

Comparative cytogenetics of two phyllostomids bats. A new hypothesis to the origin of the rearranged X chromosome from *Artibeus lituratus* (Chiroptera, Phyllostomidae)

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Abstract - It was carried out a cytogenetic comparison between *Phyllostomus hastatus* and *Artibeus lituratus* (Chiroptera, Phyllostomidae), using G-, C- and NOR banding techniques. The *P. hastatus* specimens presented $2n=32$ chromosomes, including 13 biarmed autosome pairs and one acrocentric. The X chromosome is submetacentric and the Y is a minute acrocentric. *A. lituratus* has $2n=30$ chromosomes in females and 31 in males specimens. The autosomes are 14 biarmed pairs and the chromosomal sexual system is XY_1Y_2 type, being the X chromosome a large submetacentric and both Y_1 (real Y) and Y_2 acrocentric elements. Several chromosomal homologies are shared between both taxa, including whole chromosomes and chromosomal arms. Based on similarity of G-banding pattern is proposed that the system XY_1Y_2 from *A. lituratus* risen by a tandem fusion event involving the stenodermatine original biarmed X chromosome with the autosome acrocentric homologous to Y_2 .

Key words: Bats, Cytogenetics, Chromosome, Phyllostomidae, Sex chromosomes, Translocation.

INTRODUCTION

The Neotropical bat family Phyllostomidae is a complex taxon encompassing about 48 genera and 148 species. Its geographic range is from the Southwestern United States and the West Indies as far as Northern Argentina (NOWAK 1996). BAKER *et al.* (1989) classify phyllostomid bats in three clades: subfamilies Desmodontinae, Vampyrinae and Phyllostominae (including the Tribes Phyllostomini, Glossophagini and Stenodermatini); the genera *Macrotus* and *Micronycteris* are placed as *incertae sedis*.

Phyllostomidae species karyotypes have been investigated since 1960 decade. PATTON and BAKER (1978), proposed the ancestral karyotype to the family with $2n=46$ chromosomes and $FN=60$, similar to that found in *Macrotus waterhousii*. These authors also support that karyotypic evolution among phyllostomids seems to lead the reduction of diploid numbers through centric fusion events with retention of the linkage groups.

The genus *Phyllostomus* (Phyllostomini) encompasses four species: *P. hastatus*, *P. elongatus* and *P. latifolius* which have karyotypes with $2n=32$ chromosomes being 14 autosome biarmed pairs and one acrocentric, while *P. discolor* has $2n=32$ but with 15 biarmed pairs (BAKER 1973, 1979; HONEYCUTT *et al.* 1980; MORIELLE 1987;

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MORIELLE and VARELLA-GARCIA 1988; RODRIGUES *et al.* 2000).

Artibeus is divided into four subgenera: *Artibeus*, *Dermanura*, *Enchistenes* and *Koopmania* (MARQUES-AGUIAR 1994). Cytogenetics studies of *Artibeus* species have demonstrated karyotypes with 30 chromosomes in females and 31 in males, the autosomes are 10 metacentric or submetacentric and 4 subtelocentric pairs (BAKER 1973, 1979; BAKER *et al.* 1981; TUCKER 1986; TUCKER and BICKHAM 1986; ARAUJO and SOUZA 1987; VARELLA-GARCIA *et al.* 1989; SOUZA and ARAUJO 1990). Such difference is well explained by the presence of a XX - XY₁Y₂ sexual system in this genus. However, *A. (D.) cinereus* has a polymorphism where populations from Central America have 2n=30-31 chromosomes (BAKER 1979; TUCKER 1986) while populations from Pará and Pernambuco States, Brazil, have 2n=30 chromosomes (SOUZA and ARAUJO 1990; NORONHA 2000). In the other hand, there are two species, *Artibeus (D.) phaeotis* and *A. (D.) watsoni* that present 2n=30 chromosomes either in male and female specimens (BAKER 1967, 1973; HSU *et al.* 1968; TUCKER 1986; TUCKER and BICKHAM 1986).

