American marsupials chromosomes: Why study them?

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Abstract

Marsupials, one of the three main groups of mammals, are only found in Australia and in the American continent. Studies performed in Australian marsupials have demonstrated the great potential provided by the group for the understanding of basic genetic mechanisms and chromosome evolution in mammals. Genetic studies in American marsupials are relatively scarce and cytogenetic data of most species are restricted to karyotype descriptions, usually without banding patterns. Nevertheless, the first marsupial genome sequenced was that of Monodelphis domestica, a South American species. The knowledge about mammalian genome evolution and function that resulted from studies on M. domestica is in sharp contrast with the lack of genetic data on most American marsupial species. Here, we present an overview of the chromosome studies performed in marsupials with emphasis on the South American species.

Key words: marsupials, cytogenetics, chromosome evolution.

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Marsupials: Evolutionary History and Taxonomy

The class Mammalia is traditionally divided into three main groups: Prototheria, comprising only three species of extant monotremes, Metatheria, in which all marsupials are grouped, and Eutheria, that reunites all the other 18 mammalian orders.

Besides typical mammalian features, as homeothermy, hairs and mammary glands, marsupials have biological characteristics that allowed their separation as a distinct group. Among them, which include dental, osteological, cranial and brain features, those related to the reproductive system are the most important. These include a bifid reproductive tract in females, which have double vagina and uterus, and an incomplete placenta. The gestation time is relatively short and the newborns are in an almost embryonic stage. The marsupium or pouch, used to name the group, is not its most important feature and is absent in several species (Stonehouse, 1977; Nowak, 1991).

A recent estimate which included molecular data from almost all extant mammalian groups led Bininda-Emonds et al. (2007) to propose that the split of Monotrema from the common mammalian ancestor occurred at 166.2 million years ago (mya), that the lineage of the marsupials would have diverged at 147.7 mya and that the four recognized placental superorders would have originated around 100 mya. The diversification of the marsupials was dated at 82.5 mya.

Regarding their evolutionary history, the traditional hypothesis was that marsupials originated during the Cretaceous in North America. From there, they would have migrated to South America, Antarctica and Australia (Clemens, 1977). Marsupial fossils were also found in Europe, Asia and North Africa, where there are no extant representatives of the group, indicating a broader geographical distribution in the past (Nowak, 1991). The discovery of the metatherian fossil Sinodelphys szalayi in northeastern China in a site dated at 125 mya changed this picture (Luo et al., 2003). Eutherian fossils have also been found in the same site, including Eomaia, the earliest known member of the eutherian lineage. Thus the marsupial-placental split must have been no later than 125 mya. Sinodelphys was found to be basal in the metatherian tree, which led Luo et al. (2003) to propose a Eurasian origin for both eutherians and metatherians. From Eurasia, marsupials would have migrated to North America and later to South America and then Antarctica and Australia.

The Australian groups of marsupials are phylogenetically and immunologically more closely related between them than they are to any American group. This led to the suggestion that they originated from a single ancestral stock that would have reached Australia in the beginning of the Tertiary (64 mya), coming from South America via Antarctica (Dawson, 1983). The phylogenetic relationship between the South American monito del monte Dromiciops gliroides and the Australasian marsupials is of special in-
The Chromosomes of American Marsupials

Marsupials were one of the first mammalian groups to have their chromosomes studied. In his review, Hayman (1990) listed the diploid numbers of 178 species, which varies from $2n = 10$ in *Pseudocheirus cupreus* to $2n = 32$ in *Aepyprymnus rufescens*, both from Australia.

From the 94 currently recognized American marsupial species (Wilson and Reeder, 2005), 45 had their karyotypes reported, most of them without banding patterns (Table 1). Three diploid numbers have been found in American marsupials: $2n = 22$ in species of *Didelphis*, *Chironectes*, *Philander* and *Lutreolina*, $2n = 18$ in species of *Monodelphis*, and $2n = 14$ in all other species studied, which belong to the genera *Caluromys*, *Gracilinanus*, *Marmosa*, *Metachirus*, *Thylamys*, *Micoconus*, *Marmosops*, *Tlacuatzin*, *Dromiciops*, *Caenolestes*, *Lestoros* and *Rhyconoestes*.

The current available chromosome data for American marsupials are detailed in Table 1. In this Table, the fundamental number (FN) seems to be variable both within the same species, for instance *Caluromys lanatus*, and among species with the same diploid number, as the *Monodelphis* species. A closer examination of the literature reveals that there is no real variation in autosomal arms in each of the species studied by each author. The reason for the difference is that some authors counted the short arms of the acrocentric or subtelocentric chromosomes, while others did not. When these short arms are not included, the FN count is very constant and equal to 20 in all species with $2n = 14$, except for all the *Marmosops* species, the caenolestids and the microbiotherid, which exhibit a FN = 24. The difference in the FN between these two karyotypic forms is due to the two smaller autosome pairs, which are acrocentric in the FN = 20 and metacentric/submetacentric in the FN = 24 complements. The same explanation applies to the $2n = 18$ *Monodelphis*: all the species studied presented two clearly biarmed elements, resulting in a FN = 20.

This observation means that no real variation was observed between the conventionally stained autosomes of specimens of the same species collected in different localities and studied by various authors. Moreover, no variation in the autosomes seems detectable among species with each of the three diploid numbers ($2n = 14$, 18 and 22), except for the species with $2n = 14$ and FN = 24.

Great intraspecific conservation was also observed in the sex chromosomes. Many apparent differences are due to the nomenclature used by various authors (SM or M; T or A) or by technical difficulties because of the low quality of the chromosome preparations, especially in the older reports. The sex chromosomes are always the smallest ones in the karyotypes of the American marsupials studied and some species have very small Y chromosomes, considered as dot-like by some or as very small A by others. Due to their small size, determining their morphology may be dif-

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The American marsupial fauna is predominantly found in the American continent (Wilson and Reeder, 2005). These seven orders reunite 331 extant species, from which 237 inhabit Australasia and 94 occur in North America, *Dromiciops gliroides*, would have originated in South America.

The American marsupials are believed to have reached that continent at the end of the Cretaceous (64 mya) and there they experienced an extensive radiation, which is suggested by the present diversity of the group in Australia. The oldest metatherian fossils known from Australia are from a single site dated at around 55 mya. The recent finding of fairly complete fossils from the metatherian *Djarthia murgonensis* led Beck et al. (2008) to propose that *Djarthia* was a member of Australidelphia. A phylogenetic analysis led to the conclusion that *Djarthia* is the most plesiomorphic known australidelphian and may represent the ancestral morphotype of the Australian marsupial radiation. Beck et al. (2008) thus concluded that, contrary to the long held view, the South American microbiotheres may have resulted from back dispersal from marsupials from eastern Gondwana.

