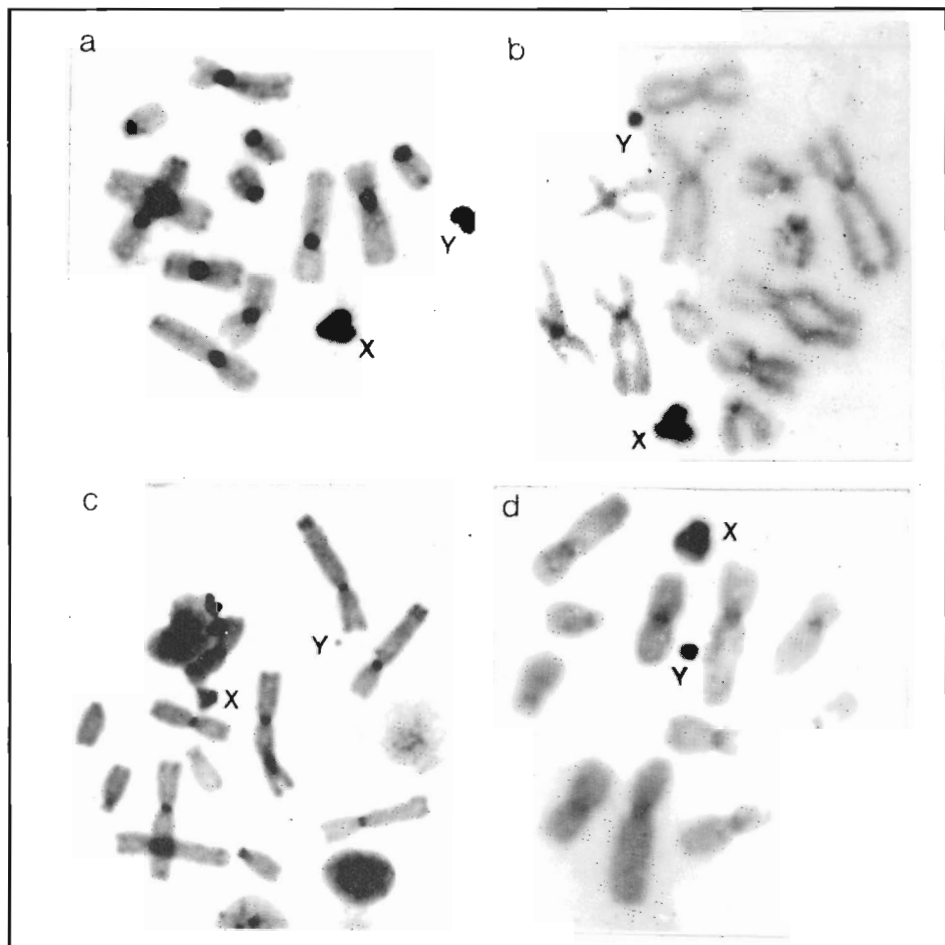


Figure 3 - C-banded karyotypes of males of: a) *Marmosa cinerea*; b) *Caluromys philander*; c) *Marmosa murina*; d) *Caluromys lanatus*.

DISCUSSION

To date, 71 species of marsupials belonging to the family Didelphidae have been identified, 66 being found in South America. Chromosomal analysis made in this family after the conventional Giemsa staining showed a high karyotypical stability, especially within each genus. This was most evident when only the autosomes were considered. A comparison made between conventionally stained karyotypes of the

