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Karyotype and chromosomal polymorphism of an undescribed *Akodon* from Central Brazil, a species with the lowest known diploid chromosome number in rodents

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Abstract. The diploid chromosome number of 2n = 10 found in a new species of *Akodon* (Cricetidae, Rodentia) from two localities of the state of Mato Grosso, Central Brazil, represents the lowest chromosome number known for rodents. One female with nine chromosomes due to sex chromosome monosomy (2n = 9,XO) was also found. The karyotype comprises two large metacentric pairs (1 and 2); one large polymorphic pair (3), which could be acrocentric, submetacentric, or heterozygous as a result of a pericentric inversion; and one minute metacentric pair (4). The sex determination is of the XX/XY type. CBG, GTG, and RBG banding patterns, Ag-NORs, and meiotic data are presented. Fluorescence in situ hybridization with a $(TTAGGG)_7$ repeat as a probe revealed interstitial telomeric bands (ITBs) in two of the large pairs.

Akodon is a widespread genus in South America and one of the largest, most complex, and taxonomically poorly understood among rodents. There are around 45 species in the genus (Musser and Carleton, 1993), which includes morphologically very similar species, such as *A. cursor* and *A. montensis*, with 2n = 14–16 and 2n = 24–26, respectively (Christoff, 1997).

Akodontine karyotypes present a wide range of diploid numbers, the majority being characterized by moderate to low chromosomal and fundamental numbers (Gardner and Patton, 1976). A remarkable degree of intraspecific and intrapopulational polymorphism due to Robertsonian events, pericentric inversions, supernumerary chromosomes, and sex-chromosome variability have been described (Yonenaga-Yassuda et al., 1979; Kasahara and Yonenaga-Yassuda, 1984; Fagundes et al., 1997).

Recently, morphological comparisons of craniodental structures and cranial dimensions associated with cytogenetic data in samples of *Akodon* from eastern Brazil allowed the chromosomal characterization of five species: *A. cursor* (2n = 14–16), *A. montensis* (2n = 24–26), *A. lindberghi* (2n = 42), *A. serrensis* (2n = 46) and *Akodon* sp. (2n = 44). The karyotypes were important markers in clarifying the systematic problem involving those species (Christoff, 1997).

Heretofore, the lowest chromosome numbers for rodents (2n = 14–16; FN = 18–26) were described for *A. cursor* from different localities of Brazil. This species has a high level of chromosomal polymorphism, with at least 24 different karyotypes due to pericentric inversions involving three pairs of chromosomes and a complex rearrangement of the largest pair (Yonenaga, 1972; Yonenaga-Yassuda, 1979; Sbalqueiro and Nascimento, 1996; Fagundes et al., 1997).

Here we describe the lowest diploid number for rodents, one still undescribed species of *Akodon* from two localities of the state of Mato Grosso, Brazil. Twenty-eight specimens presented 2n = 10,XX or XY (FN = 14–16), and one female had 2n = 9,XO (FN = 14). Autosomal polymorphism due to a pericentric inversion in pair 3 and the X monosomy resulted in four different karyotypic constitutions in the species. CBG, GTG, and RBG banding, Ag-NOR staining, fluorescence in situ hybridization (FISH) with telomeric probes, and a meiotic analysis were performed.

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Materials and methods

Specimens

Twenty-six specimens (13 males and 13 females) of *Akodon* sp. collected at Gaúcha do Norte (13°11′00″ S, 57°23′36″ W) and two males and one female from Vila Rica (9°54′28″ S, 51°12′13″ W) were examined cytogenetically. Both localities are in the state of Mato Grosso in Central Brazil. These regions are geographically separated by nearly 450 km and situated in a transitional area between the Amazon rain forest and the Cerrado dry forest. The climate in both regions is highly seasonal, with a rainy season lasting from November to March and a dry season from June to August. The animals were collected during April to May and September to October, 1997.

The specimens are deposited in the Museu de Zoologia da Universidade São Paulo (MZUSP) collection, in the state of São Paulo, Brazil.

