

## The multiple sex chromosome system of American leaf-nosed bats (Chiroptera, Phyllostomidae)<sup>1</sup>

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**Abstract.** Karyological examination of 26 species of bats from the family Phyllostomidae revealed two Y chromosomes ( $XY_1Y_2$ ) in six species involving three genera. All females are of the XX constitution. Meiotic studies demonstrate the origin of the additional Y in *Carollia* to be the homolog of an autosome translocated to the X element. The origin of an additional Y in *Artibeus* is best explained as a separate event. In one species of *Artibeus* (*A. turpis*) a centric fusion of the two Y elements is emphatically suggested.

### Introduction

Deviations from the standard XX/XY sex determination system are sporadic in the class Mammalia. In several isolated cases an XX/ $XY_1Y_2$  system was found. These include two marsupials, *Potorous tridactylus* (SHARMAN *et al.*, 1950; WALEN and BROWN, 1962; SHAW and KROOTH, 1964) and *Protemnodon bicolor* (SHARMAN, 1961; MOORE and GREGORY, 1963), an insectivore, *Sorex araneus* (BOVEY, 1949; SHARMAN, 1956) and a rodent, *Gerbillus gerbillus* (MATTHEY, 1954; WAHRMAN and ZAHAVI, 1955).

This report presents several additional cases of the XX/ $XY_1Y_2$  sex determination system, found in the American leaf-nosed bats, with a discussion on the phylogeny of this family, Phyllostomidae.

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### Materials and methods

Other than two male specimens of *Carollia perspicillata* that were collected by Mr. ROBERT DOOLEY, all specimens were collected by one of us (R.J.B.). These were from the 11 localities listed in the table. All animal specimens were deposited in the Department of Zoology, University of Arizona.

The lung tissues of *Carollia perspicillata* were used to initiate cell cultures for cytological preparations. Testicular tissues of these specimens were fixed directly for squash preparations. For all other specimens, the animals were injected with Velban (0.05% per gram of body weight) for three hours, and cells from bone marrow were used for air-dried slides (PATTON, 1967). With the exception of *Carollia perspicillata*, where meiotic cells were studied, sex chromosomes were determined on the basis of the heteromorphic pair which remained after all other chromosomes had been satisfactorily paired in the male complement. Of the heteromorphic elements found in the male, the one that occurred twice in the female complement was considered the X, and the chromosome(s) that appeared only in the male complement was considered the Y('s).

### Results

#### *The chromosomes of phyllostomids*

The table summarizes the cytological data of the present study. The diploid number varies from 16 (*Choeronycteris mexicana*) to 46 (*Macrotus waterhousii*). The diploid number of 16 is the lowest known in Eutherian mammals.

The three genera under consideration show an XX/XY<sub>1</sub>Y<sub>2</sub> sex chromosome complex. Other species, except those whose males have not been studied, exhibit the regular XX/XY sex chromosome condition. All species, except *Trachops cirrhosus*, had a distinctly biarmed X element. Of these species, 17 had a small acrocentric Y, whereas six had a biarmed Y element.

#### *Genus Choeroneiscus*

Five male specimens of *C. godmani* were examined cytologically. All showed 19 chromosomes (Fig. 1). Since no female specimen was available, the X chromosome is presumed to be a medium-sized submetacentric, which can be identified without difficulty. The two Y chromosomes differ considerably in size. The small Y<sub>2</sub> chromosome, an acrocentric, is also unique in this karyotype. The Y<sub>1</sub> element, a relatively large acrocentric, may be confused with one pair of the