Marsupials have been traditionally grouped in the order Marsupialia, but the recognition of their great diversity is reflected in the increasing number of taxa proposed by different authors (Nowak, 1991; Wilson and Reeder, 2005). More recently new classifications were proposed mainly based on molecular data and the most current includes ten orders, seven of which with living representatives (Wilson and Reeder, 2005). These seven orders reunite 331 extant species, from which 237 inhabit Australasia and 94 occur in the American continent (Wilson and Reeder, 2005). The American marsupial fauna is predominantly found in Central and South America, as the only marsupial species in North America, *Didelphis virginiana*, is a relatively recent invader, derived from the South American *D. marsupialis* (Dawson, 1983).

Three marsupial orders each including one extant family are found in the American continent. Among them, the family Didelphidae, order Didelphimorphia, is the richest in number of taxa and has the broadest geographical distribution, with representatives in the three Americas. It has 87 species belonging to 17 genera (*Caluromys*, *Caluromysios*, *Chironectes*, *Didelphis*, *Glorinia*, *Gracilinanus*, *Hyladelphys*, *Lestodelphys*, *Lutreolina*, *Marmosa*, *Marmosops*, *Metachirus*, *Micoconus*, *Monodelphis*, *Philander*, *Thylamys* and *Tlacuatzin*). Six species, belonging to three genera (*Caenolestes*, *Lestoros* and *Rhyconolestes*), form the family Caenolestidae, order Paucituberculata, whose representatives inhabit high altitude regions, usually in the Andes; the family Microbiotheriidae, order Microbiotheria, has as its sole extant representative *Dromiciops gliroides*, which has only been found in southern Chile and in the adjacent region of Argentina (Wilson and Reeder, 2005).

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Table 1 - American marsupial species karyotyped.

