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Chromosomal banding patterns in *Akodon arviculoides* (2n=14), *Akodon* sp. (2n=24 and 25), and two male hybrids with 19 chromosomes

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Abstract

Chromosomal polymorphism resulting from two pericentric inversions in *Akodon arviculoides* (2n=14) has been described (YONENAGA, 1972*a*). In this paper the banding patterns are presented and identification of the inverted segments of the autosomal pairs 2 and 3 is made. The karyotype of *Akodon* sp., which varies in diploid number (2n=24 and 25), is described and shown to be due to the presence of a small submetacentric chromosome in the 2n=25 individuals. The karyotypes of two 2n=19 males studied show that they are hybrids between *Akodon arviculoides* (2n=14) and *Akodon* sp. (2n=24).

Cytogenetic studies of the South American *Akodon* rodents were reported by BIANCHI et al. (1971). Constitutive heterochromatin, G-bands, and Robertsonian rearrangements in the chromosome of *Akodon molinae* were studied by BIANCHI et al. (1973) in a population with a varying diploid number (2n=42, 43, and 44) due to polymorphism of chromosome 1. This variation was considered to result from a combination of Robertsonian and non-Robertsonian chromosomal rearrangements.

YONENAGA (1972*a*) has described chromosomal polymorphism in *Akodon arviculoides* (2n=14). Mitotic chromosome measurements as well as meiotic observations supported the hypothesis of pericentric inversions in two different autosomes. A karyotype of *Akodon arviculoides cursor* with 2n=24 has been described by CESTARI and IMADA (1968) as

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including a metacentric X chromosome. However, it is clear now that this was due to misidentification and that the X chromosome is actually an acrocentric. The same karyotype was described as *Akodon* sp. by YONENAGA (1972*b*), but in this case chromosomal variation due to the presence of a supernumerary chromosome was recorded. *Akodon arviculoides* (2n=14) and *Akodon* sp. (2n=24 and 25) have been morphologically indistinguishable up to now, and a taxonomic revision of their status is called for.

YONENAGA et al. (1976) have reviewed the cytogenetic data avaiable for Brazilian rodents, including a summary of the types of chromosomal variation found, e.g., pericentric inversions, centric fusion, supernumerary chromosomes, autosomal heteromorphism, elimination of the Y chromosome, and interspecific hybridization.

The present paper presents further information on the chromosomes of the two species of *Akodon* and describes cytogenetically two male hybrids between them.

Materials and methods

Our material included a sample of 17 specimens (11 males and 6 females) of *Akodon arviculoides* (2n=14) collected in the states of São Paulo and Rio de Janeiro; 8 specimens of *Akodon* sp., including 3 males and 2 females with 2n=24 and 2 males and 1 female with 2n=25 from São Paulo; and two male hybrids from the above species obtained in a laboratory cross.

For chromosome studies air-dried preparations of bone marrow and testes were processed according to methods previously described (YONENAGA, 1972*a*). G-bands were obtained by means of trypsin treatment (SEABRIGHT, 1972) and the ASG technique (EVANS et al., 1971); the fluorescence banding was performed according to CASPERSSON et al. (1970) with modifications.

Results

Chromosome complements in Akodon arviculoides *(2n=14)*

In the present sample it was possible to identify the inverted segments through banding techniques. In a total of 17 specimens we found two females and one male with an acrocentric pair 2 and a metacentric pair 3, one female and three males with acrocentric pairs 2 and 3, two males and two females heterozygous for pair 3 with an acrocentric pair 2, one male heterozygous for pair 2 with an acrocentric pair 3, one female and three

