CARYOLOGIA Vol. 57, no. 1: 1-9, 2004

Meiotic analyses of the sex chromosomes in Carolliinae-Phyllostomidae (Chiroptera): NOR separates the XY_1Y_2 into two independent parts

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Abstract - Meiotic analyses of the sex chromosomes were made in two different species belonging to the subfamily Carollinae (Phyllostomidae, Chiroptera): *Rhinophylla pumilio* and *Carollia perspicillata*. These species have different sex chromosome systems. Through banding techniques, chromosomes homologies between *Carollia perspicillata* and *Rhinophylla pumilio* were not found, possibly because *Carollia* species have highly rearranged chromosomes. Probably, *Rhinophylla* is better associated to Phyllostomini tribe. Sex vesicle pairing behavior at the meiotic prophase was analyzed in both species. *R. pumilio* had a simple sex chromosome system (XX/XY), the XY pair showed an asynchronic behavior compared to the autosomal bivalents. The sex vesicle showes the X and Y pairing in the pseudoautosomal region. Moreover, a folding of the X-asynaptic axis on itself and condensed differentiated axes were also identified. In *C. perspicillata*, with a multiple sex chromosome system (XY_1Y_2), the segments Xp-Y_1 and Xq-Y_2 paired independently from each other. Compared to the autosomes, the sex vesicle showed a precocious pairing behavior. In *C. perspicillata*, a NOR stained with silver was localized in the X rearranged chromosome. Possibly, this region separates the XY_1Y_2 trivalent in two independent parts: one involving the X and Y_2 autosomal with transcriptional activity, and the other corresponding to the original X and Y sex pair.

Key words: Carolliinae, Meiosis, genic activity, translocations, NOR.

INTRODUCTION

A The subfamily Carolliinae (Phyllostomidae-Chiroptera) constitutes a group of frugivorous Neotropical bats widely distributed throughout the New World. This subfamily is composed of two genera, *Carollia* and *Rhinophylla* and is characterized by an intrageneric karyotypic stability, with multiple XX/XY_1Y_2 and simple XX/XY chromosomal sexual determination systems (BAKER *et al.* 1989).

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According to RUSSEL (1963), SOLARI (1994) and DELOBEL *et al.* (1998), the X chromosome has an inactivation center responsible for a phenomenon called spreading effect of the chromatin inactivation, where it is probable that inhibition occurs in genic transcription in the autosomal regions translocated to the X chromosome in mammals.

In some species that present translocations in sexual chromosomes, such as the mouse with Searle translocation (T(X;16) 16 H) inactivation of the sexual chromosomes occurs together with the translocated autosomal region (READER and SOLARI 1969; SOLARI

1971). The hypothesis of inactivation of the X chromosome assumes that in all heterogametic organisms the X chromosome is normally deactivated during a critical stage of spermatogenesis. The same hypothesis suggests that the inactivation center of the X chromosome (Xq3 in humans) leads to the inactivation of X (sexual chromatin) in somatic cells and to the formation of the sex vesicle in spermatogenesis (SOLARI 1994).

In *Gerbillus* sp. (RANTOMPONIRINA *et al.* 1986) and Stenodermatinae (NORONHA *et al.* 2001), careful description of the structure of the sexual pair in composite and multiple systems during meiosis in male, showed the presence of an intercalated heterochromatic region that acts as an "isolator", allowing the independent behavior of the true (inactive) and autosomal (active) sexual segments. In some species of mammals, the sex vesicle (XY body) is associated with the nucleolar material in certain stages of the meiotic prophase, such as, for example, in the mouse *Mus musculus* (SOLARI, 1994). Nonetheless, few species present the nucleolar organizer region (NOR) located in a sexual chromosome, such as the bat *Carollia perspicillata* (X chromosome) (VARELLA-GARCIA *et al*. 1989), *Canis familiaris* (PATHAK *et al*. 1982) and the primate *Callithrix jacchus* (NAGAMACHI and FERRARI 1986) and *Hylobates syndactylus* (LEDBETTER 1981).

Through use of optical microscopy we intended to describe the meiotic behavior of the sex chromosomes comparing the two systems of the chromosomal sex determination of the Carolliinae, Phyllostomid bats.

MATERIAL AND METHODS

The sample for this study is made up of 6 exemplars (5M, 1F) of *Carollia perspicillata* and 2 (M) of *Rhinophylla pumilio*, collected at five localities in Pará (Table 1).

❶ Submitted to the conventional meiosis technique, following EICHER (1966): the cells were stained with Giemsa.
❷ Submitted to the synaptonemic complex technique, following VERMA and BABU (1995): the synaptonemic complexes were marked with silver nitrate.
❸ The mitotic cells were extracted, according to FORD and HAMERTON (1956) and fibroblast cell lines, tissue culture. The metaphasic chromosomes were submitted to the NOR technique, following HOWELL and BLACK (1980); the nucleolar organizer regions were marked with silver nitrate.

The selected cells were photographed in a Carl Zeiss III photomicroscope, 100X immersion lens, optovar 1,25, with a green filter, ASAs 3,2 (DIN 6) and 6,3 (DIN 9). The films employed were Agfa Copex Pan and Imagelink HQ, developed and fixed with Kodak D76 developer and Kodak fixer, respectively. The images were copied on Kodabrome Print F3 paper (Kodak) and digitized.

RESULTS

Carollia perspicillata – This species presented $2n$=20/21 and a multiple system of chromosomal sexual determination of the XX/XY_1Y_2 type. Analysis of the NORs in the mitotic cells, allowed us to identify them only in the secondary interstitial constriction of the long arm

Table 1 – Species identified, with references to the techniques applied and collection site.