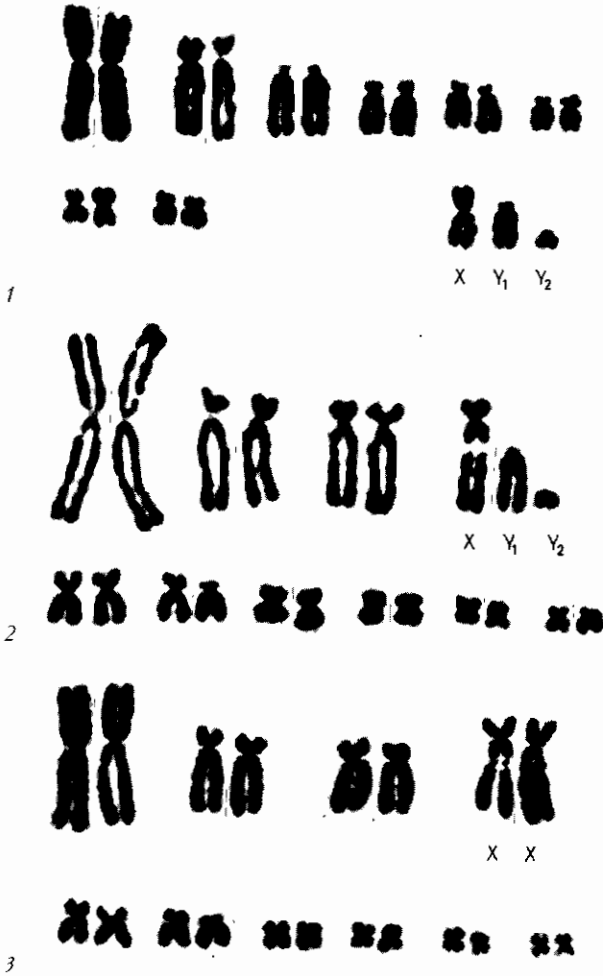


Fig. 1. Karyotype of a male *Choeroniscus godmani*,  $2n = 19$ .

Fig. 2. Karyotype of a male *Carollia perspicillata*,  $2n = 21$ .

Fig. 3. Karyotype of a female *Carollia subrufa*,  $2n = 20$ . Note a piece of chromatin within the secondary constriction of the X chromosome.

*Collection and cytological data of the*

	Sex	Locality	No. of specimens	2n
Chilonycterinae				
<i>Pteronotus dayi</i> GRAY	♀	7	1	38
	♂	11	1	38
<i>P. parnellii</i> (J. A. ALLEN)	♂	4	1	38
	♀	7,9	2	38
<i>P. psilotus</i> (DOBSON)	♀	7	1	38
	♂	11	1	38
Phyllostominae				
<i>Micronycteris megalotis</i> GRAY	♂	9	1	40
<i>Macrotus waterhousii</i> GRAY	♂	4	1	46
<i>Phyllostomus discolor</i> WAGNER	♂	7	1	32
	♀	7,9	3	32
<i>Trachops cirrhosus</i> (SPIX)	♂	7,9	3	30
	♀	7	2	30
Glossophaginae				
<i>Glossophaga commissarisi</i> GARDNER	♂	7	2	32
	♀	7	2	32
<i>G. soricina</i> (PALLAS)	♂	4,5	10	32
	♀	5,7,9	7	32
<i>Anuora geoffroyi</i> GRAY	♂	7	2	30
<i>Choeronycteris mexicana</i> TSCHUDI	♀	6	1	16
<i>Choeroniscus godmani</i> (THOMAS)	♂	7	5	19
<i>Leptonycteris sanborni</i> HOFFMEISTER	♂	1	1	32
	♀	7	1	32
Carollinae				
<i>Carollia perspicillata arteca</i> SAUSSURE	♂	12	2	21
<i>C. subrufa</i> (HATTN)	♂	3,7,9	5	21
	♀	3,7,9	6	20

*bat species examined in this study*

	Sex	Locality	No. of Specimens	2n
<b>Sternoderminae</b>				
<i>Sturnira lilium</i> (GEOFFROY)	♂	2,3	4	30
	♀	2,3	2	30
<i>S. ludovici</i> ANTHONY	♂	3	1	30
	♀	3	1	30
<i>Uroderma bilobatum</i> PETERS	♂	8,9	4	44
	♀	9	1	44
<i>Vampyrops helleri</i> PETERS	♂	7,9	2	30
<i>Chiroderma villosum</i> PETERS	♂	7	1	26
	♀	7	2	26
<i>Artibeus jamaicensis</i> LEACH	♂	3,4,10	6	31
	♀	4,9	3	30
<i>A. lituratus</i> (LICHENSTEIN)	♂	2,7,10	5	31
	♀	2,3	3	30
<i>A. turpis</i> (MILLER)	♂	9	1	30
	♀	9	1	30
<i>A. toltecus</i> (SAUSSURE)	♂	3	1	31
	♀	2,3,7	3	30
<i>Inchisthenes bartii</i> (THOMAS)	♀	7,8	2	30
<i>Centurio senex</i> GRAY	♀	7	1	28