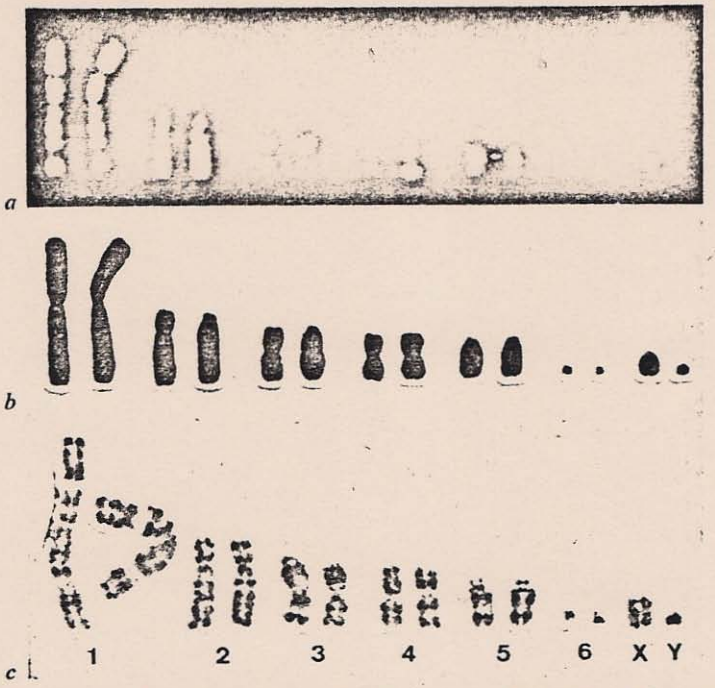

Fig. 1. Karyotypes of *Akodon arviculoides* (2n=14). *a.* Q-banding of a male heterozygous for pairs 2 and 3. *b.* The same karyotype with conventional Giemsa stain. *c.* G-banding of a male heterozygous for pair 3 with a submetacentric pair 2.

males heterozygous for pairs 2 and 3, and, finally, one male heterozygous for pair 3 with a submetacentric pair 2. Among 17 specimens studied, 10 were heterozygous for at least one pericentric inversion.

Figures 1*a* and *b* show the fluorescence banding pattern and karyotype of an *Akodon arviculoides* male heterozygous for pairs 2 and 3. Homology of the bands can be perceived in pair 2 and even more clearly in pair 3. The G-bands obtained by means of ASG and trypsin show good agreement. The G-band patterns in *Akodon arviculoides,* with its low diploid number (14), is very characteristic, especially in pair 1, which amounts to 37 % of the haploid set (YONENAGA, 1972*a*). G-bands present more subdivisions than Q-bands. Figure 1*c* shows the G-bands of a specimen heterozygous for pair 3 with a submetacentric pair 2.