Chromosome preparation and banding

Chromosome preparations were obtained in the field from bone marrow and spleen after in vivo colchicine treatment. Fibroblast cultures from ear biopsies of eight specimens were established in the laboratory using Dulbecco's modified Eagle's medium (MEM) supplemented with 20% fetal bovine serum. CBG and GTG-banding patterns and Ag-NOR staining were obtained for 13 individuals, using routine techniques. RBG-banding was performed on chromosome preparations of one male and one female after in vitro BrdU incorporation (Dutrillaux and Couturier, 1981). Testicular chromosome spreads from three specimens were also obtained.

Length measurements

Each chromosome length was estimated as a percentage of the length of the female haploid set in 10 conventionally stained metaphase spreads from four individuals. The mean of the values for the two homologs was taken as the relative length of each chromosome per cell.

FISH

For localization of telomeric sequences, TTAGGG heptameres were synthesized in both sense and antisense orientation (GIBCO BRL) and used as probe for FISH experiments after 3′-end labeling with biotin-11-dUTP (Boehringer Mannheim). Slides from six specimens were treated with RNase and denatured for 2 min at 75 °C in 70% formamide, 2 × SSC. The probe was denatured for 10 min at 75 °C and was applied at a concentration of 25 ng/µl in 50% formamide, 2 × SSC, 40 mM sodium phosphate (pH 7.0), 0.1% SDS, and 10% dextran sulfate. Hybridization was performed overnight at 37 °C. The slides were washed twice (5 min each) in 50% formamide, 2 × SSC at 42 °C, twice (5 min each) in 2 × SSC at 42 °C, and three times (5 min each) in buffer at room temperature. Hybridization signals were detected by incubation with fluorescein isothiocyanate (FITC)-labeled anti-biotin, and the slides were counterstained with propidium iodide in fluorescence antifade solution. Chromosome signals were visualized using a Zeiss Axiophot microscope equipped with an FITC filter and photographed on Kodak Ektachrome 400 color film.

Results

Mitotic chromosomes

A diploid number of 2n =10 was found in a sample of 28 specimens of *Akodon* sp. The fundamental number was variable, ranging from 14 to 16, due to a pericentric inversion of pair 3. One female from Gaúcha do Norte presented monosomy X (2n = 9,XO; FN = 14).

The karyotype included two large metacentric pairs (1 and 2); a polymorphic pair 3, which could be a homozygous submetacentric (3SM), homozygous acrocentric (3A), or heterozygous acrocentric/submetacentric (3H); and a dot-like metacentric pair 4. The X was acrocentric and the Y a small subtelocentric (Fig. 1). Among the 26 specimens from Gaúcha do Norte, the frequencies of the different morphologies of pair 3 were: 3A = 0.46, 3H = 0.46, and 3SM = 0.08. In the sample from Vila Rica, the female had an acrocentric pair 3 (3A), whereas both males were heterozygous (3H).

CBG banding revealed conspicuous blocks of constitutive heterochromatin in the pericentromeric regions of pair 1 and the X chromosome, whereas pair 2 presented a very small amount of pericentromeric heterochromatin, and pairs 3 and 4 and the Y chromosome had no positive C-bands (Fig. 2a). All of the chromosome pairs were identified after GTG and RBG banding, which allowed the delimitation of the segment of pair 3 involved in the pericentric inversion of pair 3 (Fig. 2b, c).

The Ag-NOR bearing chromosomes were identified by sequential RBG banding and silver staining. The Ag-NORs were located interstitially in the short and long arms of chromosome 1, at the telomeric region of 2p, and in one of the arms of the minute metacentric pair 4 (Fig. 3). Some metaphases presented a secondary constriction in pair 1 (1p) which corresponded to a nucleolus organizer region. Forty-seven metaphases from two individuals were analyzed, and Ag-NORs ranged from two to six per cell, with a mean of 3.75 and a mode of 4. Associations of NOR-bearing chromosomes were not observed (Fig. 3).

Length measurements

The largest pairs, 1 and 2, corresponded to about 35% and 32% of the haploid set, respectively; pair 3 was about 24% and the minute metacentric pair 4 about 2% of the haploid set. The X chromosome represented 7% of the haploid set, whereas the Y was about 3%.