## Key to localities:

1. Tequesquitengo – Cueva del Cerro, Morcos, Mexico.
2. Oaxtepec, Morelos, Mexico.
3. Ojo de Agua del Río Atoyac, near Potrero, Veracruz 461M, Mexico.
4. 231 km S from Mexico City by Highway 95, Guerrero, Mexico.
5. 157 km S from Mexico City by Highway 95, Guerrero, Mexico.
6. Ojo de Agua de Mexicapán, 3.4 km N. Teloloapan, Guerrero 148M, Mexico.
7. 24 miles W. Cintalapa, Chiapas 750M, Mexico.
8. Puente Mosquito Carretera Arriaga a Tapachula Chiapas, Mexico.
9. km number 184 on Highway 200 N. Huxilta, Chiapas, Puente Bada Ancho, Mexico.
10. Río Piaxtla on Highway 15, Sinaloa, Mexico.
11. Alamos, Sonora, Mexico.
12. Tuxtla, Veracruz, Mexico.

subtelocentric autosomes. However, the second arm of  $Y_1$  is a short, knoblike structure, whereas that of the autosome is considerably more prominent.

The long arm of the  $Y_1$  chromosome is longer than the long arm of the X.

#### *Genus Carollia*

Two species, *C. perspicillata* and *C. subrufa*, were analyzed. Only two male specimens of the former were available for cytological studies, but 11 specimens of the latter were examined.

The karyotypes of both species are identical (Figs. 2 and 3). The female individuals have a diploid number of 20 (XX) and the males 21 ( $XY_1Y_2$ ). The X chromosome is a large subtelocentric.



*Fig. 4.* Diakinesis of *Carollia perspicillata*. Note nine autosomal bivalents and the sex trivalent.

*Fig. 5.* Metaphase I of *Carollia perspicillata*. Note nine autosomal bivalents and the sex trivalent.

*Fig. 6.* Cut-out trivalents of the sex chromosomes of *Carollia perspicillata* showing similarity in synapsis behavior.

Its long arm has a deep, conspicuous secondary constriction near the centromere. The  $Y_1$  chromosome is a long acrocentric, and is slightly longer than the distal segment of the X beyond the secondary constriction; the  $Y_2$  is a small acrocentric (Fig. 2).

The X chromosome of one female specimen of *C. subrufa* had a special feature: the secondary constriction seems to have two compartments, *viz.*, a small piece of chromatin was present in the middle of the constriction (Fig. 3). However, no other specimens possessed such a characteristic.

The homology between the sex chromosomes can be traced by their pairing behavior in prophase of spermatocytes. Figures 4 and 5 represent complete diakinesis and metaphase I, respectively, taken from aceto-orcein squash preparations of testes of *Carollia perspicillata*. The sex trivalent is clearly demonstrated in each case. The  $Y_1$  chromosome and the long arm of the X synapse perfectly, but the  $Y_2$  is attached end-to-end to the short arm of the X. Figure 6 presents seven cut-out trivalents from different cells to show the consistency of the configuration. Thus, the long arm of the X distal to the secondary constriction is homologous to  $Y_1$ . The behavior of the  $Y_2$  is typical of the ordinary Y chromosomes in most mammals with the XY-type of sex determination.

### Genus *Artibeus*

All autosomal complements of the four species, *A. jamaicensis*, *A. lituratus*, *A. toltecus* and *A. turpis*, are morphologically indistinguishable from one another. The diploid number is 30 (XX) for all female individuals and 31 for males of the first three species ( $XY_1Y_2$ ). The diploid number of *A. turpis* is 30 (XY).

The autosomes consist of ten pairs of submetacentric and metacentric and four pairs of subtelocentric elements. The X chromosome is a large subtelocentric. It is difficult to distinguish unequivocally from the large subtelocentric autosomes. The several species of *Artibeus* can be distinguished cytologically only by the morphology of the Y chromosome or Y chromosomes.

*A. jamaicensis*. Two acrocentric Y chromosomes. The  $Y_1$  chromosome is approximately one third the length of the long arm of the X, and the  $Y_2$  chromosome is a minute (Fig. 7).

*A. lituratus* and *A. toltecus*. Both species possess two Y chromo-

somes,  $Y_1$  and  $Y_2$ . Both Y chromosomes are small. It appears that the  $Y_1$  chromosome of *lituratus* is slightly larger than its  $Y_2$  (Fig. 8), but the two Y's are almost equal in size in *toltecus* (Fig. 9). However, the distinction is rather vague.

*A. turpis*. Classic XX/XY sex determination system. The Y chromosome is a small submetacentric (Fig. 10).

Since no testicular material was saved for studies on meiosis, homology between the sex elements remains to be determined.