| Species | Referred as | 2n | FN | X | Y | Banding and FISH | Provenience | Reference |
|---------|-------------|----|----|---|---|------------------|-------------|-----------|
| *Family Didelphidae, Order Didelphimorpha* | | | | | | | |
| *Caluromys derbianus* | | 14 | 24 | A | T | | Nicaragua | Biggers et al., 1965 |
| | | 14 | 24 | A | A | | Cartago, Costa Rica; Chiapas, Mexico | Reig et al., 1977 |
| *Caluromys lanatus* | | 14 | 24 | SM | - | | Iquitos, Peru | Hayman and Martin, 1974 |
| | | 14 | 24 | A | A | | Loreto, Peru | Reig et al., 1977 |
| | | 14 | 22 | M | - | G, C, Ag-NORs | San Diego, Zoo, USA | Rohe and Hayman, 1985 |
| | | 14 | 22 | SM | A | G, Ag-NORs | Amazonas, Brazil | Casartelli et al., 1986 |
| | | 14 | 20 | A | D | G, Ag-NORs | Rondônia, Brazil | Souza et al., 1990 |
| | | 14 | 22 | A | M | | Pando, Bolivia | Palma and Yates, 1996 |
| | | 14 | 20 | SM | D | Ag-NORs | Goiás, Brazil | Pereira et al., 2008 |
| *Caluromys philander* | | 14 | 24 | A | - | | not specified | Hayman and Martin, 1974 |
| | | 14 | 24 | SM | A | | Simla, Trinidad; Guárico, Aragua and Miranda, Venezuela | Reig et al., 1977 |
| | | 14 | 20 | A | D | G, C, Ag-NORs | Pernambuco, Brazil | Souza et al., 1990 |
| | | 14 | 20 | A | A | G, Ag-NORs, FISH (Tel; rDNA) | São Paulo, Brazil | Svartman and Vianna-Morgante, 1998, 1999, 2003 |
| | | 14 | 20 | A | D | Ag-NORs | São Paulo, Brazil | Pereira et al., 2008 |
| *Gracilinanus agilis* | | 14 | 24 | SM | SM | | La Paz, Bolivia | Palma and Yates, 1996 |
| | | 14 | 24 | M | A | | Goiás and Minas Gerais, Brazil | Carvalho et al., 2002 |
| *Gracilinanus emiliae* | | 14 | 24 | M/A* | A | C, Ag-NORs, FISH (Tel) | Goiás, Brazil | Carvalho and Mattevi, 2000; Carvalho et al., 2002 |
| | | 14 | 24 | SM | A | Ag-NORs | Goiás, Brazil | Pereira et al., 2008 |
| *Gracilinanus microtarsus* | | 14 | 24 | SM | A | C, Ag-NORs, FISH (Tel) | Rio Grande do Sul, Brazil | Carvalho and Mattevi, 2000; Carvalho et al., 2002 |
| | | 14 | 20 | A | A | | Bahia, Brazil | Pereira and Geise, 2007 |
| | | 14 | 24 | M | - | | São Paulo, Brazil | Pereira et al., 2008 |
| *Marmosa mexicana* | | 14 | 24 | A | D | | Nicaragua | Biggers et al., 1965 |
| *Marmosa marina* | | 14 | 24 | M | A | | Villavicencio, Colombia | Hayman and Martin, 1974 |
| | | 14 | 24 | M | A | | Loreto, Peru; Bolivar, Venezuela | Reig et al., 1977 |
| | | 14 | 20 | A | D | G, C, Ag-NORs | Pernambuco, Brazil | Souza et al., 1990 |
| | | 14 | 24 | M/A* | A | C, Ag-NORs, FISH (Tel) | Amapá, Goiás and Tocantins, Brazil | Carvalho and Mattevi, 2000; Carvalho et al., 2002 |
| | | 14 | 20 | A | - | C, FISH (Tel) | Ceará and Mato Grosso, Brazil | Pagnozzi et al., 2002 |
| | | 14 | 22 | A | - | Ag-NORs | Tocantins, Brazil | Lima, 2004 |
| | | 14 | 20 | A | D | Ag-NORs | Espírito Santo, Brazil | Paresque et al., 2004 |
| | | 14 | 20 | SM | A | Ag-NORs | Goiás and Tocantins, Brazil | Pereira et al., 2008 |
| *Marmosa robinsoni* | | 14 | 20 | M | T | | Guárico and Apure, Venezuela | Reig, 1968 |
| | | 14 | 24 | SM | A | | Guárico, Apure and Miranda, Venezuela | Reig et al., 1977 |
| *Marmosops dorothea* | | 14 | 24 | M | A | | La Paz, Bolivia | Palma and Yates, 1996 |
| *Marmosops fuscatus* | | 14 | 24 | M | A | | Northern Venezuela | Reig and Sonneschein, 1970 |
| *Marmosops incanus* | | 14 | 24 | M | A | | São Paulo, Brazil | Svartman and Vianna-Morgante, 1998, 1999, 2003 |
| | | 14 | 24 | M | A | C, Ag-NORs | Bahia and Minas Gerais, Brazil | Carvalho et al., 2002 |
| | | 14 | 24 | M | - | C, FISH (Tel) | Bahia and São Paulo, Brazil | Pagnozzi et al., 2002 |
| | | 14 | 24 | M | A | | Espírito Santo, Brazil | Paresque et al., 2004 |
| | | 14 | 24 | M | A | | Bahia, Brazil | Pereira and Geise, 2007 |
| *Marmosops noctivagus* | | 14 | 24 | SM | UN | | La Paz, Bolivia | Palma and Yates, 1996 |
| *Marmosops parvidens* | | 14 | 24 | SM | - | | La Paz, Bolivia | Palma and Yates, 1996 |
| | | 14 | 24 | SM | A | Ag-NORs | Goiás, Brazil | Carvalho et al., 2002 |
| | | 14 | 24 | M | A | C, FISH (Tel) | Mato Grosso, Brazil | Pagnozzi et al., 2002 |
| Species             | Referred as                      | 2n | FN  | X | Y | Banding and FISH | Provenience* | Reference                          |
|---------------------|----------------------------------|----|-----|---|---|------------------|-------------|------------------------------------|
| *Marmosops paulensis* |                                  |    |     |   |   |                  |             |                                    |
|                     |                                  | 14 | 24  | M | A | Ag-NORs          | São Paulo, Brazil | Pereira et al., 2008               |
| *Metachirus nudicaudatus* |                                | 14 | 20  | A | - | Kasmera, Venezuela | Hayman and Martin, 1974 |
|                     |                                  | 14 | 20  | A | C | Meta, Colombia    | Yunis et al., 1973  |
|                     |                                  | 14 | 24  | A | A | Loreto and Ayacucho, Peru; Trujillo and Mérida, Venezuela | Reig et al., 1977 |
|                     |                                  | 14 | 20  | A | - | La Paz, Bolivia   | Palma and Yates, 1996 |
|                     |                                  | 14 | 20  | A | A | G, C, Ag-NORs, FISH (Tel; rDNA) | São Paulo, Brazil | Svartman and Vianna-Morgante, 1998, 1999, 2003 |
|                     |                                  | 14 | 20  | A | A | Mato Grosso, Brazil | Pagnozzi et al., 2002 |
|                     |                                  | 14 | 20  | A | A | Espírito Santo, Brazil | Paresque et al., 2004 |
|                     |                                  | 14 | 20  | A | - | São Paulo, Brazil | Pereira et al., 2008 |
| *Micoureus alstoni*  | *Marmosa alstoni*                | 14 | 20  | A | A | San Jose, Costa Rica | Reig et al., 1977 |
|                     | *Micoureus constantiae*          | 14 | 20  | A | A | Pando, Bolivia    | Palma and Yates, 1996 |
| *Micoureus demerarae* |                                    | 14 | 20  | A | - | Rancho Grande, Venezuela | Hayman and Martin, 1974 |
|                     | *Marmosa cinerea*                | 14 | 24  | M | A | Ag-NORs          | Loreto, Peru; Aragua, Bolivia, Amazonas and Mérida, Venezuela | Reig et al., 1977 |
| *Micoureus cinereus* |                                  | 14 | 20  | A | A | G, C, Ag-NORs     | Amazonas, Brazil | Casartelli et al., 1986 |
|                     |                                  | 14 | 20  | A | A | Pernambuco, Brazil | Souza et al., 1990 |
|                     |                                  | 14 | 20  | A | A | La Paz, Bolivia   | Palma and Yates, 1996 |
|                     |                                  | 14 | 20  | A | A | São Paulo, Brazil | Svartman and Vianna-Morgante, 1998, 1999, 2003 |
|                     |                                  | 14 | 20  | A | A | Bahia, Ceará, Goiás, Mato Grosso and São Paulo, Brazil | Pagnozzi et al., 2000 |
|                     |                                  | 14 | 20  | A | A | Goiás and Rio Grande do Sul, Brazil | Carvalho and Mattevi, 2000; Carvalho et al., 2002 |
|                     |                                  | 14 | 20  | A | A | Espírito Santo, Brazil | Paresque et al., 2004 |
|                     |                                  | 14 | 20  | A | A | São Paulo, Brazil | Pereira et al., 2008 |
| *Micoureus paraguayanus* |                               | 14 | 20  | A | - | São Paulo, Brazil | Pereira et al., 2008 |
| *Thylamys elegans*   | *Marmosa elegans*                | 14 | 24  | SM | D | Aconcagua, Chile  | Reig et al., 1972 |
|                     | *Marmosa elegans*                | 14 | 22  | SM | A | Aconcagua, Chile; Huancavelica and Arqueipa, Peru | Reig et al., 1977 |
| *Marmosa elegans*    |                                  | 14 | 24  | A | - | Coquimbo, Chile   | Fernandez-Donoso et al., 1979 |
|                     |                                  | 14 | 20  | UN | A | Cochabamba and Santa Cruz, Bolivia | Palma and Yates, 1996 |
|                     |                                  | 14 | 20  | ST | - | Rio Loa, Pichidangui and Las Melosas, Chile | Spotorno et al., 1997 |
| *Thylamys macrurus*  | *T. macrura*                     | 14 | 20  | A | - | Concepción, Paraguay | Palma, 1995 |
| *Thylamys pallidior* |                                  | 14 | 20  | A | UN | Chuaquisaca and Tarija, Bolivia | Palma, 1995 |
|                     |                                  | 14 | 20  | A | UN | Chuaquisaca, Bolivia | Palma and Yates, 1996 |
| *Thylamys pusillus*  | *Marmosa pusilla*                | 14 | 24  | M | D | Buenos Aires, Argentina | Reig et al., 1977 |
|                     |                                  | 14 | 20  | SM | - | Tarija, Bolivia    | Palma and Yates, 1996 |
| *Thylamys velutinus* |                                  | 14 | 24  | SM | A | Goiás, Brazil     | Carvalho et al., 2002 |
| *Monodelphis americana* |                               | 18 | 22  | T  | - | Paraíba, Brazil   | Langguth and Lima, 1988 |
|                     |                                  | 18 | 22  | A | A | C, FISH (Tel)    | Ceará, Brazil | Pagnozzi et al., 2002 |
|                     |                                  | 18 | 32  | A | A | Espírito Santo, Brazil | Paresque et al., 2004 |
|                     |                                  | 18 | 32  | A | - | São Paulo, Brazil | Pereira et al., 2008 |
| *Monodelphis breviceps* |                               | 18 | 20  | A | D | Aragua and Zulia, Venezuela | Reig and Bianchi, 1969 |
| *M. orinoci*        |                                  | 18 | 30  | A | A | Aragua, Merida and Zulia, Venezuela | Reig et al., 1977 |
|                     |                                  | 18 | 30  | A | A | Guaráico, Venezuela | Reig et al., 1977 |
|                     |                                  | 18 | 22  | A | D | Pando, Bolivia    | Palma and Yates, 1996 |
|                     |                                  | 18 | 30  | A | D | C, Ag-NORs, FISH (Tel) | Carvalho and Mattevi, 2000; Carvalho et al., 2002 |
### Table 1 (cont.)

