Chromosomal Variability in Akodon sp. (Rodentia, Cricetidae)

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In the genus Akodon approximately 15 South American species have been studied cytogenetically heretofore. Large variations in diploid numbers from 2n=14 in A. arviculoides (Yonenaga 1972) to 2n=52 in A. xanthorhinus (Bianchi et al. 1971) have been found. Furthermore, chromosome polymorphisms due to numerical and structural rearrangements both within the same population and among different populations have been described (Bianchi and Contreras 1967, Bianchi et al. 1973, Yonenaga et al. 1975, Yonenaga-Yassuda 1979).

Yonenaga et al. (1975) reported a case of interspecific hybridization between A. arviculoides (2n=14) and Akodon sp. (2n=24), two morphologically indistinguishable species. In the hybrids (2n=19), homology of G-band patterns of some chromosomes or chromosomal segments of these two species was established. Homology of several G-banded chromosomes from A. molinae (2n=42), A. azarae (2n=38) and A. obscurus (2n=34) was also found (Bianchi et al. 1976).

Yonenaga et al. (1975) described the Q-band patterns of Akodon sp. and a variation in the chromosome number (2n=24, 25) due to a supernumerary chromosome. A summary of cytogenetic data on two cases of mosaicism due to the presence of supernumeraries and Y chromosome elimination from somatic cells was presented by Yonenaga et al. (1976).

This paper presents additional data on the chromosomal variability in Akodon sp. as found in specimens collected from several regions in the state of São Paulo, Brazil.

Material and methods

The sample comprises 18 males and 12 females which were collected from Itapetininga, Iguape, Salesópolis, Guararema, Caçapava, Taubaté and São Paulo, in the state of São Paulo.

For chromosome analysis air-dried preparations of bone marrow, spleen, thyme and testis were made after “in vivo” colchicine 0.1% treatment (1 ml per 100 g of weight, for 2 hours); 0.075 M KCl was used as hypotonic solution for 15
minutes at 37°C; the fixative was 3:1 methanol: acetic acid. Conventional staining was done with buffered Giemsa 2%, pH 6.8. G-bands were obtained by trypsin treatment (Seabright 1971) and C-bands, according to the method described by Sumner (1972).

Results

Somatic chromosomes

Fifteen males and 11 females had a 2n=24 karyotype which included 9 pairs of large and medium sized metacentrics and submetacentrics, 1 pair of small acrocentrics and 1 pair of minute metacentrics; the X chromosome was usually found as a medium sized acrocentric and the Y chromosome was always a minute acrocentric. Six specimens (4 males and 2 females), however, showed a slightly different X chromosome which had an enlarged short arm (X marker). No female was homozygote for this X marker. A G-banded karyotype of a male is presented in Fig. 1.

One male and 1 female had a 2n=25 karyotype with a small extra submetacentric chromosome which was interpreted as a supernumerary. The female also had an X marker.

Two males showed mosaicism. One mosaic (2n=25/26) had one or two supernumerary chromosomes in bone marrow, spleen and thymic cells (Figs. 2a, 2b). The supernumerary appeared uniformly stained after G-banding. The frequencies of 2n=25 and 2n=26 cells were, respectively, 40% and 60%, in 165 cells studied.
The second case presented 2n=23/24 in spleen and bone marrow. The 2n=24 cells had a normal XY constitution while the 2n=23 cells lacked the Y chromosome. The frequency of the XO and XY cells were about 80% and 20%, respectively, in 60 cells studied. Both mosaics showed an acrocentric X without an enlarged short arm.

Figs. 2-3. 2a-b, G-banded metaphases of a male Akodon sp. with mosaicism. a, 2n=25 cell. b, 2n=26 cell. Arrows indicate the supernumerary chromosomes. 3, G-banded diagram of metaphase chromosomes of Akodon sp.

The precise identification of all chromosomes in Akodon sp. could be made only in the banded karyotype (Fig. 1). A simplified diagram of the main G-band patterns of Akodon sp. chromosomes is shown in Fig. 3.
Figs. 4–6. 4, C-banded karyotype of a male Akodon sp. (2n = 24). The X chromosome is an acrocentric. 5, C-banded X chromosomes of a female Akodon sp., showing heteromorphism. 6a–b, a, G- and b, C-banded meiotic chromosomes of a male Akodon sp. (2n = 24) showing 12 bivalents. The sex chromosome pair is indicated by an arrow.
The C-band patterns of the somatic chromosomes are presented in Fig. 4. Constitutive heterochromatin was distributed at the pericentromeric regions of the autosomes and of the acrocentric X chromosome, and throughout the Y. The X marker did not show any heterochromatin (Fig. 5). In some cells telomeric hetero-

chromatin was noticed in pairs 2 and 10 and this was even more evident in meiotic cells (Fig. 6b).

**Meiotic chromosomes**

Meiotic analysis of 2n=24 males disclosed a condensed sex vesicle at pachytene, 12 bivalents at diplotene and metaphase I, and 12 dyads at metaphase II. Some
meiotic preparations were treated for obtaining G- and C-bands (Figs. 6a, 6b). The bivalents 1 and 2 which could be well analysed showed the typical G-bands as presented by the corresponding somatic chromosomes.