Chromosome complements in Akodon *sp. (2n=24 and 25)*

Two males and one female of *Akodon* sp. present 2n=25 and three

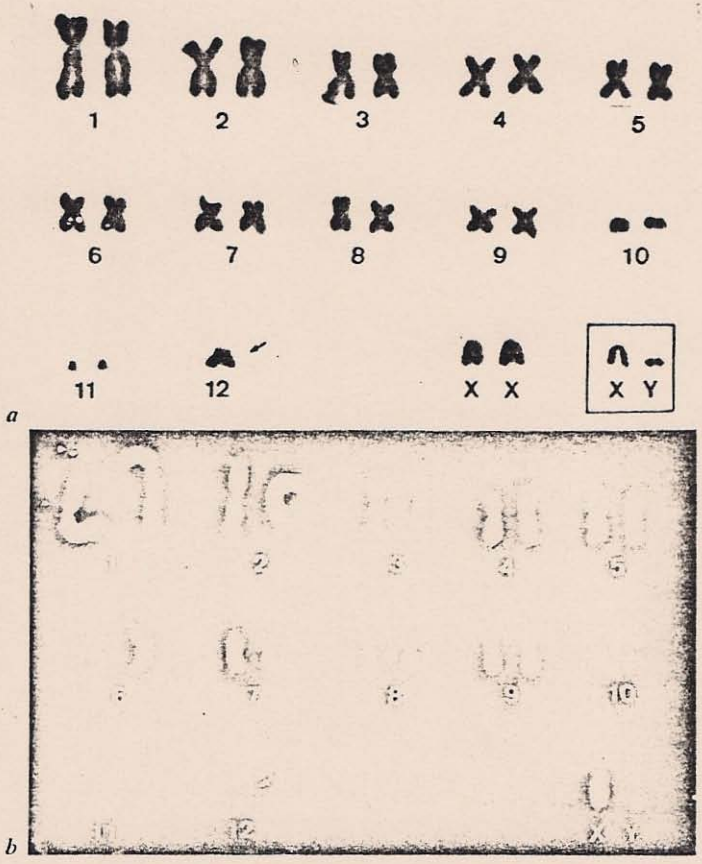

Fig. 2. a. Karyotype of *Akodon* sp. female (2n=25) showing one supernumerary chromosome indicated by an arrow. Inset: sex chromosomes of the male. *b.* Q-banding of *Akodon* sp. male (2n=25). The supernumerary chromosome is indicated by an arrow.

males and two females showed 2n=24. Figure 2*a* presents the chromosome complement of the species arranged in order of decreasing size, except for the small odd submetacentric chromosome. Some of the autosomes are not identifiable morphologically.

The fluorescence banding patterns of a male *Akodon* sp. (2n=25) is presented in fig. 2*b*. The Q-bands allow identification of each chromosome.

Meiotic analysis disclosed 12 bivalents at diplotene for the 2n=24 males and 12 bivalents (including the sex-chromosome pair) and one

Fig. 3. a–d. Meiosis of *Akodon* sp. (2n=25): *(a)* pachytene showing 11 autosomal bivalents, the sex-chromosome pair, and the univalent indicated by arrow; *(b)* diplotene with the univalent indicated by an arrow; *(c)* metaphase II with 12 chromosomes; *(d)* metaphase II with 13 chromosomes, the supernumerary indicated by an arrow. *e, f.* Meiosis of *Akodon* sp. (2n=24): *(e)* diplotene showing 12 bivalents including the sex-chromosome pair; *(f)* metaphase II with 12 chromosomes, including the Y chromosome.

univalent (corresponding to the odd submetacentric) for the 2n=25 males (fig. 3). These last specimens presented at metaphase II 12 and 13 dyads. Sex determination is XX:XY, and the odd chromosome does not participate in the sex-chromosome bivalent; it forms an univalent. Since this chromosome is present in both sexes and can be absent in some individuals apparently without affecting viability, it is adequate to describe it as a supernumerary chromosome.

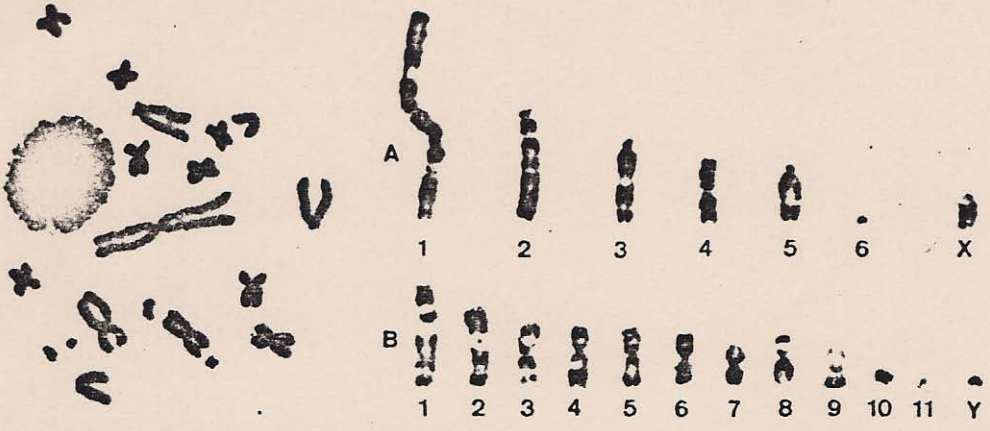

Fig. 4. Metaphase of the male hybrid between *Akodon arviculoides* (2n=14) and *Akodon* sp. (2n=24) showing 19 chromosomes.

Fig. 5. G-banding of the hybrid with 19 chromosomes: (A) the haploid set of *Akodon arviculoides* (2n=14) and (B) the haploid set of *Akodon* sp. (2n=24).

