Sex-chromosome Variability in *Zygodontomys lasiurus* (Rodentia, Cricetidae)

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Specimens of *Zygodontomys lasiurus* have been collected in different regions of Brazil such as the State of Sao Paulo (Yonenaga 1972, 1975), Amazon region (Barroso and Barros 1978), and the State of Pernambuco, in the Northeast region (Maia and Langguth, in press). The basic karyotype (2n=34) includes 32 autosomes consisting of 15 acrocentric pairs and one small metacentric pair. The X chromosome is a medium sized acrocentric morphologically undistinguishable from the autosomes of equal size, and the Y is a small submetacentric. Some female specimens from Sao Paulo had slightly different karyotype having heteromorphic sex chromosome pair (Yonenaga 1972, 1975). One of the X chromosomes presented a subterminal centromere while the other had 1/3 of the length of the first probably due to a gross deletion in the long arm. A polymorphism (2n=34, 33) due to a centric fusion between autosomes was also found in specimens from Pernambuco (Maia and Langguth 1981).

This paper presents further data on the sex chromosomes in *Z. lasiurus* from the State of Sao Paulo. The G- and C-banding patterns were studied and the silver stained nucleolus organizer regions (NORs) were detected in the karyotype.

Material and methods

Thirteen males and thirteen females were collected in different localities of the State of Sao Paulo (Table 1).

For cytogenetic studies, air-dried preparations of bone marrow, spleen and testis were made after "in vivo" 0.1% colchicine treatment (1 ml/100 g of weight) for 2 hours. In some specimens where colchicine could not be injected intraperitoneally, the bone marrow was treated "in vitro" with colchicine. In both cases, 0.075 M KCl was used as hypotonic solution for 15 minutes at 37°C, and 3:1 methanol and acetic acid as fixative. Conventional staining was done with buffered Giemsa 2%, pH 6.8; G-bands were obtained after trypsin treatment (Seabright 1971) and C-bands, according to the method described by Sumner (1972). NORs were detected using the method described by Bloom and Goodpasture (1976) modified

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Results

Twenty five specimens showed $2n=34$ chromosomes and one female was a mosaic of the $2n=33/34$ type. The sex chromosome constitution of all specimens is presented in Table 1. Twelve males and five females showed acrocentric X chromosomes. One male and two females had one acrocentric X marker chromosome $(X_m)$ with enlarged short arm, while two other females were homozygous for this chromosome (Fig. 1). Three females presented a sex chromosome pair (Fig. 1) consisting of an $X$ marker $(X_m)$ and a small submetacentric chromosome $(X_d)$. The $X_d$ chromosome which corresponds nearly to $1/3$ of the acrocentric $X$ is morphologically similar to the $Y$ chromosome. The sex chromosome pair $X_mX_d$ is, therefore, undistinguishable from that of a male with a $X_mY$ constitution (Fig. 2). In the mosaic female, among 81 metaphases studied, 65% showed $2n=33$ and 35% $2n=34$, with a $X_mO$ and a $X_mX$ sex chromosome constitution, respectively.
The G-band analysis allowed the identification of each chromosome pair (Fig. 1). A diagram of the main G-band patterns is shown in Fig. 3. 

*Z. lasiurus* presented a small amount of constitutive heterochromatin at the pericentromeric region of some chromosomes (Fig. 4). No metaphase, nevertheless, showed conspicuous C-band patterns. Maybe this is due to the nature of the constitutive heterochromatin which in this species is not easily banded by the usual techniques.

Silver stained metaphase spreads from four specimens scored for NORs showed a maximum of 11 and a minimum of 2 NORs, some of them in close association. The NORs are located at the telomeres on the long arms of the chromosomes and, less frequently, on the short arms (Fig. 5). Although the NOR bearing chromosomes
have not been identified, it seems that in *Z. lasiurus* there are at least 4 pairs with NORs on the long arm and 2 pairs on the short arm.