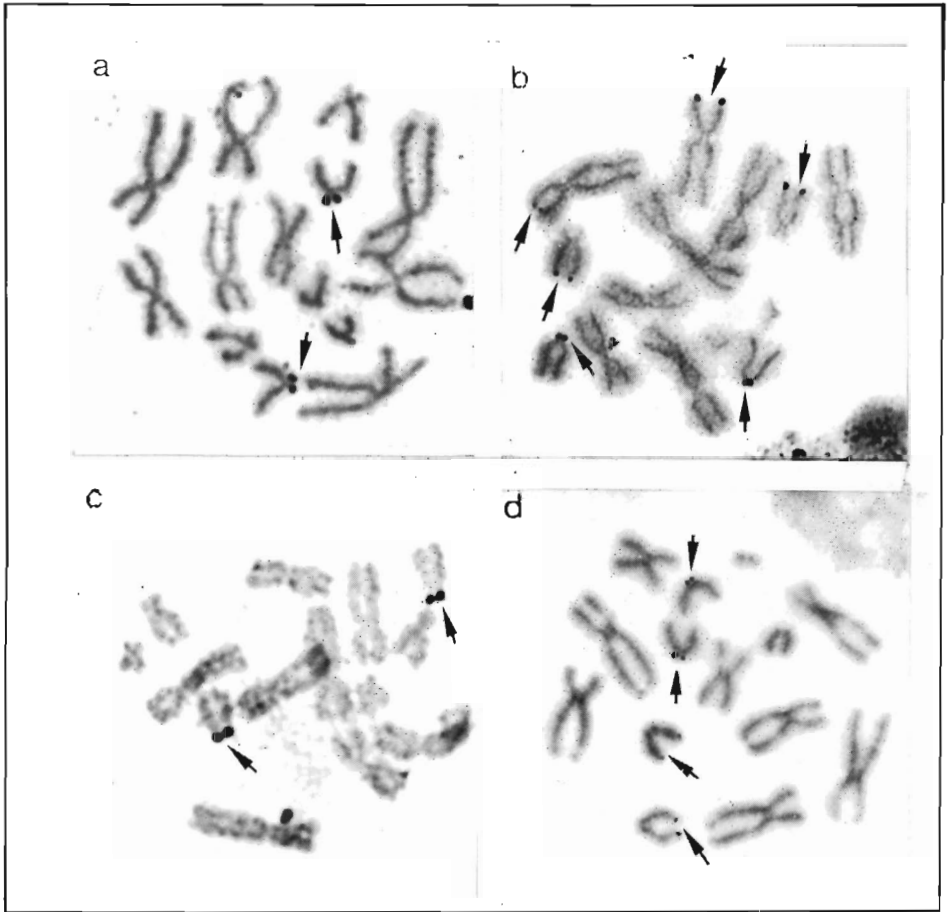


Figure 4 - Ag-NOR stained karyotypes of: a) *Caluromys philander* (female); b) *Marmosa murina* (female); c) *Caluromys lanatus* (female); d) *Marmosa cinerea* (male).

species studied here and those published on the same taxa (Reig et al., 1977) has shown some differences in the sex chromosomes: a) in our sample the X is always acrocentric whereas in Reig's list this chromosome is submetacentric in *C. philander* and metacentric in *M. cinerea* and *M. murina*. This variation may have been caused by rearrangements such as pericentric inversions or by mechanisms of addition or deletion of heterochromatic segments; b) the Y chromosome - commonly found as a typical acrocentric in the didelphids - was punctiform in the specimens of *M. murina*,

C. philander and *C. lanatus* we studied, differing from those of the same taxa collected in Peru and Venezuela.

Rofe and Hayman (1985) compared the G bands of 15 species belonging to 5 families of marsupials from Australia and South America, all with $2n = 14$, and found a great similarity of patterns. Based on this assumption, didelphids with $2n = 14$ may be elected as the group most closely related to the ancestral stock of this family, those with $2n = 18$ and $2n = 22$ being considered as derivatives. This fact led these authors to point out that chromosomal evolution in marsupials probably occurred through centric fissions rather by fusions.

In contrast with the evidence of a conserved G-banding pattern in the autosomal complement of didelphids with $2n = 14$ (Rofe and Hayman, 1985), $2n = 18$ (Merry *et al.*, 1983; Langguth and Lima, 1987) and $2n = 22$ (Yonenaga-Yassuda *et al.*, 1982), an extensive interspecific variation of C bands and NORs have been described by some of these authors. Our data corroborate both findings, since a high degree of G band homoeology is found among the autosomes and variation of C bands and NORs are evident. The variation of autosomal C bands is not related to differential location of the blocks of constitutive heterochromatin in these species, but to the amount of pericentromeric heterochromatin. This fact could be ascribed to the loss or acquisition of repetitive DNAs or even to some kind of mechanism that could account for the changes in the state of the chromatin.

The number of NORs varied from 2 to 6 per cell. These values were within the range of 2 to 8 NORs described for didelphids (Yonenaga-Yassuda *et al.*, 1982; Rofe and Hayman, 1985; Casartelli *et al.*, 1986). No intraindividual variation of NORs was detected in 20 cells of each specimen analysed, contrasting with reported data from *Philander opossum* and *Didelphis marsupialis* (Yonenaga-Yassuda *et al.*, 1982).

The location of NORs in the species we studied allows some speculation on rDNA cistron evolution. According to Hsu *et al.* (1975), karyotypes with a single NOR can be identified as the ancestral form in a taxon. It could be considered that *Caluromys* represents the most primitive form, in terms of NOR distribution (only one bearing pair), and this would imply that evolutionary changes would have progressed through a double step that would have led first, as a single event, to an increase in the number of rDNA loci, in both species of *Marmosa*, followed by an additional increase in *M. murina*. These changes could be involved in the process of speciation of didelphids.

In conclusion, this paper has reinforced the concept that apart from C banding and NOR patterns, didelphids, are chromosomically very stable, a situation quite different from the high degree of chromosome variability observed in many other groups of mammals. Reig *et al.* (1977) emphasized that this uniformity could be interpreted as a reflection of the stability of the regulatory mechanism of gene action

in these animals, which would be controlled by a strict system of gene arrangement within each chromosome. This property would have been acquired early in the radiation of marsupials.

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RESUMO

Quatro espécies de marsupiais didelfídeos (*Marmosa murina*, *M. cinerea*, *Caluromys philander* e *C. lanatus*) foram estudados cariotipicamente, mostrando um complemento similar de $2n = 14$, $NF = 20$. Os resultados mostraram uma grande homeologia nos padrões de bandas G entre as mesmas. Com respeito aos padrões de heterocromatina constitutiva e regiões organizadoras de nucléolos (RONs) ocorre uma considerável diferença.

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