The multiple sexual system XX-XY₁Y₂ from *Artibeus* was originated through a translocation

of the X chromosome with a small autosome acrocentric (KASAHARA and DUTRILLAUX 1983; TUCKER and BICKHAM 1986). It is a consensus that the translocated autosome corresponds to the short arm of the X chromosome, since this region has homology of C- and RBA bands with the free autosome element Y₂ (TUCKER 1986). This is also supported with meiotic studies that demonstrate an autosome-like meiotic pairing between the chromosomes X and Y₂ where is detected a chiasma (TUCKER and BICKHAM 1986; SOLARI and PIGOZZI 1994).

TUCKER (1986) proposed a hypothesis to explain the origin of stenodermatine sex chromosomes. She argues that the Phyllostomidae original X chromosome could be an acrocentric or telocentric element that firstly undergone a translocation resulting in a insertion of autosomal material within the long arm of the X. Such event is tentatively explained by the presence of an intermediate replicating band on the long arm of the X in both stenodermatine and phyllostomine species. However, it is not provided an explanation for the destiny of the autosomal segment homologous to the translocated material. Sequentially, another X-autosome translocation give rise to the XY₁Y₂ condition found in the modern stenodermatine species.

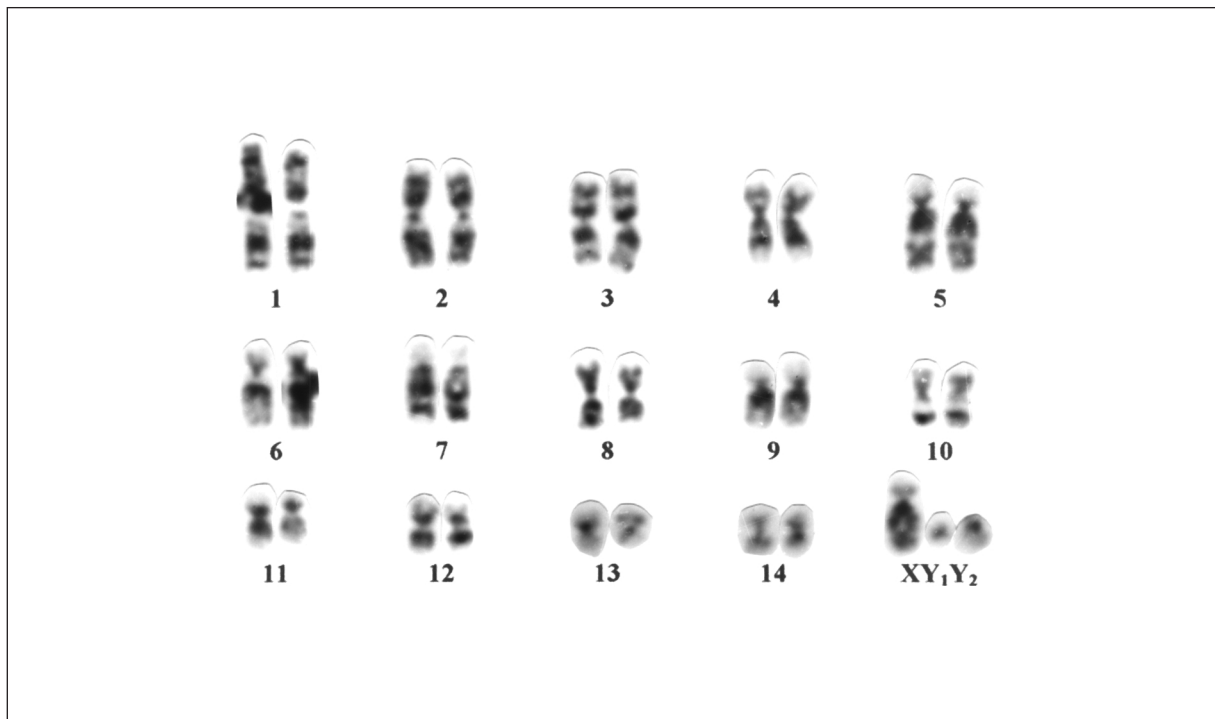


Fig. 1 – G-banded karyotype of *Artibeus lituratus*.

Table 1 – Chromosomes and chromosomal arms shared between *P. hastatus* and *A. lituratus*.