FISH

Hybridization signals were observed at the telomeres of all chromosomes. Additionally, chromosome 1 showed a strong signal at the pericentromeric region, as well as interstitial telomeric bands (ITBs) in the short arm (1p). Another strong ITB was also found in the long arm of pair 3 (Fig. 4). Some metaphases showed a weak signal in the distal portion of 3q.

Meiotic chromosomes

Analysis of diakinesis of three males revealed four autosomal bivalents, with the largest pairs presenting chiasmatic associations and the sex pair typical end-to-end pairing in all diakineses analyzed (Fig. 5). The individual that was heterozygous for a pericentric inversion in pair 3 had four bivalents whose configuration did not differ from those observed in the homozygotes 3A or 3SM.

Discussion

The *Akodon* sp. that we examined had an exceptional karyotype of 10 chromosomes (FN = 14–16). A high level of polymorphism was detected with four different karyomorphs in a sample of 29 specimens.

The karyotype had four pairs of autosomes and one sex-chromosome pair. The three largest pairs represented 91% of the haploid set, and they have probably been involved in complex rearrangements to give rise to a karyotype with the lowest diploid chromosome number observed so far among

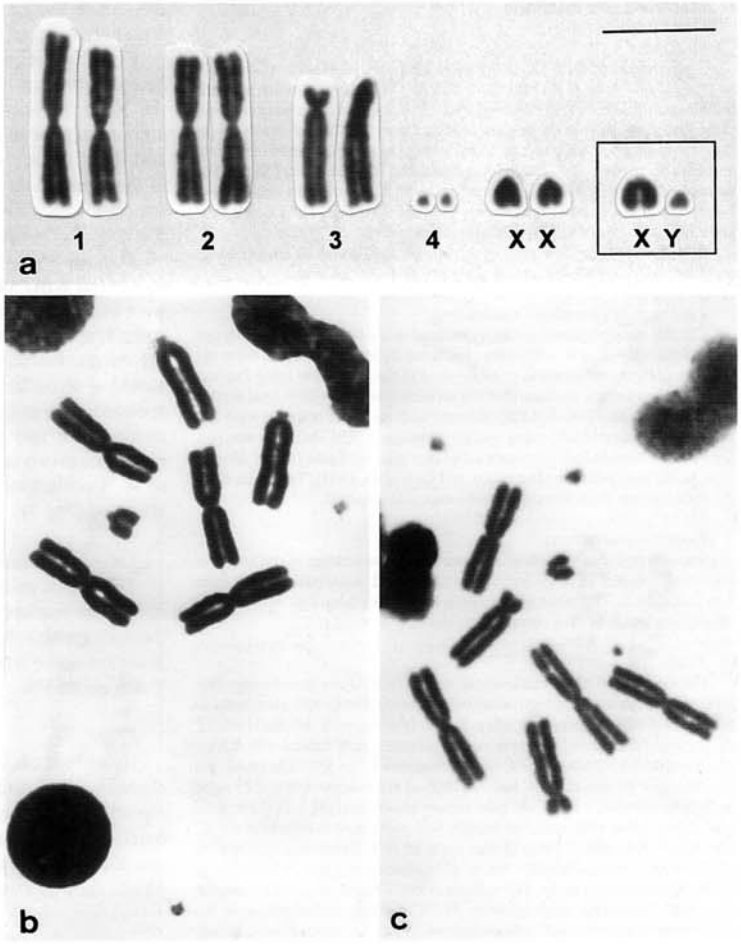


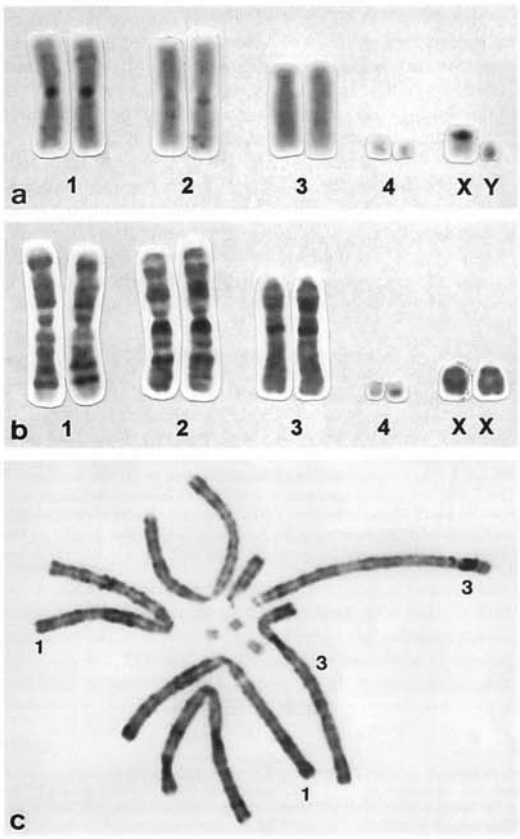
Fig. 1. Conventionally stained chromosomes of *Akodon* sp. (**a**) Karyotype of a female, 2n = 10, with a heterozygous chromosome pair 3 (3H); inset: XY chromosome pair. (**b**) Female metaphase with 2n = 9,XO and a homozygous acrocentric pair 3 (3A). (**c**) Male metaphase, 2n = 10, with a homozygous submetacentric pair 3 (3SM). Bar = 10 μm.

rodents. The minute autosome represents only 2% of the haploid set.