| Speciesa | Referred as | 2n | FN | X | Y | Banding and FISH | Provenienceb | Reference |
|-----------|-------------|----|----|---|---|------------------|--------------|-----------|
| *Monodelphis dimidiata* | | 18 | 20 | A | D | Buenos Aires, Argentina | Reig and Bianchi, 1969 |
| | | 18 | 30 | A | A | Buenos Aires, Argentina | Reig *et al.*, 1977 |
| | | 18 | 32 | SM | D | Rio Grande do Sul, Brazil | Carvalho *et al.*, 2002 |
| | | 18 | 20 | A | A | Buenos Aires, Argentina | Palma and Yates, 1996 |
| | | 18 | 28 | A | D | Goiás, Brazil | Carvalho and Mattevi, 1999, 2003 |
| | | 18 | 20 | A | A | Chuquisaca and Santa Cruz, Bolivia | Carvalho *et al.*, 2002 |
| | | 18 | 22 | A | A | São Paulo, Brazil | Sinha and Kakati, 1976 |
| *Monodelphis domestica* | | 18 | 20 | A | G, C, NOR | Laboratory bred, USA | Pathak *et al.*, 1993 |
| | | 18 | 24 | A | A | Chaquisaca and Santa Cruz, Bolivia | Palma and Yates, 1996 |
| | | 18 | 20 | A | A | São Paulo, Brazil | Yonenaga-Yassuda *et al.*, 1982 |
| | | 18 | 24 | SM | D | Goiás, Brazil | Carvalho and Mattevi, 1999, 2003 |
| | | 18 | 30 | A | D | Espírito Santo, Brazil | Pereira *et al.*, 2007 |
| | | 18 | 20 | A | A | Bahia, Brazil | Carvalho *et al.*, 2002 |
| | | 18 | 20 | A | A | Tocantins, Brazil | Pereira *et al.*, 2008 |
| *Monodelphis kunsi* | | 18 | 30 | SM | SM | Tarija, Bolivia | Palma and Yates, 1996 |
| | | 18 | 30 | SM | A | Goiás, Brazil | Carvalho and Mattevi, 2000; Carvalho *et al.*, 2002 |
| *Monodelphis rubida* | | 18 | 32 | A | A | Ag-NORs | São Paulo, Brazil | Pereira *et al.*, 2008 |
| *Chironectes minimus* | | 22 | 20 | A | A | Rio Camoto, Venezuela | Hayman and Martin, 1974 |
| | | 22 | 20 | A | A | Tarija, Bolivia | Palma and Yates, 1996 |
| | | 22 | 20 | A | UN | Goiás, Brazil | Carvalho and Mattevi, 1999, 2003 |
| | | 22 | 20 | A | A | São Paulo, Brazil | Yonenaga-Yassuda *et al.*, 1982 |
| *Didelphis albiventris* | | 22 | 20 | A | A | Buenos Aires, Argentina; Mérida, Venezuela; Huana-co, Peru | Reig *et al.*, 1977 |
| | | 22 | 20 | A | A | São Paulo, Brazil | Yonenaga-Yassuda *et al.*, 1982 |
| | | 22 | 20 | A | A | Uruguay (six localities) | Seluja et al., 1984 |
| | | 22 | 20 | A | A | São Paulo, Brazil | Casartelli *et al.*, 1986 |
| | | 22 | 20 | A | A | Goiás and Tocantins, Brazil | Carvalho and Mattevi, 2000; Carvalho *et al.*, 2002 |
| *Didelphis aurita* | | 22 | 20 | A | A | Bahia, Brazil | Carvalho *et al.*, 2002 |
| | | 22 | 20 | A | A | Espírito Santo, Brazil | Pereira *et al.*, 2004 |
| *Didelphis marsupialis* | | 22 | 20 | SM | T | Philadelphia, USA | Biggers *et al.*, 1965 |
| | | 22 | 20 | A | A | San Jose, Costa Rica; San Luis Potosí, Veracruz and Chiapas, Mexico; Loreto, Peru; Aragua, Miranda and Merida, Venezuela | Reig *et al.*, 1977 |
| | | 22 | 20 | A | A | São Paulo, Brazil | Yonenaga-Yassuda *et al.*, 1982 |
| | | 22 | 20 | A | A | Amazonas and São Paulo, Brazil | Casartelli *et al.*, 1986 |
| | | 22 | 20 | A | A | La Paz, Bolivia | Palma and Yates, 1996 |
| | | 22 | 20 | A | A | São Paulo, Brazil | Svartrman and Vianna-Morgante, 1998, 1999, 2003 |
| | | 22 | 20 | A | A | Mato Grosso, Brazil | Pagnozzi *et al.*, 2002 |
| | | 22 | 20 | A | A | Tocantins, Brazil | Lima, 2004 |
| | | 22 | 20 | A | A | Tocantins, Brazil | Pereira *et al.*, 2008 |
| *Didelphis virginiana* | | 22 | 32 | M | A | C | Texas, USA | Painter, 1922 |
| | | 22 | 32 | M | A | G, C, 1H-timidine replication | Sinha *et al.*, 1972 |
| | | 22 | 32 | M | A | G, C | not specified | Sinha and Kakati, 1976 |
Table 1 (cont.)