Species	Chromosomal Segments														
	5	6	8	9	10	4*	1q	1p	2q	2p	3q	3p	7q	X	Y
<i>P. hastatus</i>	5	6	8	9	10	4*	1q	1p	2q	2p	3q	3p	7q	X	Y
<i>A. lituratus</i>	4	8	10	11	12	2*	1p	6q	7q	3p	1q	5q (prox)	9q	Xq	Y ₁

* Chromosomes modified by pericentric inversion

In this paper, is performed a karyotypic comparison between *Phyllostomus hastatus* (Phyllostomini) that is assumed to have a primitive condition of Sex chromosomes (PATTON and BAKER 1978) and *Artibeus lituratus* (Stenodermatini) that has the derived one (XY₁Y₂ system).

MATERIAL AND METHODS

Were studied four specimens of *Phyllostomus hastatus* (1M, 3F) from Municipality of Peixe-Boi (1°11'S - 47°19'W) and two of *Artibeus lituratus* (1M, 1F) from Cotijuba Island (1° 28'S - 48° 29' W), both localities from Pará State, Brazil. The Voucher specimens were placed in the Mammal Collection of the Museu Paraense Emílio Goeldi, and the access numbers are: *P. hastatus* (26331, 26332, 26333, 26334), *A. lituratus* (26336, 26337). Metaphases were obtained from fibroblast culture and bone marrow preparation (BAKER and QUMSIYEH 1988). Slides were prepared by air drying method, G-banding was processed by incuba-

tion in 2xSSC solution at 60° C during few minutes and stained with Wright's Stain (Sigma) during two minutes and thirty seconds. C-banding procedure was according to SUMNER (1972) and Ag-NOR staining was following HOWELL and BLACK (1980). Some metaphases G-banded were photographed and afterward submitted to C-banding treatment (G-C sequential banding).

RESULTS

Karyotypes

Phyllostomus hastatus specimens presented $2n=32$ chromosomes and FN=58, with 14 pairs of biarmed autosomes and one acrocentric; the X chromosome is medium metacentric or submetacentric and the Y is a small acrocentric. G-, C- and NOR banding results are in agreement with literature data (figures published in RODRIGUES *et al.* 2000).

