Conservation of chromosomal location of nucleolus organizer in American marsupials (Didelphidae)

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Abstract
The distribution and expression of nucleolus organizer regions (NORs) were analyzed in seven species of marsupials representative of the three karyotypes (2n = 14, 18 and 22) found in the American family Didelphidae. Analyses comprised silver-staining of NORs and fluorescence in situ hybridization with an rDNA probe. In addition to confirming the variability in number and distribution of NORs in Didelphidae, we demonstrated the conserved location of NORs on one autosome pair in the three karyotypes. In Monodelphis domestica (2n = 18), the NOR on the X chromosome was not inactivated in females.

Introduction
Marsupials represent an extreme example of karyotypic conservation in which a ‘basic karyotype’ with 2n = 14 occurs in most of the roughly 270 living species that inhabit Australasia and the American continent. Rofe and Hayman (1985) stressed the interspecific variability in the amount of constitutive heterochromatin, size of sex chromosomes, and the number and location of Ag-NORs, in contrast with the conservation of G-banding patterns in 15 marsupial species with 2n = 14. NORs may be located at secondary constrictions, as in Australian Macropodidae, or may be less conspicuous and only detectable after specific staining. There are species with NORs on one or more autosome pairs, on the sex chromosomes and autosomes, restricted to the X and Y chromosomes or to the X chromosome (Hayman, 1990).

To date, the study of NORs in American marsupials has been limited to silver-staining analysis, a technique that reflects NOR expression. The distribution of Ag-NORs was described in 11 species, comprising the three known diploid numbers (2n = 14, 18 and 22) (Fernandez-Donoso, Berrios & Pincheara, 1979; Yonenaga-Yasuda et al., 1982; Merry, Pathak & Vandeberg, 1983; Seluja et al., 1984; Casartelli, Rogatto & Ferrari, 1986; Souza, Maia & Santos, 1990). Ag-NORs varied from two to nine and were located on autosomes, except for the NOR on the X chromosome of Monodelphis domestica. NORs were never observed in association with conspicuous secondary constrictions. In situ hybridization, that allows the detection of ribosomal cistrons independently of their activity, has not been performed.

In the present study, we analyzed the distribution and activity of NORs in seven species of Didelphidae, belonging to different genera and representative of the three diploid numbers found in this family, through fluorescence in situ hybridization with an rDNA probe, and silver-staining.

Materials and methods
NOR distribution was analyzed after in situ hybridization of rDNA and silver-staining in 13 specimens belonging to seven genera of Didelphidae collected in different localities in Brazil (Table 1).

Chromosome preparations were obtained directly from bone marrow or from kidney or tail cells, cultured in DMEM/L-15 1:1 (Gibco-BRL/Interlab), supplemented with 20% of fetal bovine serum (Interlab).
Table 1. Number and distribution of NORs in Didelphidae

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimens</th>
<th>2n/FN*</th>
<th>No. of NORs</th>
<th>NORMs location</th>
<th>No. of cells analyzed</th>
<th>Origin of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>*</td>
<td></td>
<td></td>
<td>In situ hybridization</td>
<td>AgNO₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marmosops incanus</td>
<td>1M</td>
<td>14/24</td>
<td>2</td>
<td>6p</td>
<td>39</td>
<td>CC</td>
</tr>
<tr>
<td>Metachirus nudicaudatus</td>
<td>1M</td>
<td>14/20</td>
<td>2</td>
<td>5p or 6p</td>
<td>43</td>
<td>CC</td>
</tr>
<tr>
<td>Caluromys philander</td>
<td>2M</td>
<td>14/20</td>
<td>2</td>
<td>6p</td>
<td>85</td>
<td>BM</td>
</tr>
<tr>
<td>Micoureus demerarae</td>
<td>1M</td>
<td>14/20</td>
<td>6</td>
<td>5pq; 6p</td>
<td>60</td>
<td>CC</td>
</tr>
<tr>
<td>Monodelphis domestica</td>
<td>1M/2F</td>
<td>18/20</td>
<td>4</td>
<td>5p; Xp</td>
<td>84</td>
<td>CC, BM</td>
</tr>
<tr>
<td>Philander opossum</td>
<td>2F</td>
<td>22/20</td>
<td>4</td>
<td>5p; 7q**</td>
<td>122</td>
<td>CC, BM</td>
</tr>
<tr>
<td>Didelphis marsupialis</td>
<td>1M/2F</td>
<td>22/20</td>
<td>8</td>
<td>Autosomal</td>
<td>125</td>
<td>BM</td>
</tr>
</tbody>
</table>