Chromosome complements in the hybrids

In two male specimens of a same litter initially classified as *Akodon arviculoides,* we found 2n=19 in 80 metaphases (fig. 4). The Q, ASG, and trypsin band patterns showed many unpairable chromosomes. Even chromosomes with similar morphology showed no homologous bands (fig. 5, but compare fig. 6). Careful comparison of the bands showed that these 19 chromosome specimens are hybrids exhibiting the complete haploid sets of both parental species, *Akodon arviculoides* (2n=14), with autosome pairs 2 and 3 acrocentric, and *Akodon* sp. (2n=24). It was possible to determine the origin of all chromosomes, with the exception of the minute metacentric pair and the sex-chromosome pair, which are identical in both species.

Differentiation between the two species resulted from thorough rearrangement without noticeable gain or loss in the amount of the genetic material. Indeed, in a sample of 10 metaphases from the hybrid, the total length of the autosomes did not differ in the two haploid sets (table I; $t = 0.7; P = 0.5$).

The testicular histology of one hybrid showed some seminiferous tubules lined only by Sertoli cells and striking vacuolation and other tubules with spermatocytes but no spermatids or spermatozoa (fig. 7). A section of the epididymis did not show spermatozoa.

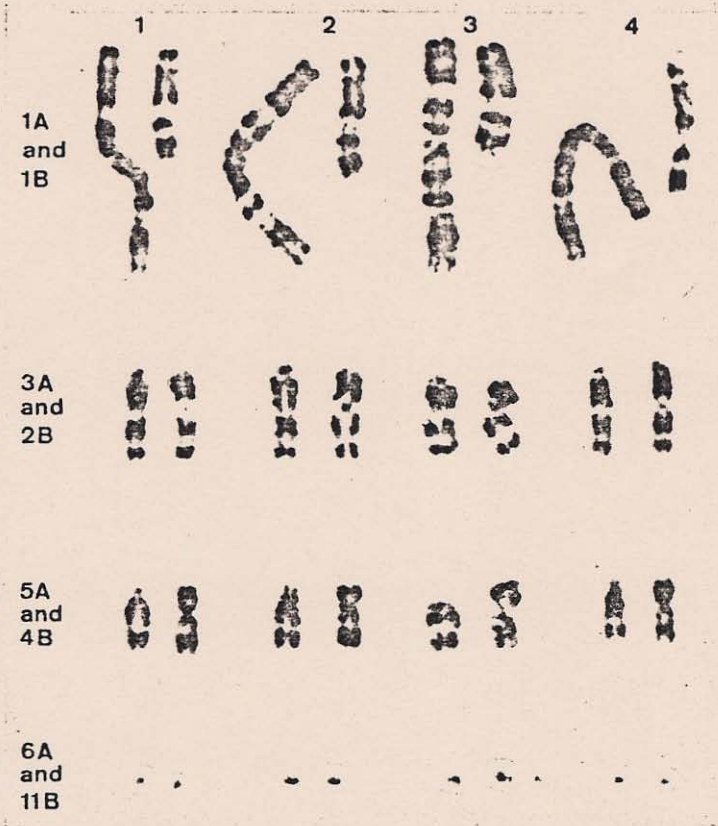

Fig. 6. Partial karyotypes of four metaphases of the hybrid comparing the G-bands of four chromosomes from the two haploid sets (A and B).

Air-dried preparations of the testis showed some meiotic activity. Many primary spermatocytes in leptotene and pachytene stages were seen (fig. 8). At pachytene some chromosome threads seemed to be paired, but others were in an univalent condition. No sex vesicle was present. Stages corresponding to diplotene or diakinesis were absent. There were cells with 19 extremely condensed figures. It was not possible to decide if they were spermatogonial cells with contracted chromosomes or some abnormal phase of meiosis I with 19 univalents. On the other hand, typical spermatogonial metaphases with 19 morphologically identifiable chromosomes were also present. Furthermore, there were many polyploid cells in which the chromosomes were extremely condensed. Some of them presented

Table I. Absolute length (in millimeters on the photographs) of the haploid autosomal sets, contributed by *Akodon arviculoides* and *Akodon* sp., in the same metaphases of 10 cells of the hybrid.