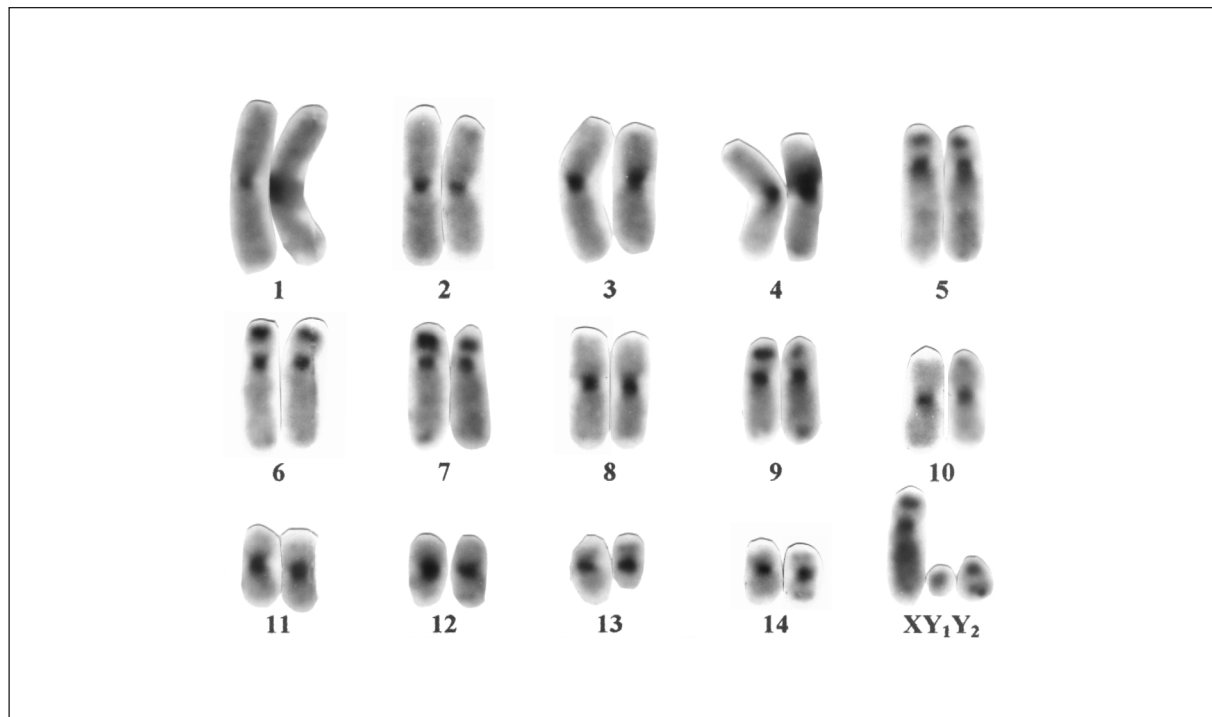


Fig. 2 – C-banded karyotype of *Artibeus lituratus*.

Artibeus lituratus specimens have $2n=30$ chromosomes (female) and 31 (male), the FN=56. The autosomes are 14 biarmed pairs, the X chromosome is large submetacentric, Y_1 (true Y) and Y_2 are acrocentrics. Figs. 1, 2 and 3 illustrate G-, C- and NOR banding, respectively. These data are congruent to literature data (BAKER 1979; KASAHARA and DUTRILLAUX 1983; SOUZA and ARAUJO 1990). Constitutive heterochromatin is located on the centromeric region of all chromosomes and at the distal region of the short arm in the pairs 5, 6, 7, 9 and X chromosome. A little interstitial C-band at the long arm of the X chromosome was observed. Ag-NOR technique revealed a range of three to six labelling and the NOR are located on the distal region of the short arm in the pairs 5, 6 and 7.

Chromosome comparison between P. bastatus and A. lituratus

Both species share several chromosomal homologies assumed based on similarity of G-banding pattern (Table 1). Five autosomes and Y chromosomes are shared integrally, conserved without rearrangements (Fig. 4a, d). The chromosome 4 from *P. bastatus* is tentatively identified as #2 from *A. lituratus*, but was changed by a pericentric inversion (Fig. 4b). Several chromo-

somal arms from *P. bastatus* were identified in *A. lituratus* constituting different combinations (Fig. 4c). The X chromosome from *P. bastatus* has the same banding pattern than Xq from *A. lituratus*, when compared one segment inverted in relation to the other (Fig. 4d).

DISCUSSION

Assuming an ancestral phyllostomid karyotype as found in *Macrotus waterhousii*, that has $2n=46$, including eight biarmed and 14 acrocentric autosome pairs (PATTON and BAKER 1978), is reasonable to suggest that the biarmed chromosomes shared between *P. bastatus* and *A. lituratus* could be primitive characters and possibly would be present in the ancestral karyotype. The shared chromosomal arms had been retained as acrocentric elements which had undergone independent fusion at the lineages Phyllostomini and Stenodermatini, resulting in the actual different arm combinations.

The proposed homology between *P. bastatus* X chromosome and *A. lituratus* Xq segment (Fig. 4d) permits some considerations about the origin of the composite Sex chromosome of the later. The literature data had demonstrated a



Fig. 3 – Metaphase silver-stained of *Artibeus lituratus*. The arrows show the NOR labels.

possible route of evolution to Sex chromosomes from stenodermatine species. TUCKER (1986) assumed that the original stenodermatine X chromosome could be an acrocentric element as typified in *Micronycteris hirsuta* (Phyllostominae), it was proposed that the actual composite stenodermatine X had been originated by a centric fusion involving the acrocentric X element with an small autosome acrocentric (Y_2 homologue).

If one considers the karyotype of *M. hirsuta* as representative of Phyllostominae, and admits an acrocentric shape as the ancestral condition for the stenodermatine X chromosome how was proposed, then the X element in the karyotypes of other phyllostomine species should be an acrocentric. However, this is not verified. According to BAKER (1979) within all phyllostomine species cytogenetically studied only *M. hirsuta* has an acrocentric X chromosome.