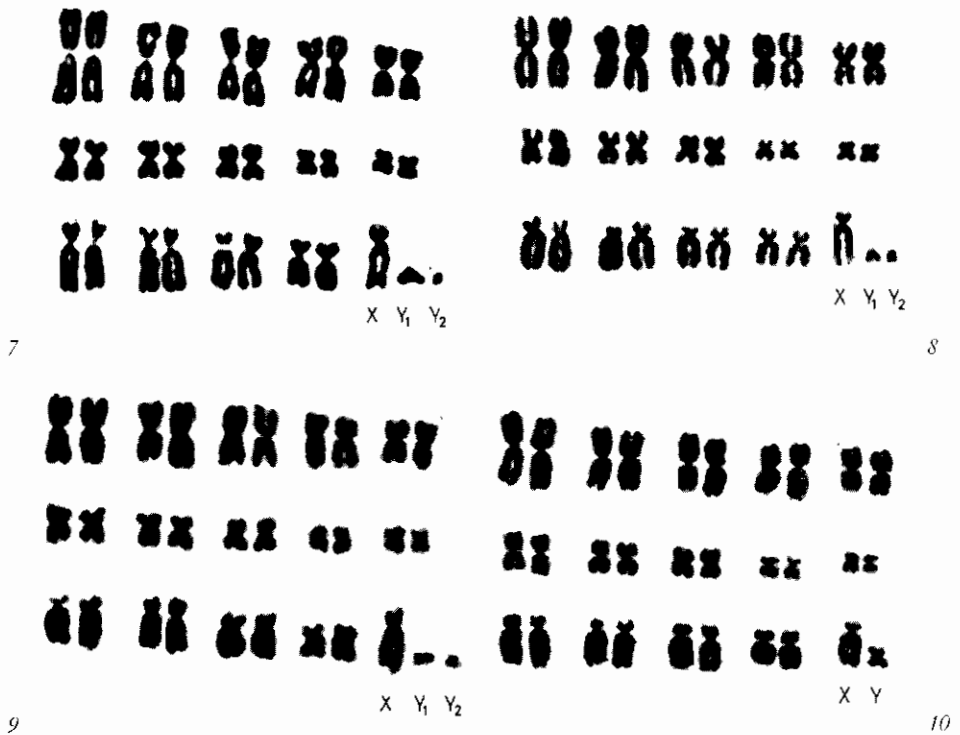


Fig. 7. Karyotype of a male *Artibeus jamaicensis*,  $2n = 31$ .

Fig. 8. Karyotype of a male *A. lituratus*,  $2n = 31$ .

Fig. 9. Karyotype of a male *A. toltecus*,  $2n = 31$ .

Fig. 10. Karyotype of a male *A. turpis*,  $2n = 30$ .

### Discussion

Translocations, especially the Robertsonian type, are thought to be one of the most common processes in mammalian chromosomal evolution. Robertsonian translocation (or fusion) is the formation of a bivalent chromosome from two acrocentric elements. If the fusion is between two autosomes, the diploid number is reduced by two and the fundamental number (FN) remains the same. When such a fusion occurs between an autosome and the sex chromosomes, both the diploid number and the fundamental number are reduced by two. If the fusion involves the X element but not the Y, the diploid number is reduced by two in the female but by only one in the male. The FN will be reduced by two in both sexes.

Naturally, a translocation between an autosome and an X does not happen frequently. Thus only a few cases have been found in the class Mammalia, and most of these do not occur in the same family. BOARGANKAR (1967) reported a case in *Echinops telfairi* O. THOMAS (Tenrecidae, Insectivora), where the population is polymorphic in regard to the sex chromosome condition, *viz.*, both XX/XY and XX/XY<sub>1</sub>Y<sub>2</sub> karyotypes coexist.

Our finding that six species (three genera) in one family possess this XX/XY<sub>1</sub>Y<sub>2</sub> constitution is unique. From the chromosome morphology of female *Choeronycteris* and the close taxonomic relationship between *Choeronycteris* and *Choeroniscus*, we consider a 17-chromosome male *Choeronycteris* a distinct possibility.

The *Carollia* case, like *Potorous*, is a classic example of X-autosome translocation. A number of bat species have a medium-sized metacentric or submetacentric X (*e.g.*, *Pteronotus*, *Phyllostomus*, *Glossophaga*). This is probably a more primitive form. Probably the original X of *Carollia* was a medium-sized, almost metacentric chromosome. The translocation apparently involved one of the arms (possibly the slightly longer one) of the original X. After the translocation, the arms of the X became distinctly unequal in length. Of interest is the deep secondary constriction on the new X at or about the juncture of translocation. Again the situation is similar to that of *Potorous*. It is possible that the long arm of the original X carried a terminal nucleolus organizer. The fusion resulted in an interstitial nucleolus organizer.