| Speciesa | Referred as | 2n | FN | X | Y | Banding and FISH | Provenienceb | Reference |
|----------|-------------|----|----|---|---|------------------|-------------|-----------|
| Lutreolina crassicaudata | 22 | 32 | M | A | A | San Luis Potosí, Nayarit, | Reig et al., 1977 |
|          |             |    |    |    |    | Chiapas and Yucatán, |             |
|          |             |    |    |    |    | Mexico; Louisiana and |             |
|          |             |    |    |    |    | Texas, USA |             |
| Philander frenatus | P. frenata | 22 | 20 | A | A | A/M | Santa Catarina and | Carvalho and Mattevi, 2000; |
|          |                 |    |    |    |    | Rio Grande do Sul, Brazil |         |
|          |                 |    |    |    |    | Sao Paulo, Brazil | Reig et al., 1977 |
|          |                |    |    |    |    | Buenos Aires, Argentina | Saez, 1938c |
|          | P. frenata     | 22 | 20 | A | A | A | Espírito Santo, Brazil | Paresque et al., 2004 |
|          | Philander mclhennyi | 22 | 20 | A | A | A | São Paulo, Brazil | Pereira et al., 2008 |
|          |                |    |    |    |    | Goiás, Brazil | Pagnozzi et al., 2002 |
|          |                |    |    |    |    | Loreto, Peru | Reig et al., 1977 |
| Philander opossum | 22 | 20 | T | T | T | Nicaragua | Biggers et al., 1965 |
|          |             |    |    |    |    | Cartago, Costa Rica; Chia- | Reig et al., 1977 |
|          |             |    |    |    |    | pas, Mexico; Loreto and |             |
|          |             |    |    |    |    | Ayacucho, Peru; Barinas, |             |
|          |             |    |    |    |    | Venezuela; São Paulo and | Yonenaga-Yassuda et al., 1982 |
|          |             |    |    |    |    | Rio de Janeiro, Brazil | Palma and Yates, 1996 |
|          |             |    |    |    |    | Beni, Chuquisaca and Santa |             |
|          |             |    |    |    |    | Cruz, Bolivia |             |
|          |             |    |    |    |    | Espírito Santo and São Pau- | Swartman and Vianna-Morgante, 1998, 1999, 2003 |
|          |             |    |    |    |    | lo, Brazil |            |
|          |             |    |    |    |    | Goiás, Brazil | Carvalho and Mattevi, 2000; |
|          |             |    |    |    |    | Goiás, Brazil | Carvalho et al., 2002 |
|          |             |    |    |    |    | Loreto, Peru | Pereira et al., 2008 |
| Tlacuatzin canescens | Marmosa canescens | 22 | 20 | A | D | D | Mexico (four localities) | Engstrom and Gardner, 1988 |
| Family Caenolestidae, Order Paucituberculata | | | | | | | |
| Caenolestes fuliginosus | C. obscurus | 14 | 24 | A | D | not specified | Hayman et al., 1971d |
|          |            |    |    |    |    | Cauc, Colombia | Hayman et al., 1971 |
| Lestoros inca | 14 | 24 | A | D | D | Cuzco, Peru | Hayman et al., 1971 |
| Rhyncholestes raphanus | 14 | 24 | A | A | A | Osorno, Chile | Gallardo and Patterson, 1987 |
| Family Microbiotheriidae, Order Microbiotheria | | | | | | | |
| Dromiciops gliroides | D. australis | 14 | 24 | A | A | A | Valdivia, Chile | Reig et al., 1972 |
|          | D. australis | 14 | 24 | A | A | A | Valdivia, Chile | Reig et al., 1977 |
|          | D. australis | 14 | 24 | A | A | - | Valdivia, Chile | Fernandez-Donoso et al., 1979 |
|          | D. australis | 14 | 24 | A | UN | UN | Valdivia, Osorno and Concepción, Chile | Gallardo and Patterson, 1987 |
|          | D. australis | 14 | 24 | ST | D | G | Valdivia and Osorno, Chile | Spotorno et al., 1997 |

2n - diploid number; FN - fundamental number (number of autosomal arms); D - dot-like; A - acrocentric; T - telocentric; SM - submetacentric; M - metacentric; FISH - fluorescent in situ hybridization: Tel - telomere probe; rDNA - ribosomal DNA probe, Gen - whole genomic DNA probe; * - polymorphism of the X chromosome; UN - undistinguishable; a - current taxonomic names according to Wilson and Reeder, 2005; b - the provenience corresponds to states, departments or provinces in each country indicated; c - apud Hayman, 1990; d - in the original paper two Caenolestes species (C. obscurus and C. fuliginosus) were described with identical karyotypes. They were likely the same species as C. obscurus is now considered a synonym of C. fuliginosus.

The only cases of real intraspecific sex chromosome variation appear to be those of the X chromosomes of Gracilinanus emiliae, G. microtarsus, Marmosa murina and Thylamys elegans. Different morphologies were reported for these chromosomes by several authors (Table 1). Additionally, one specimen of Philander frenatus had a metacentric Y, in contrast with the acrocentric form found otherwise in this species (Carvalho et al., 2002).

The Y chromosome was reported as undistinguishable or absent in males of Thylamys pallidior...
additional large distal heterochromatic blocks were present.

Marmosops incanus has a 2n = 13 (Gallardo and Patterson, 1987). Nevertheless, the Y chromosome of this species was also reported as dot-like in bone marrow cells in other studies (Reig et al., 1997; Spotorno et al., 1997) and was likely missed in the former report.

**Chromosome Banding In American Marsupials**

G-banding was performed in 14 species of American marsupials (Sinha and Kakati, 1976; Yonenaga-Yassuda et al., 1982; Merry et al., 1983; Rofe and Hayman, 1985; Casartelli et al., 1986; Souza et al., 1990; Pathak et al., 1993; Seluja et al., 1984; Spotorno et al., 1997; Svartman and Vianna-Morgante, 1999) (Table 1). No intraspecific variation was observed in these studies, which reinforced the conservation of the karyotypes extending it to G-banded chromosomes. Moreover, species with the same diploid number showed a striking similarity in their banded chromosomes with small karyotypic differences attributed to inversions and variation in the amount of constitutive heterochromatin (Yonenaga-Yassuda et al., 1982; Rofe and Hayman, 1985; Casartelli et al., 1986; Souza et al., 1990; Pathak et al., 1993; Seluja et al., 1984; Spotorno et al., 1997; Svartman and Vianna-Morgante, 1999). The comparison of G-banded karyotypes of species with 2n = 14, 18 and 22 allowed to establish a complete homeology between all the autosomal arms of species with 2n = 14, 18 and 22 (Svartman and Vianna-Morgante, 1998, 1999). This observation reinforced the role played by Robertsonian rearrangements in the karyotypic evolution of American marsupials (Svartman and Vianna-Morgante, 1998, 1999).

C-banding patterns were obtained in 22 species of American marsupials (references in Table 1) and, although intraspecific variation seemed absent, differences in constitutive heterochromatin contents and location were reported among species with the same diploid numbers. Most of the ten 2n = 14 species studied presented pericentromeric heterochromatin in all autosomes and on the X chromosome (two species of Caluromys, two of Gracilinanus, Marmosa murina and Thylamys elegans). In Metachirus nudicaudatus only small pericentromeric C-bands were observed in the two smaller autosomes (pairs 5 and 6) and on the X chromosome. Large blocks of heterochromatin were present in the autosomes and on the X chromosome of Marmosops incanus and M. parvidens. In M. incanus two additional large distal heterochromatic blocks were present, one in each arm of the metacentric X chromosome (Svartman and Vianna-Morgante, 1999; Carvalho et al., 2002; Pagnozzi et al., 2002). Micourus demerarae had large heterochromatic blocks in the pericentromeric regions of the four largest autosome pairs (pairs 1-4) and very little pericentromeric C-bands in the two smallest autosomes (pairs 5 and 6). Besides the pericentromeric C-band, the acrocentric X chromosome of this species also presented a distal heterochromatic block (Casartelli et al., 1986; Souza et al., 1990; Svartman and Vianna-Morgante, 1999; Pagnozzi et al., 2000; Carvalho et al., 2002). The Y chromosome was described as entirely heterochromatic in all the species.