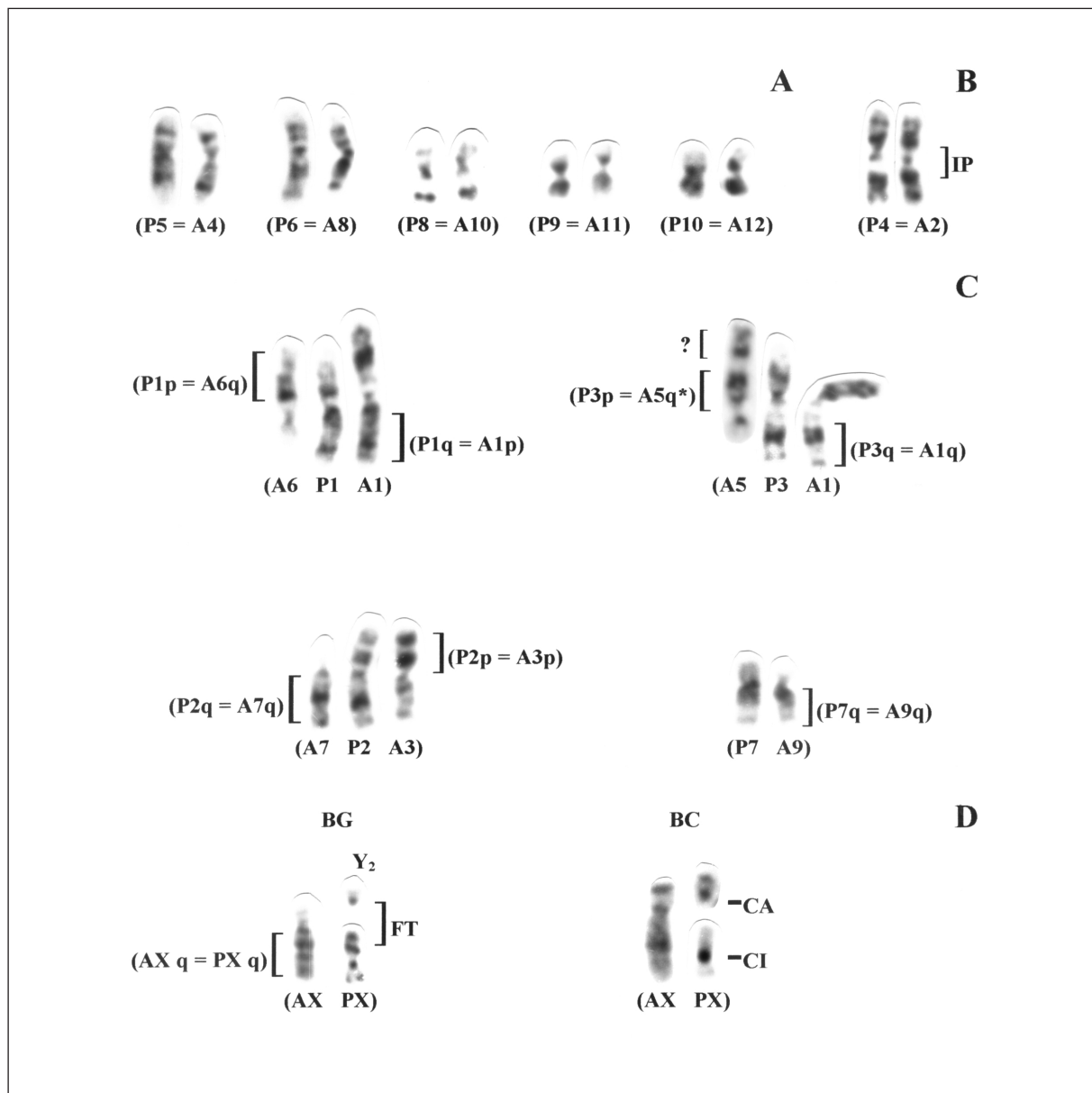


Fig. 4 – Chromosomal homologies shared between *Phyllostomus bastatus* and *Artibeus lituratus*. A) Whole chromosomes conserved; B) chromosome modified by pericentric inversion; C) shared chromosomal arms; D) G- and C-banding patterns of the X chromosomes from *P. bastatus* (PX) and *A. lituratus* (AX) and chromosome Y_2 , is proposed that PX in inverted position is homologue to the long arm of AX. The letter P indicates chromosomes and segments of *P. bastatus* while A indicates those of *A. lituratus*; IP (pericentric inversion), FT (tandem fusion), CA (active centromere), CI (inactivated centromere).