F = Female; M = Male; p = Short arm; q = Long arm; CC = Cell culture; BM = Bone marrow; FN = Fundamental number.
* Short arms of subtelocentric chromosomes not included.
** According to Yonenaga-Yasuda et al. (1982).

NOR expression was analyzed after silver-nitrate staining (Howell & Black, 1980). Probe HM456, which contains part of the 18S and 28S rDNA of Xenopus laevis (Meunier-Rotival et al., 1979), was used for in situ hybridization. Probe hybridization and detection were performed according to Viegas-Péquignot (1992).

Results and discussion

NOR distributions in species representing the three diploid numbers found in Didelphidae (2n = 14, 18 and 22) are shown in Table 1.

**NORs in the karyotypes with 2n = 14**

The karyotypes of the four species with 2n = 14 include three pairs of large submetacentric autosomes (pairs 1–3), one pair of medium metacentrics (pair 4) and two small pairs (pairs 5 and 6). The two smallest autosomes pairs are acrocentric in Caluromys philander, Metachirus nudicaudatus and Micoureus demerarae (= Marmosa cinerea), and submetacentric in Marmosops incanus. With the exception of the metacentric X of the latter species, sex chromosomes are acrocentric, with interspecific variation in size (Svartman & Vianna-Morgante, 1999).

In situ hybridization with the rDNA probe revealed ribosomal cistrons just on the short arms of a small autosomes pair in C. philander, M. incanus and M. nudicaudatus (Figure 1(a and b)). This was identified as pair 6 in the two former species based on the analysis of the same cells after DAPI staining. In M. incanu, interstitial telomere sequences are present at the pericentromeric region of pair 5, but not of pair 6, which is of similar size (Svartman & Vianna-Morgante, 1998). This allowed further confirmation of pair 6 as the NOR-bearing chromosome. Pairs 5 and 6 of M. nudicaudatus are of similar size and morphology, which prevented precise identification of the NOR-bearing autosomes. In M. demerarae, hybridization signals were present on the short arms and telomeric region of the long arms of pair 5 and on the short arms of pair 6, totaling six NORs (Figure 1(c)).

In the three species with NORs on one pair of autosomes, both of them were active in all cells, as demonstrated by silver-staining (Figures 2(a and b)). In M. demerarae, the only species with 2n = 14 presenting more than two NORs, the number of active NORs per cell varied: out of 60 cells analyzed, 47 showed activity of the six NORs, 11 had four Ag-NORs and two presented five Ag-NORs (Figure 2(c)).

This is the first description of NORs for M. incanu and M. nudicaudatus. In C. philander, Souza, Maia and Santos (1990) reported the presence of two Ag-NORs as herein described, although the authors had classified the NOR-bearing autosomes as pair 5.

In M. cinerea (= M. demerarae), Casartelli, Rogatto and Ferrari (1986) and Souza, Maia and Santos (1990) reported the presence of four Ag-NORs. In the specimen that we analyzed, a total of six NORs was observed with both techniques and the difference is probably due to variations in NOR activity.

**NORs in the karyotype with 2n = 18**

The karyotype of M. domestica (2n = 18) includes four large submetacentric/metacentric auto-