The 2n=25/26 male also had two cell lines in the gonads (Fig. 7a, 7b, 7c, 7d). Among 68 cells at diplotene or metaphase I, 35 had one supernumerary as a univalent; 33 had two supernumeraries which appeared in 27 cells as two univalents and as a bivalent in the remaining 6 cells. At metaphase II we found 24 cells with no supernumeraries, 14 cells with one supernumerary, and 2 cells with two supernumeraries.

The meiotic analysis of the 2n=23/24 male did not reveal an XO cell line in the gonads. Six spermatogonial metaphases showed a normal XY constitution. In 25 cells at diplotene or metaphase I, 12 bivalents, including the sexual pair, were always found.

**Discussion**

The inter and intraindividual chromosome number variations in *Akodon* sp. (2n=24, 2n=25, 2n=25/26) are due to the presence of 1 or 2 small supernumerary chromosomes which can be identified even in a non-banded karyotype. This variability is probably due to reduced mechanical stability (John and Lewis 1968) which leads such chromosomes to non-disjunction or anaphase-lag during mitosis and meiosis. Accordingly, the 2n=25/26 individual must have originated from a 2n=26 zygote by the loss of one supernumerary in an early cell division. However, a non-disjunction of the supernumerary in a 2n=25 zygote can not be completely ruled out, even though, a 2n=24 cell line with no supernumerary has not been detected. These cells may have been eliminated in competition with 2n=25 and 2n=26 cells or they may occur in a tissue not investigated.

Two supernumerary chromosomes were seen in some metaphase II cells resulting from non-disjunction. This could represent an accumulation mechanism as described by Patton (1977) in *Perognathus baileyi*. This being true, individuals with more than two supernumeraries are expected to appear.

The role of supernumerary chromosomes is not clear, but some effects on morphological traits, fertility, vigor and chiasma frequency are ascribed to them (Jones 1975). Even an evolutionary significance is suggested by the wide occurrence of supernumeraries among vertebrates (Yonenaga et al. 1976). In a recent report (Assis et al. 1978), nucleolar organizing regions were described at the telomeres of both the long and short arms of a supernumerary chromosome in *Akodon* sp. (2n=25). This finding is indicative that, at least in this species, such a chromosome has a role in nucleolar organization.

The X chromosome in *Akodon* sp. is polymorphic due to the presence of an enlarged short arm in some specimens. G-band analysis suggests that there is a true addition of chromosome material since the banding patterns are similar on the long arms of both X types.

Variations in size and morphology of the X chromosome in the same species are described among rodents and, in general, they are the result of an increase or
reduction in the amount of constitutive heterochromatin visualized by C-bands (Mascarello et al. 1974, Baverstock et al. 1977). This is not the case in our specimens since the marker X failed to show any C-bands either in the enlarged short arm or at the pericentromeric region. On the otherhand, a pericentromeric C-band was present in the acrocentric X of this species. The short arm of the X marker could be euchromatic resulting from a translocation or a duplication of a small segment, whereas, it could also be heterochromatic and not detected by C-banding technique. Indeed, biochemical changes which affect the affinity of heterochromatin to the Giemsa staining appears to cause reduction or even the absence of C-bands (Yosida and Sagai 1975).

The X chromosome variation found in Akodon sp. is similar to that of the polymorphic autosome no. 1 of Notomus alexis (Baverstock et al. 1977). One of the chromosome forms is relatively larger than the others. This form has no short arm and shows no C-bands, while all the others show variable amounts of heterochromatin on their short arms. The origin of such a type of variation is, according to the authors, an enigma.

The male specimens with a 2n=23/24 mosaicism must have resulted from somatic elimination of the Y chromosome, giving rise to an XO/XY chromosomal constitution. Meiotic analysis discarded the hypotesis of a translocation between the Y and an autosome since a typical sex bivalent with end-to-end pairing was observed in diplotene and metaphase I cells.

Elimination of sex chromosomes from somatic cells is found in some species of marsupials and eutherian mammals. It has been thought to represent an uncommon and extreme manifestation of the inactivation mechanism (Hayman and Martin 1965, Bianchi 1973). The elimination of the Y chromosome in Akodon sp. is a sporadic event and apparently does not affect the fertility of the specimen.

Summary

In a sample of 30 specimens of Akodon sp. (Rodentia, Cricetidae), diploid numbers of 2n=24 and 2n=25 along with two mosaic cases with 2n=25/26 and 2n=23/24 were found. The inter and intraindividual chromosome variability of 2n=24, 2n=25 and 2n=25/26 was due to the presence of supernumerary chromosomes. The 2n=23/24 mosaic resulted from elimination of the Y chromosome in somatic cells, the gonads showing a normal XY constitution. Heteromorphism of the X chromosome was also observed. G- and C-band patterns of mitotic and meiotic chromosomes are described.

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