Additionally, since in the genus *Micronycteris* there is a high karyotypic variation, and all species except *M. hirsuta* have biarmed X-chromosome, we accept that the “acrocentric X” possibly is an autapomorphic character of that species.

We argue that primitive condition for phyllostomid X chromosome would be a biarmed shape as found in *M. waterhousii*, *P. discolor* and *P. hastatus* (PATTON and BAKER 1978; BAKER 1979; RODRIGUES *et al.* 2000). Herein is tentatively proposed that the stenodermatine original X chromosome could be similar to the biarmed X from *P. hastatus*, and the rearrangement involving the autosome homologue to Y2 could be a *tandem* fusion (centromere-telomere) followed by centromere inactivation (Fig. 5). The presence of a positive interstitial C band at the long arm of *A. lituratus* X chromosome may be interpreted as indicative of reminiscent material from the inactivated X-centromere.

If the start point of the evolution of phyllostomid Sex chromosomes was a biarmed X chromosome, the hypothesis here discussed (*tandem* fusion hypothesis) is more parsimonious since it requires only one rearrangement from the ancestral to modern states, while the centric fusion hypothesis requires an additional evolu-

tionary step, necessary to produce the intermediary acrocentric shaped X chromosome.

Additional comparative studies of phyllostomid karyotype with new molecular cytogenetic tools, under a phylogenetic framework, are expected to provide a better understanding of the evolutionary changes involved in the origin of the stenodermatine sexual systems.

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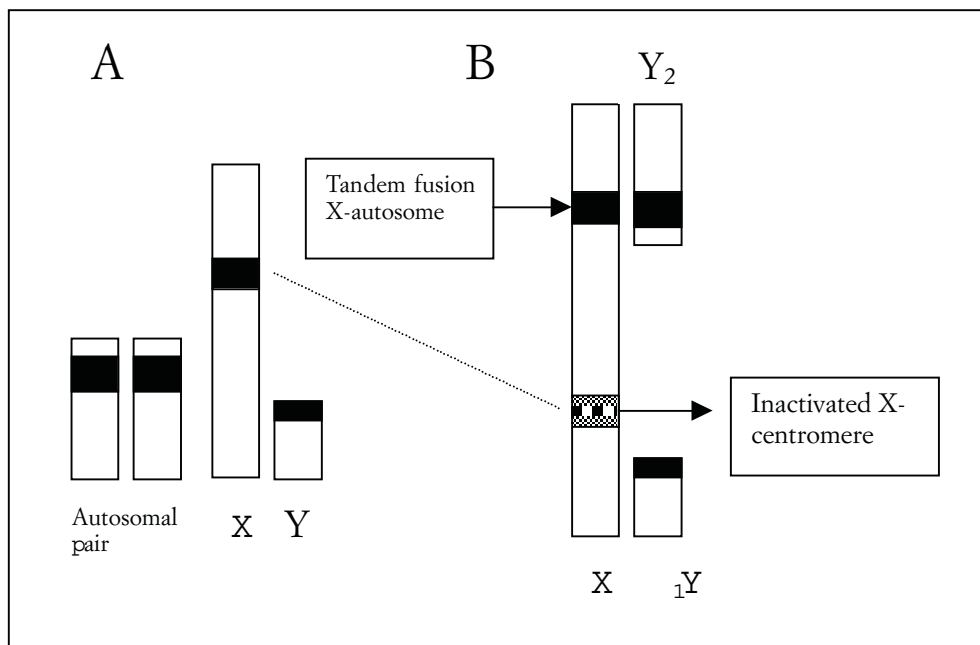


Fig. 5 – A hypothetical scheme illustrating an alternative route to origin of stenodermatine X chromosome. A) Primitive condition of the XY pair, it is assumed that original stenodermatine X is biarmed and similar to chromosomes from *Macrotus* and *Phyllostomus*. B) The derived stenodermatine X, modified by a *tandem* fusion with an autosome acrocentric.

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