Cell	*A. arviculoides* set (6 autosomes)	*A.* sp. set (11 autosomes)	Difference
1	11.2	11.8	+0.6
2	11.3	11.6	+0.3
3	10.8	10.9	+0.1
4	10.8	10.3	−0.5
5	10.6	11.3	+0.7
6	8.3	8.2	−0.1
7	9.2	9.3	+0.1
8	11.7	11.6	−0.1
9	10.6	10.1	−0.5
10	10.9	11.2	+0.3
Mean	10.54	10.63	+0.9

pairing of some elements, and others showed pronounced degeneration (fig. 9).

Discussion

Rodents seems to go through a phase of "karyotypic explosion." Many instances of chromosomal polymorphism have been disclosed in Brazilian rodents (YONENAGA et al., 1976). The most illustrative examples of pericentric inversions were found in *Akodon arviculoides* (2n=14). To explain the great frequency of heterozygotes for such inversions in the population, we suggested that it could be related to a mechanism of cytogenetic control of the litter number, which would be adaptive in relatively adverse ecological conditions (YONENAGA et al., 1976). A similar hypothesis was proposed by VORONTSOV (1973) to explain the mechanism of sex determination in the rodent *Ellobius lutescens*, with 2n=17 in both sexes.

The chromosomal polymorphism in *Akodon* sp. (2n=24 and 25) is due to the presence of a submetacentric supernumerary chromosome. Its characteristics are in accordance with those considered as typical for

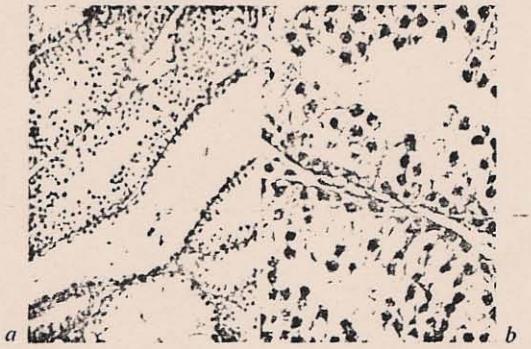

Fig. 7. Testicular histology in the hybrid. *a.* Some seminiferous tubules show striking vacuolation, while others show typical stages of spermatogenesis but a lack of spermatozoa. *b.* Spermatogenesis seen with higher magnification.

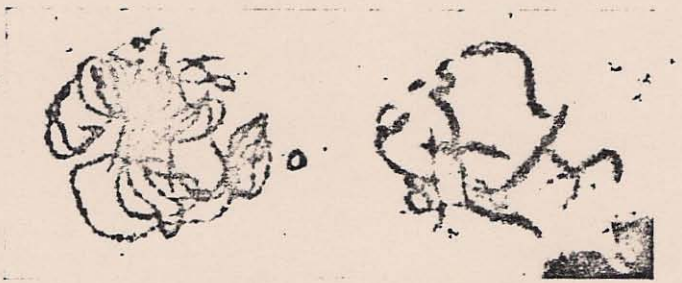

Fig. 8. Pachytene cells from the hybrid.

supernumeraries in general: small size, variation in number within individuals, reduced mechanical stability (JOHN and LEWIS, 1968). Supernumerary chromosomes were described in the rodents *Reithrodontomys megalotis* (SHELLHAMMER, 1969), *Rattus rattus* (GROPP et al., 1970; RAMAN and SHARMA, 1974), *Rattus rattus diardii* (YONG and DHALIWAL, 1972), *Rattus rattus bruneusculus* (PATHAK, 1971), and *Perognathus baileyi* (PATTON, 1972).

The role of supernumerary chromosomes is not clear. They could influence chiasma frequency (JOHN and HEWITT, 1966), act as centromere donators in processes of centric fission, or represent a result of repeated duplications of a small heterochromatin autosomal segment (WHITE, 1973). Some aspects of their influence on fertility and vigor, ecological adaptation, chiasma frequency, and chromosome pairing were discussed by MUNTZING (1974).