Analysis of male meiosis revealed a condensed sex vesicle at pachytene. At diplotene and metaphase I, 16 autosomal bivalents plus the sexual with end-to-end pairing between X and Y were observed. In one male, however, the sex chromosomes appeared as univalents in 57 cells (Fig. 6) while in 3 cells the X and Y chromosomes were seen in an end-to-end association. The counting of 50 cells at metaphase II in this male showed an abnormal segregation of the sex chromosomes. Twenty nine cells had 18 chromosomes probably including both X and Y chromosomes, 16 cells had 17 chromosomes, and 5 cells had 16 chromosomes without X or Y.

![Fig. 4. C-banded metaphase of a male *Zygodontomys lasiurus* (2n=34). The arrow indicates the Y chromosome.](image)

**Discussion**

The enlarged short arm of X marker chromosome (Xm) in *Z. lasiurus* is probably due to the addition of material, since the G-band patterns on the long arm are identical to those on the acrocentric X. Intraspecific variation in size and morphology of X chromosomes is usually due to different amounts of constitutive heterochromatin (Mascarello *et al.* 1974, Baverstock *et al.* 1977). Nevertheless, the enlarged short arm of the X that was found in some specimens of the rodent *Akodon* sp. (2n=24, 25) was euchromatic (Kasahara and Yonenaga-Yassuda, 1982). We could not obtain a definite pattern of C-bands in the Xm chromosome of *Z. lasiurus*.
and it is not possible, therefore, to decide about the nature of the material on its short arm.

Figs. 5a–d. Silver-stained chromosomes of *Zygodontomys lasiurus* from 4 metaphases bearing: a—11; b—10; c— and d—8 NORs. Associated NORs are observed in a and b. The arrows indicate NORs on the short arms.

Fig. 6. Metaphase I of *Zygodontomys lasiurus* (2n=34) showing 16 autosomal bivalents and 2 univalents corresponding to early dissociated X and Y chromosomes.
The $X_mX_d$ constitution was previously found in three among 13 females of *Z. lasiurus* and this heteromorphism was considered by Yonenaga (1972, 1975) as resulting from a gross deletion in one of the X chromosomes ($X_d$) plus an alteration in the arm proportion of the other ($X_m$). The most probable interpretation is that the additional arm of the $X_m$ chromosome does not represent an inert material but active genes that are result of a duplication; these genes would compensate the deleted portion of the $X_d$, allowing a normal development for $X_mX_d$ females. In this way, the $X_d$ chromosome must always occur together with the $X_m$ but never with the acrocentric X. In fact, taking into account the frequency of the different types of X chromosomes (Table 1), the occurrence of $XX_d$ females would be expected but they have never been found. Probably these females have a very low viability due to the monosomy of part of the X chromosome. On the other hand, the $X_mX_m$ and $X_mX$ females and $X_mY$ male of our sample, bearing an excess of genetic material, have no problem of viability. The occurrence of the $X_mO/X_mX$ mosaic would also favour the duplication/deletion hypothesis. The

Table 1. Sex-chromosome constitutions of *Zygodontomys lasiurus* collected in the State of São Paulo, Brazil

<table>
<thead>
<tr>
<th>Sex-chromosome constitution</th>
<th>2n</th>
<th>Number of animals</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>34</td>
<td>5</td>
<td>Itapetininga</td>
</tr>
<tr>
<td>XY</td>
<td>34</td>
<td>12</td>
<td>Itapetininga, Caçapava, Americana, Taubaté, Campinas, Guararema</td>
</tr>
<tr>
<td>$X_mX$</td>
<td>34</td>
<td>2</td>
<td>Taubaté, Nova Odessa</td>
</tr>
<tr>
<td>$X_mY$</td>
<td>34</td>
<td>1</td>
<td>Itapetininga</td>
</tr>
<tr>
<td>$X_mX_m$</td>
<td>34</td>
<td>2</td>
<td>Itapetininga, Guararema</td>
</tr>
<tr>
<td>$X_mX_d$</td>
<td>34</td>
<td>3</td>
<td>Itapetininga, Guararema</td>
</tr>
<tr>
<td>$X_mO/X_mX$</td>
<td>33/34</td>
<td>1</td>
<td>Itapetininga</td>
</tr>
</tbody>
</table>