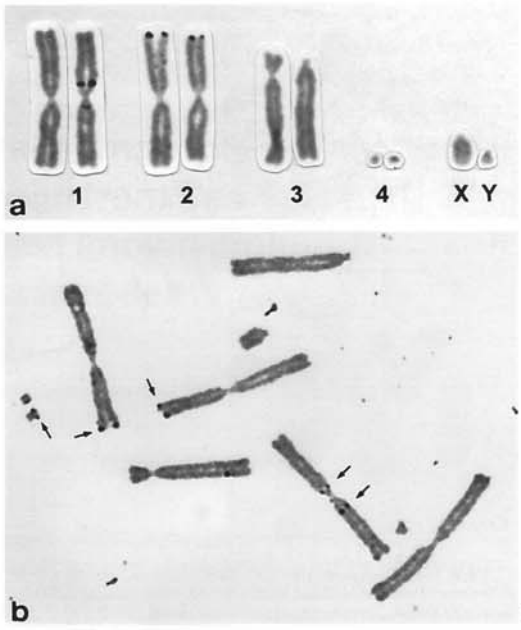
Autosomal polymorphism was the result of a pericentric inversion. This variation was observed in both localities from which samples were collected, and it is remarkable that two of the three individuals collected in Vila Rica were heterozygous for the inversion. The frequencies of acrocentric homozygous (3A) and heterozygous (3H) forms (46% each) were higher than the frequency of the submetacentric homozygous (3SM) form. Despite the lack of data on synaptonemal complex formation, the conventionally stained diakineses of the heterozygous specimens did not differ from those of the homozygous ones. This suggests the presence of such mechanisms as heterosynapsis or synaptic adjustment that suppress meiotic stress and maintain the polymorphism throughout the populations. A number of studies on the synaptonemal complex in heterozygous carriers of pericentric inversions have shown the occurrence of heterosynapsis with chiasma suppression as a meiotic strategy for the maintenance of this sort of rearrangement in some species, including *A. cursor* (Greenbaum and Reed, 1984; Hale, 1986; Fagundes, 1993).

We also observed monosomy X in one *Akodon* sp. female. The X chromosome of mammals represents around 5% of the genome, but in this species it corresponded to about 7%, and the difference is probably due to the addition of constitutive heterochromatin. Monosomy of sex chromosomes, as well as the occurrence of XY females, seems to be more frequent than previously supposed in this genus. Recently, Fagundes (1997) described a sample of 63 specimens of *A. montensis,* from the state of São Paulo, Brazil, in which five females were XY and one was XO; 10% of the population, therefore, had sex-chromosome abnormalities.