Four of the five Monodelphis species studied (M. brevicaudata, M. dimidiata, M. domestica and M. kusi) presented pericentromeric heterochromatin in all autosomes and on the X chromosome and the tiny Y chromosome appeared completely heterochromatic. Monodelphis americanana showed a different pattern, with pericentromeric heterochromatin restricted to the X chromosome and a completely heterochromatic Y (Merry et al., 1983; Svartman and Vianna-Morgante, 1999; Carvalho et al., 2002; Pagnozzi et al., 2002).

In all species with 2n = 22 studied the Y chromosome appeared heterochromatic. The most common pattern observed was the presence of C-bands in the pericentromeric regions of all autosomes and on the X chromosome, which was the case in Chironectes minimus, Didelphis albiventris, D. marsupialis and Philander frenatus. Pericentromeric heterochromatin was restricted to the X chromosome in Didelphis marsupialis, D. virginiana and Lutreolina crassicaudata. The two latter species presented distinctive features on their X chromosomes which had C-banded blocks. This was also the case for the X chromosome of Philander opossum (Sinha et al., 1972; Sinha and Kakati, 1976; Yonenaga-Yassuda et al., 1982; Seluja et al., 1984; Casartelli et al., 1986; Svartman and Vianna-Morgante, 1999; Carvalho et al., 2002; Pagnozzi et al., 2002).

The variation in X chromosome size among species including the three diploid numbers was attributed to variations in the contents of constitutive heterochromatin (Yonenaga-Yassuda et al., 1982; Souza et al., 1990; Svartman and Vianna-Morgante, 1999).

Genome comparisons among Philander opossum (2n = 22), Micourus demerarae and Marmosops incanus (2n = 14) were extended through FISH (fluorescent in situ hybridization) using total genomic DNAs as probes (Svartman and Vianna-Morgante, 1999). In this study, interspecific conservation of the euchromatin among the three karyotypes was reinforced, while the heterochromatin was shown to be species-specific, not only in amount, but also in content.

Ag-NORs were studied in a total of 24 species of American marsupials. Thirteen of the species analyzed had a 2n = 14 (Caluromys lanatus, C. philander, Gracilinanus...
emiliae, G. microtarsus, Marmosa murina, Marmosops incanus, M. parvidens, M. paulensis, Metachirus nudicaudatus, Micoureus demerarae, M. paraguayensis, Thylamys elegans and Dromiciops australis; references in Table 1). All of these species presented Ag-NORs in the short arms of chromosome 6 (6p). In Dromiciops gliroides one additional NOR was observed in 5p (Fernandez-Donoso et al., 1979) and in the two analyzed Micoureus species there were six NORs located on 5pq and 6p (Carvalho et al., 2002; Svartman and Vianna-Morgante, 2003).

Five species of Monodelphis, all with 2n = 18, had their Ag-NORs described (M. brevicaudata, M. dimidiata, M. domestica, M. kunsi and M. rubida). Four of them presented a single NOR-bearing pair, which was 5p in three species (M. dimidiata, M. kunsi and M. rubida) and Xp in M. brevicaudata. Monodelphis domestica was the only species with 2n = 18 to present four NORs, located on 5p and Xp (references in Table 1). It is possible that all Monodelphis species have the same pattern observed in M. domestica (5p and Xp) and that they were not detected because of the few specimens analyzed and because Ag-NORs reflect activity in the previous interphase and are thus variable. It would be interesting to perform FISH with an rDNA probe in order to determine the location of NORs in all the Monodelphis species. In Monodelphis domestica there is no inactivation of the NOR on the X chromosomes of the females, which are both active in every cell analyzed (Merry et al., 1983; Svartman and Vianna-Morgante, 2003). This may also be the case in M. brevicaudata (Carvalho et al., 2002).

Three species with 2n = 22 presented four Ag-NORs located on 5p7q (Lutreolina crassicaudata, Philander opossum and P. frenata), while Chironectes minimus had a single Ag-NOR on 5p. In Didelphis albiventris the maximum number of Ag-NORs reported was six and they were located at the tip of the long arm of pairs 4, 5 and 6 (Seluja et al., 1984). Didelphis marsupialis had eight NORs as shown by FISH with an rDNA probe (Svartman and Vianna-Morgante, 2003), but the number of Ag-NORs reported was highly variable, possibly reflecting differences in activity between samples (Yonenaga-Yassuda et al., 1982; Casartelli et al., 1986; Carvalho et al., 2002; Svartman and Vianna-Morgante, 2003; Lima, 2004).

Besides Monodelphis domestica and D. marsupialis, other five species of American marsupials representing the three diploid numbers known in the group were studied after FISH with an rDNA probe (Svartman and Vianna-Morgante, 2003). The results obtained, combined with the demonstration of G-banding homeologies between all the autosomal arms of the three diploid numbers (2n = 14, 18 and 22), led to the conclusion that at least one NOR-bearing pair is the same in the three different 2n complements. Thus, the NOR-bearing chromosome 6 of the 2n = 14 karyotype corresponds, respectively, to chromosomes 5 and 7 in the species with 2n = 18 and 22, which also have NORs (Svartman and Vianna-Morgante, 2003).

Karyotype Evolution In Marsupials

The preponderance of a conserved karyotype with 14 chromosomes in animals of almost all marsupial families from the American and Australian faunas was interpreted as evidence that this was a conserved complement, possibly present in a common ancestor. Martin and Hayman (1967) and Hayman and Martin (1969) suggested that a karyotype with 14 chromosomes would be the “ancestral karyotype” present in an ancestral stock. It was suggested that the evolution proceeded from a karyotype with 14 chromosomes, which would have undergone centric fissions originating the second mode, a complement with 22 chromosomes, which in turn, would have given rise to the karyotypes with intermediate diploid numbers through centric fusions (Hayman, 1990). A karyotypic evolution through centric fissions from the basic karyotype with 2n = 14 was also suggested to explain the 2n = 18 and 2n = 22 in American marsupials (Reig et al., 1977).

An opposing view was held by Sharman (1973), who suggested that the second modal number of 2n = 22 would be ancestral as it was present in the more plesiomorphic American didelphids and because chromosome fissions are rarer and more difficult to accept. This author thus proposed that chromosome fusions would explain the occurrence of marsupial karyotypes with diploid numbers lower than 2n = 22.