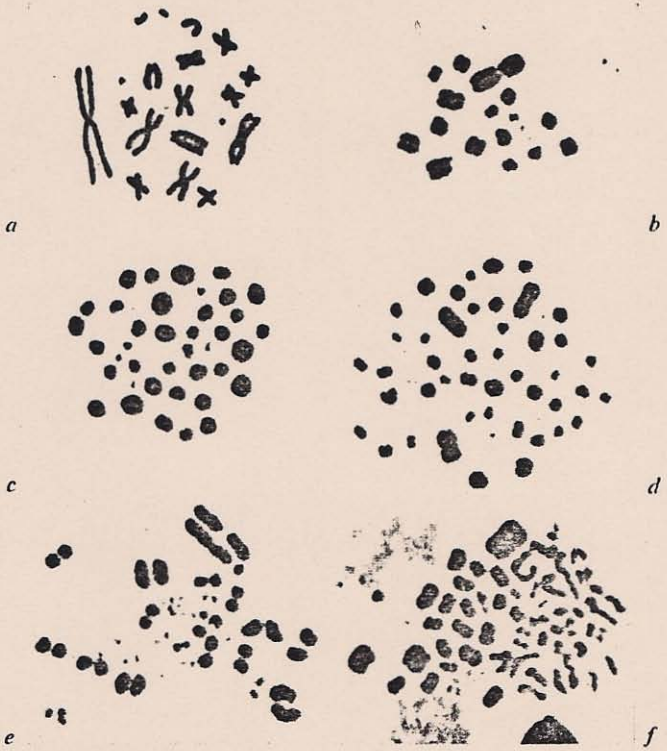

Fig. 9. Meiosis of the hybrid: *(a, b)* spermatogonial metaphases; *(c, d)* polyploid cells; *(e, f)* abnormal cells.

YONENAGA et al. (1976) advanced the hypothesis that supernumerary chromosomes could have an evolutionary significance similar to that of duplications (OHNO, 1970). If they are homologous to parts of regular chromosomes and well tolerated, they would provide the individuals bearing them with partial trisomy (or tetrasomy, in the case of a pair of supernumerary chromosomes) involving loci for which mechanisms of dose regulation have been established. Such a situation would give the species the opportunity for rapid evolution through "exploratory" point mutations in the redundant loci, which might lead to newly differentiated adaptive loci and the tendency for the supernumerary chromosome to be retained as a new pair of regular chromosomes.

The hybrids indicate a close afinity between the two species of *Akodon,* which explains why they are so similar taxonomically. The great difference between their karyotypes contrasts with their phenotypic similitude and

illustrate dramatically the explosive nature of karyotypic evolution in this rodent group.

The coexistence in cells of these hybrids of the two haploid sets of the parent species provided an ideal opportunity for comparing their total haploid chromosome length as well as their chromosome banding patterns. The total autosome length proved to be almost exactly the same (table I) in the two haploid sets. The banding patterns, on the other hand, are different in the two sets, but tentative correspondence can be set for some chromosomes. There is good homology between the G-band patterns of the following (fig. 6): (1) short arms of chromosome 1A and the 1B with a pericentric inversion, (2) chromosome 3A and the 2B with a pericentric inversion, and (3) chromosome 5A and the 4B with a pericentric inversion; in addition, chromosomes 6A and 11B are identical. It is interesting to recall that one of the polymorphisms (fig. 1) found in *Akodon arviculoides* (2n=14) refers to a pericentric inversion identical with the one in chromosomes 3A and 2B of the hybrid. It is difficult to establish any other direct correspondence in the remaining chromosomes, although the overall lengths of the two genomes are equal.

The present material is peculiar in that karyotypic evolution resulted from translocations and inversions rather than Robertsonian events or duplications and deletions. The cytogenetic analysis suggests that the hybrid is sterile. The germ cells fail to form gametes either because pachytene spermatocytes degenerate or because synapsis does not occur.

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