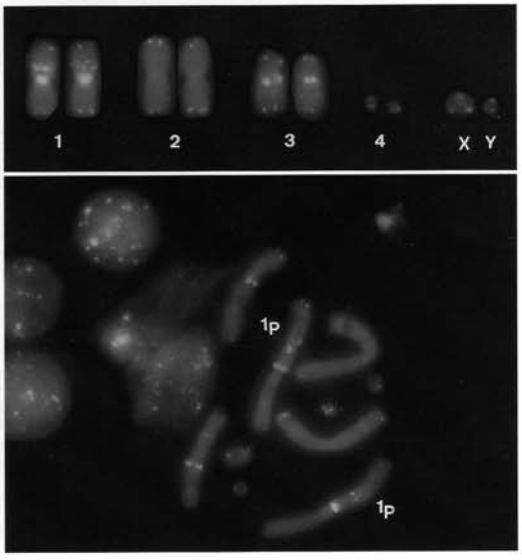
The karyological similarity between *Akodon* sp. (2n = 9, 10) and *A. cursor* (2n = 14–16), led us to believe that they are relat-



2



3



4a

4b

Fig. 2. Banding patterns of *Akodon* sp., 2n = 10. (**a**) CBG banding of male metaphase cell. (**b**) GTG banding of female metaphase cell. (**c**) RGB banding of male metaphase cell.

Fig. 3. NOR-bearing chromosomes in *Akodon* sp., 2n = 10. (**a**) Karyotype showing Ag-NORs at 1p and 1q in one homolog and in the telomeric region of 2p. (**b**) Metaphase cell with a total of five Ag-NORs, located at 1p and 1q, at 2p in both homologs of pair 2, and one homolog of pair 4 (arrows).

Fig. 4. Result of FISH with telomeric $(TTAGGG)_7$ probes. Telomeric signals are evident at all chromosome telomeres, as well as in the interstitial telomeric bands (ITBs) at 1p and 3q. (**a**) Karyotype. (**b**) Metaphase cell preparation.

ed species. *Akodon cursor,* as well as *Akodon* sp., presents a remarkable example of chromosomal polymorphism, with pericentric inversions involving pairs 2, 3, and 5 and a complex rearrangement including a fusion and a pericentric inversion in pair 1 (Fagundes et al., 1997).

Our FISH data revealed conspicuous ITBs at the pericentromeric region of pair 1 and at 1p and 3q. These ITBs could be remnant sequences of chromosomal rearrangement events, as have been suggested for some species (Meyne et al., 1990; Lee at al., 1993; Vermeesch et al., 1996).

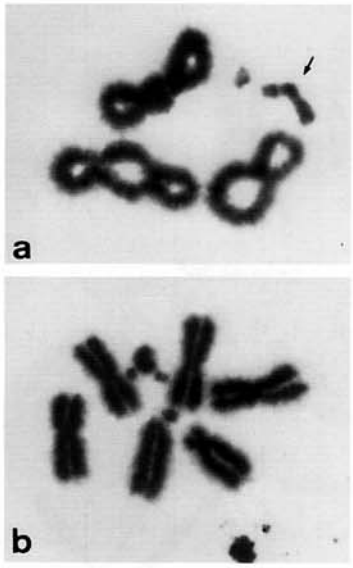


Fig. 5. Meiotic cell preparations of a male *Akodon* sp., 2n = 10, pair 3H. **(a)** Diakinesis with five bivalents, including the XY pair showing end-to-end association (arrow). **(b)** Spermatogonial metaphase cell.

The lowest and highest chromosome numbers now known for rodents range from 2n = 9 and 10, described in the present paper for a new *Akodon* species found in Central Brazil, to 2n = 102, discovered in *Tympanoctomys barrarae,* a desert member of the family Octodontidae and an ancestor of the South American hystricognath rodents (Contreras et al., 1990).

Our exceptional finding in *Akodon* sp. is in accordance with cytogenetic data on the majority of South American akodontines, which are characterized by reasonably low diploid chromosome numbers, a high frequency of pericentric inversions, the presence of a minute pair of metacentrics, and a moderate amount of sex-chromosome variability (Bianchi et al., 1971; Kasahara and Yonenaga-Yassuda, 1984; Fagundes, 1993, 1997).

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