The karyotype of *Carollia subrufa* depicted in Figure 3 may have had a paracentric inversion which involved the nucleolus organizer.

Since most specimens of this species examined had a nucleolus organizer without a piece of chromatin in the middle, the inversion may simply be an anomaly. Other than this minor difference, the two species of *Carollia* unquestionably share the same original translocation. But we are not certain about the direct phylogenetic relationship between *Carollia* and *Choeroniscus*, though the two karyotypes exhibit many similarities. Superficially, the two genera are quite different in their feeding habits (*Carollia* are fruit-eaters and *Choeroniscus* nectar-eaters). Their differences, however, may be adaptive to suit the individual feeding habits *after* the translocation occurred in a primitive ancestor. Therefore, it is not altogether outrageous to consider that the two genera are more related than other taxonomic characteristics have indicated. Nevertheless, the sex chromosomes of *Choeroniscus* and those of *Carollia* are considerably different, *e.g.*, the absence of a distinct secondary constriction in the X of *Choeroniscus*, the disproportion between the long arm of the X and  $Y_1$ , etc. Without additional evidence, homology between the sex elements of these two genera remains a question, since the possibility of parallelism cannot be eliminated. In all *Choeroniscus* examined,  $Y_1$  is always longer than either arm of the X. If the homolog of  $Y_1$  has been translocated to the X, subsequent rearrangement of either the X, the  $Y_1$  or both must have occurred, which would account for the huge size of  $Y_1$  in relationship to the arms of the X.

The situation in *Artibeus* is quite different from those in *Carollia* and *Choeroniscus*. The two Y chromosomes do not differ greatly in length, even though one may be slightly longer than the other. In all species the  $Y_1$  is considerably shorter than the long arm of the X. If the X-autosome translocation in *Artibeus* were the same as the one which gave rise to *Carollia*, numerous changes would be required to produce the present *Artibeus* karyotype. These include the elimination of the nucleolus organizer region, a shortening of the short arm of the X and, above all, a drastic reduction in size of the  $Y_1$  chromosome.

A more likely and alternative explanation is that the *Artibeus* translocation was an entirely different event from that of *Carollia*. The long arm of the original X (a distinct submetacentric) fused with a relatively small acrocentric, approximately the size of the  $Y_1$  of *jamaicensis*. Thus one translocation will produce the present *jamaicensis* sex chromosomes. Neither the original long arm of the X nor the acrocentric had terminal nucleolus organizers.

The genus *Artibeus* is interesting in itself because of the karyological differences among the species examined. The  $Y_1$  chromosome shows a reduction of size. The morphology of the *turpis* Y chromosome emphatically suggests that a Robertsonian fusion between  $Y_1$  and  $Y_2$  has taken place. If this be true, *turpis*, though having a classical XX/XY karyotype, is in reality more advanced than its sister species which possess an XX/ $XY_1Y_2$  constitution. By the same token, many closely related genera, such as *Vampyrops* and *Sturnira*, which have karyotypes indistinguishable from that of *A. turpis*, may all have had X-autosome and Y-autosome fusions in their past history. Without intermediate forms such as *A. lituratus*, the origin of the aberrations would not have been detected, and the sex-determining mechanism would have to be considered as a classical XX/XY.

Since the morphology of the X chromosomes of all members of the subfamily Stenoderminae, which undoubtedly represent one line of evolution, suggests an X-autosome fusion, this translocation must have an ancient history. The evolutionary sequence of the Y chromosome, however, appears complex. The species which possess the highest number of acrocentrics, *Uroderma bilobatum*, shows a biarmed Y, similar to that of *Artibeus turpis*. If we consider *Uroderma*, a cytologically more primitive form, two possibilities exist regarding the formation of the metacentric Y: (1) the Y-autosome translocation occurred repeatedly but independently and (2) a fission occurred in the ancestor of several species of *Artibeus* after the fusion.

Karyological features are often useful in tracing phylogenetic relationships. In cases such as *Myotis*, where 14 species had karyotypes indistinguishable from one another but different from those belonging to other genera (17 species) of Vespertilionidae, chromosome analysis offers unquestionable support to the validity of the common origin of these 14 members (BAKER and PATTON, 1967). In Phyllostomidae, chromosomal characteristics are certainly superb tools for evolutionary studies, especially if these are combined with morphological, physiological and behavioral patterns. A general discussion regarding chromosomal evolution in this family is presented by BAKER (1967) with detailed documentation of karyotypes of various species in Phyllostomidae.

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