The later demonstration that the G-banding patterns in American and Australian species with 2n = 14 were very similar, mainly differing due to intrachromosomal variations, was used to reinforce the idea that this was the marsupial “basic ancestral karyotype” and to lend support for the fission hypothesis (Rofe and Hayman, 1985; Hayman, 1990).

Recent studies using reciprocal interspecific chromosome painting to compare the chromosomes of marsupials from Australian and South American families confirmed the great conservation between chromosome segments among very divergent groups and allowed to delineate conserved segments that were differently combined in each species (Rens et al., 1999; 2001; 2003). These studies, which confirmed and extended the conclusions based on previous G-banding comparisons (Rofe and Hayman, 1985; Yonenaga-Yassuda et al., 1982; Souza et al., 1990; Svartman and Vianna-Morgante, 1999) have been frequently used in support of the fission hypothesis. Nevertheless, the demonstration of conservation does not in itself favor any of the two competing views.

The finding of interstitial telomeric sequences (ITS) in the autosomes of Didelphidae species with 2n = 14 and 2n = 18, but not in those with 2n = 22, led to the suggestion that the karyotype of South American marsupials would have proceeded through centric fusions leading to the re-
duction in diploid number from an ancestral complement with at least 22 chromosomes (Svantman and Vianna-Morgante, 1998; Carvalho and Mattevi, 2000). This hypothesis defined the idea of the “ancestral” $2n = 14$ marsupial karyotype which had prevailed for the previous three decades.

Pagnozzi et al. (2000) demonstrated that in Micoureus demerarae ($2n = 14$) autosomal pericentromeric interstitial telomeric sequences varied from 2 through 8 in specimens caught in different localities. These authors also stated that the ITS co-localized with heterochromatic blocks in M. demerarae and would thus represent part of satellite DNA sequences and not remnants of Robertsonian fusions. Pagnozzi et al. (2002) later performed the same kind of analysis in eight Didelphidae species, four with $2n = 14$, two with $2n = 18$ and two with $2n = 22$, and came to a similar conclusion. These authors claimed that other kinds of datasets, basically derived from molecular studies involving DNA sequence comparisons, supported the ancestral $2n = 14$ karyotype hypothesis and an evolution based on chromosome fissions would comply with it.

Taken together, the studies with ITS performed in American marsupials revealed conserved results in the six $2n = 22$ species analyzed (Chironectes minimus, Didelphis albiventris, D. marsupialis, Philander frenatus, P. opossum and Lutreolina crassicaudata), that presented no ITS. In three out of the four species with $2n = 18$ studied (Monodelphis brevicaudata, M. domestica and M. kunsi), ITS were conserved in the pericentromeric region of pair 1. The results in the eight $2n = 14$ species analyzed were more variable: four species had no ITS (Caluromys philander, Metachirus nudicaudatus; Marmosa murina and Gracilinanus emiliae), Gracilinanus microtarsus had ITS on pair 1, Micoureus demerarae had a maximum of eight signals on the four largest autosome pairs, Marmosops incanus had ITS on pairs 1-5 and M. parvidens presented ITS in all of its six autosomal pairs (Svantman and Vianna-Morgante, 1998; Carvalho and Mattevi, 2000; Pagnozzi et al., 2000, 2002).

It is interesting to point out that ITS were only observed in biarmed chromosomes. Another point to be stressed is that, with the exception of Marmosops incanus and Metachirus nudicaudatus studied by Svartman and Vianna-Morgante (1998), the results obtained by the other authors were from metaphases derived from bone marrow cells. These spreads present relatively condensed chromosomes when compared with those derived from cultured fibroblasts. A closer look at the results obtained by Svartman and Vianna-Morgante (1998) clearly show that the ITS detected by FISH in Marmosops incanus are close to but not inside the pericentromeric heterochromatin, as suggested by Pagnozzi et al. (2002).

Although the fusion hypothesis was clearly advanced to account for the karyotypic evolution of the three diploid numbers in American marsupials (Svantman and Vianna-Morgante, 1998; Carvalho and Mattevi, 2000) Metcalfe et al. (2004) studied the distribution of ITS and of constitutive heterochromatin in nine species of Australian marsupials to test this view. Three of the species analyzed presented the presumed “ancestral” $2n = 14$ karyotype and the other six species were Macropodinae with varying diploid numbers which had been previously extensively studied. Centromeric ITS were observed in the largest three chromosome pairs of the $2n = 14$ species and also on pair 6 in two of them. In the Macropodinae species large ITS signals were observed in almost all chromosomes and the same sites were shown to be C-band positive. Macropus agilis, the only species without extensive C-banding, presented ITS in three pairs which corresponded to fusion sites from the putative $2n = 22$ ancestral macropodine complement. The authors associated the presence of most ITS with heterochromatin, but attributed the ITS observed in Macropus agilis to be the result from previously postulated chromosome fusions. Based on these results, the authors concluded that ITS represent telomeric sequences that are part of the native satellite DNA and that they only represent remnants of rearrangements when not present in the heterochromatin, a conclusion similar to that advanced by Pagnozzi et al. (2000, 2002).

In conclusion, it seems that the support given by various authors to each of the alternative hypotheses (fission or fusion) is strongly influenced by their view on how the chromosome data should be interpreted. Thus, those who believe that chromosome data are accessory to other kinds of datasets and should be used to corroborate them favor the fission hypothesis. Contrarily, authors that tend to interpret chromosome data independently before comparison with other kinds of studies are more inclined to support the fusion hypothesis. In any case, the resolution of this debate will rely on more detailed studies aiming at a molecular characterization of the ITS in marsupials. The sequencing of these regions could reveal if their composition is strictly telomeric or if they are part of satellite DNAs located at the heterochromatin. Another possibility is to verify if the composition of the satellite DNAs present in the heterochromatin of different species is similar and contain telomere sequences embedded in them.

Marsupials and Sex Chromosomes

Among the main contributions resulting from marsupial genetics studies are those related to sex chromosomes evolution and function. Sex determination in eutherians depends on the presence of the Y chromosome. Testis development is primarily due to the SRY gene and other male characters develop later under the control of the testicular hormones. Testes development in marsupials is also determined by the Y chromosome, which bears an orthologue of the human SRY gene (Foster et al., 1992). Nevertheless, as demonstrated by Shaw et al. (1990), in marsupials the scrotum develops before the testes and thus, differently from
eutherians, scrotum differentiation is not dependent on the hormones secreted by the developed testis.