$X$—acrocentric X, $X_m$—acrocentric X with enlarged short arm, $X_d$—small submetaacentric chromosome.

loss of the acrocentric X is well tolerated probably due to the duplicated portion in the $X_m$. A XO cell line would be inviable.

An alternative hypothesis would be that $X_mX_d$ females have indeed a XY constitution like the phenotypically normal fertile females of the rodent *Myopus schisticolor* (Fredga *et al.* 1976, Gropp *et al.* 1976). The hypothesis is based on the morphological similarity between the $X_d$ and Y chromosomes even by the G-banding patterns, for a slightly darker band near the centromere is seen in both chromosomes. $X_mX_d$ and $X_mY$ metaphases are undistinguishable. If this is the correct interpretation, the $X_m$ chromosome would carry a gene mutation which would inactivate the male determining effect of the H-Y gene on the Y chromosome so that the $X_mX_d$ would be a female. Nevertheless, this hypothesis is weakened by the existence of the $X_mY$ male and of the $X_mX_m$ female, except if we consider that the $X_m$ is not always genetically identical, having different effects on the expression of male determining genes.

Undoubtedly, additional studies are required to decide about the sex determination mechanism in *Z. lasiurus*. 
The early dissociation of X and Y chromosomes during meiosis is a sporadic event affecting just one male of our sample. The spermatogenesis seems to be normal because many spermatids and spermatozoa were observed in the meiotic preparation, although some of them may be disomic or nulisomic for sex chromosomes as revealed by metaphase II counting. Early dissociation or nonpairing of X and Y chromosomes have been observed in some male specimens of Mus musculus (Beechey 1973, Rapp et al. 1977), but the causes of such behaviour have remained unknown.

The karyotype of Z. lasiurus is very distinct from other Zygodontomys species, being characterized by the high diploid numbers. Z. microtinus from Venezuela (Kiblisky et al. 1970) and Z. brevicauda from Central America (Gardner and Patton 1976) have 2n=84. Z. microtinus from Colombia (Gardner and Patton 1976) has 2n=88, and according to these authors Z. lasiurus is best classified as a member of the genus Akodon. In fact, the karyotype of Z. lasiurus shows similarity in G-band patterns to those presented by Akodon obscurus (2n=34) described by Bianchi et al. (1976) and the species shares the same pair of minute metacentric autosomes typical of Akodon group. Recently, Maia and Langguth (1981) have reviewed the taxonomic status of Z. lasiurus and based on morphological and cytogenetical data they suggested that this species really belongs to the genus Bolomys, classifying it as B. lasiurus.

Summary

Cytogenetic studies were performed on 26 specimens of the rodent Zygodontomys lasiurus (2n=34, 2n=33/34), collected in the State of Sao Paulo, Brazil. The G- and C-band patterns of mitotic chromosomes were studied and the NORs were localized in the karyotype. Two types of acrocentric X chromosomes, morphologically distinctive, were observed, one of them having an enlarged short arm (Xm). In some females the sex chromosome pair consisted of an Xm and a small submetacentric Xd similar to the Y chromosome. The possibilities that these are females bearing a deletion in one X plus a duplication in the other, or are true XY females are discussed. A chromosome mosaicism of 2n=33/34 type was detected in one female. The 2n=34 cell line had an XmX constitution, while the 2n=33 cells were XmO. Both early dissociation between X and Y chromosomes and altered segregation of such chromosomes at metaphase II were observed in one male specimen.

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References