Like most mammals, the majority of marsupials present a chromosome sex determination system of the XX:XY type. Multiple sex chromosomes systems were only described in four Australian species: in Potorous tridactylus, Wallabia bicolor and Macrotris lagotis the multiple sex chromosomes result from a translocation of an autosome to the X chromosome, whereas Lagorchestes conspicillatus presents a more complex system, involving translocations between two autosomes and both sex chromosomes (reviewed in Hayman, 1990).

Sex chromosome mosaicism resulting from their elimination in somatic tissues was described in several species. Almost all specimens from the Australian family Peramelidae (genus Echymipera, Isoodon, Perameles and Peroryctes) analyzed had one X chromosome eliminated in females and the Y chromosome eliminated in the same somatic tissues in males. The Y chromosome was absent in most cells of the bone marrow, liver and spleen of Petauroides volans (family Petauridae), but cells of the same tissue retained both X chromosomes in females (Murray and McKay, 1979).

A mechanism similar to that of P. volans, with the absence of the Y chromosome in bone marrow cells, was described in the American marsupial Dromiciops gliroides and used as evidence of its close phylogenetic relationship to Australian marsupials (Gallardo and Patterson, 1987). Nevertheless, the tiny Y chromosome of D. gliroides was probably missed by the authors, as discussed above in a previous topic.

Some cytological features of marsupial sex chromosomes are peculiar: the very small Y chromosome, which is dot-like in many species, and the small size of the X chromosome (3% of the genome) in relation to the eutherian X (5% of the genome) (Hayman et al., 1982); the absence of the synaptonemal complex between the X and Y chromosomes during meiosis, which may be due to the absence of the pseudoautosomal region in marsupials (Sharp, 1982); the presence of nucleolus organizer regions (NORs) in the X or in both sex chromosomes of some species without their inactivation on one of the X chromosomes in females, which implies in escape from inactivation and no dosage compensation for these loci (Hayman, 1990; Svartman and Vianna-Morgante, 2003); and the differential location of chiasmata in the meiosis of males and females of some species (interstitial chiasmata in males and distal chiasmata in females, resulting in a much higher recombination rate in males), as Sminthopsis crassicaudata and Monodelphis domestica (Bennett et al., 1986; Hayman et al., 1988; Samollow et al., 2004).

The absence of a synaptonemal complex between the X and Y chromosomes during the first meiotic prophase in males of the South American marsupials Thylamys elegans, Dromiciops gliroides and Rhyncholestes raphanurus, was demonstrated by Page et al. (2003, 2005). Instead, a structure called dense plate, a modification of the axial elements of the sex chromosomes, forms between the X and Y chromosomes during pachytene, thus ensuring their correct segregation.

The inactivation of the X chromosome in marsupials also presents important differences in relation to that of eutherians. In the somatic cells of eutherian females one X chromosome is randomly inactivated. This inactivation is stable through cell generations and encompasses most genes on the X chromosome. In marsupials and monotremes, there is preferential inactivation of the paternal X chromosome, which is incomplete and tissue specific (Cooper et al., 1990; Heard and Distecho, 2006). The mechanisms involved in this inactivation are just starting to be understood (Hornecker et al., 2007; Namekawa et al., 2007).

Among the studies performed in marsupials, gene mapping was especially fruitful in providing important information related to chromosome evolution in mammals, particularly about the sex chromosomes.

Marsupials were very important in the identification of the mammalian testis determining factor (TDF) on the Y chromosome. The autosomal location of the putative TDF, the ZFY gene, in marsupials, was a strong indication that this was not the TDF gene (Sinclair et al., 1988). The later finding of SRY, another candidate for TDF, on the marsupial Y chromosome, was used as evidence to corroborate its role as the mammalian TDF (Foster et al., 1992).

Mapping of human X-linked genes in marsupials resulted in important findings. Genes already mapped in the human Xq were also mapped in the marsupial and monotreme X chromosomes, defining a mammalian X-conserved region (XCR). Nevertheless, human Xp genes were located in three autosomal regions in both marsupials and monotremes (reviewed in Wilcox et al., 1996 and in Graves, 2006).

Based on the sex chromosomes mapping data and behavior in the three mammalian groups, Graves (1995) suggested that the eutherian sex chromosomes evolved through cycles of autosome additions to the ancestral X and Y chromosomes followed by the degradation of Y chromosome sequences and the recruitment of the equivalent regions on the X by the inactivation mechanism. The pseudoautosomal region on the human sex chromosomes would be a relic of the last addition and the genes on the Y chromosome without an essential function in sex determination and differentiation in males would tend to mutate or be deleted (Graves, 1995; reviewed in Graves, 2006).

Gene mapping results in marsupials contradicted the hypothesis of mammalian X chromosome conservation proposed by Ohno (1967), as only part of the X was conserved in mammals. The same results also weakened the idea proposed by Lyon (1974) that the loss of Y chromosome genes and X chromosome inactivation could have
happened in a single deletion or translocation event, as sug-
uggested by the lack of variation in size and gene content of
the mammalian X chromosome.

Gene mapping data in American marsupials were
much more limited than those from Australian species. Un-
til a decade ago, Didelphis virginiana was the only Ameri-
can marsupial species to have genes mapped by in situ
hybridization. In this case, G6PD was located on the Xp
and HPRT was mapped on the Xq (Driscoll and Migeon,
1988). These genes are in the region considered to be con-
served on the X chromosome of all species of eutheri-
ans, marsupials and monotremes already studied. They are
closely located in eutherians and more distantly located in
marsupials. In most species of Dasyuridae and Didelphidae
the X chromosome is a small acrocentric, considered to be
the marsupial ancestral X (Rofe and Hayman, 1985). The X
chromosome of D. virginiana is submetacentric and proba-
bindly originated from a pericentric inversion in the D.
marsupialis X chromosome, which would explain the loca-
tion of G6PD and HPRT in different X chromosome arms
(Driscoll and Migeon, 1988).

Recently, the lack of more detailed data from Ameri-
can marsupials changed radically with the publication of
the genome sequence of Monodelphis domestica, the first
marsupial species to be sequenced (Mikkelsen et al., 2007).
This species has been used as a model laboratory animal for
biomedical research for some decades and, although origi-
nally from South America, has been studied by scientists
worldwide. The contributions provided by the study of M.
domestica for different biological areas along the years
were recently reviewed by Samollow (2008) and include
very important data on mammalian genetics.

In conclusion, studies already performed in marsupi-
als, particularly in Australian species, have demonstrated
the great potential provided by the group for the under-
standing of basic genetic mechanisms in mammals. Genetic
studies in American marsupials are relatively scarce and
cytogenetic data of most species are restricted to karyotype
description, usually without banding patterns. We hope to
see this situation change by having South American re-
searchers interested in studying marsupials, which are not
only an important component of our biodiversity, but a trea-
sure trove of information on genome function and evolu-
tion.

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