Species	N° (MPEG)	N° (UFPA)	Sex	Collection site
Carollia perspicillata[a]	26435	AL 13 ❶	M	MPEG Park, Belém, Pará, Brazil
	26465	AL 46 ❶	M	Mosqueiro Island, Belém, Pará, Brazil
	26489	AL 71 ❸	F	Mosqueiro Island, Belém, Pará, Brazil
	26496	AL 77 ❶❸	M	Campus of the UFPA, Belém, Pará, Brazil
	27499	AL 195 ❷	M	Campus of the UFPA, Belém, Pará, Brazil
	27519	AL 215 ❷	M	MPEG Park, Belém, Pará, Brazil
Rhinophylla pumilio[b]	26508	AL 93 ❶	M	Santa Rosa, Vigia, Pará, Brazil
	27279	AL129 ❶	M	Vila de Tacajós, Sta. Izabel, Pará, Brazil

a – species with multiple XY_1Y_2 sexual system. b – species with simple XY sexual system.
The specimens were collected from natural populations and identified by Dr. Suely Marques-Aguiar, of the Zoology Department (MPEG) and are part of the collection at the Museu Paraense Emílio Goeldi.

of the X chromosome, with two stainings in the females (Fig. 1a) and one in the male (Fig. 1b).

Through meiotic analysis of the male, 9 autosomal bivalents and one sexual trivalent formed by XY_1Y_2 were observed. In the initial stages of meiosis a typically condensed sex vesicle is formed (Figs. 2a and 2b). In the diplotene and diacinesis, the formation of chiasmas in all the extension of the 9 autosomal bivalents was observed (Fig. 2c); while in the sexual trivalent the formation of at least three chiasmas in the distal half of the long arm of X with its homologue Y_2 (Fig. 2d) and point to point pairing of Y_1 with the extremity of the short arm of X were visualized (Figs. 2e and 2f). In metaphase I, with 9 bivalents and one sexual trivalent, one may observe a more precocious behavior of the sexuals, with the Y1 open in relation to the X axis, while Y2-X are still paired in a manner similar to that of the biva-

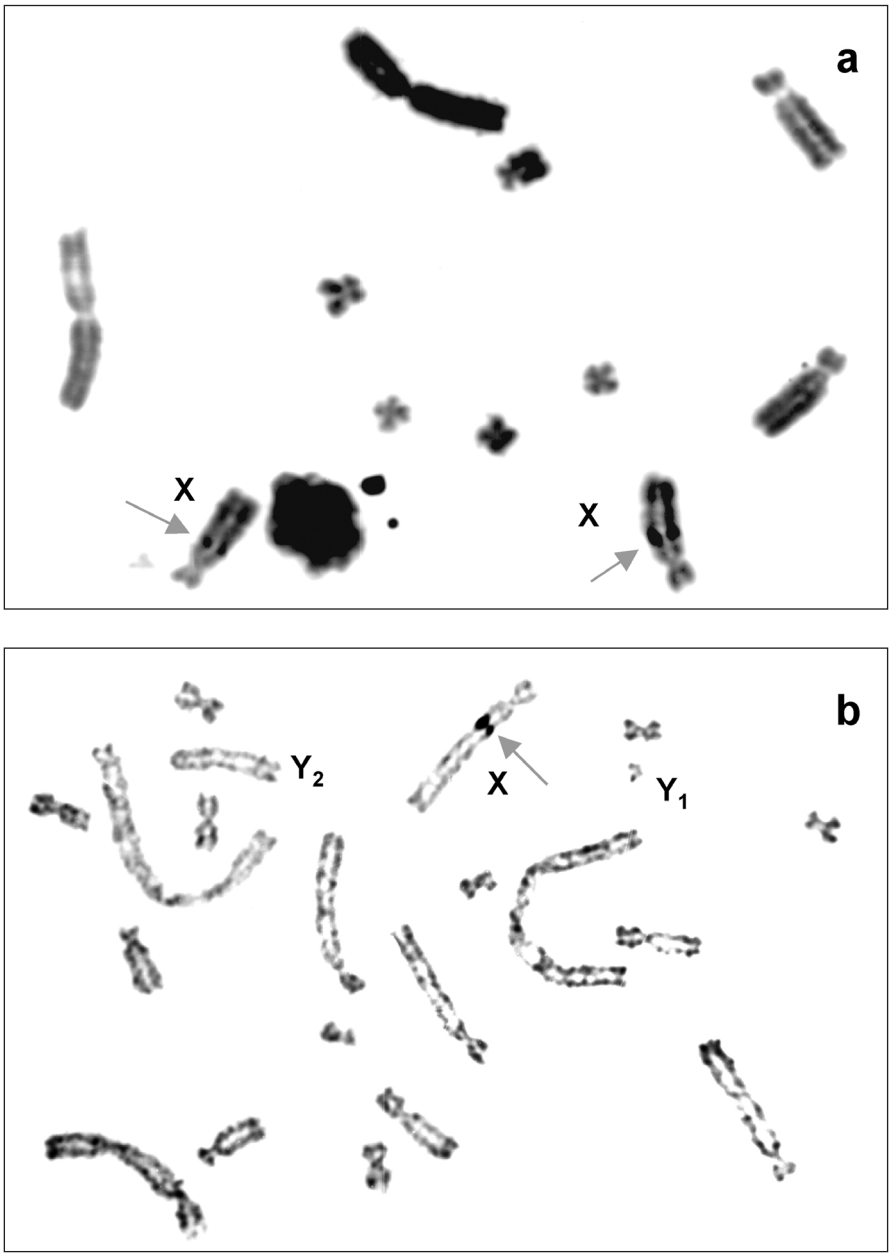

Fig. 1 – Ag-NOR staining in the interstitial region of the long arm of the X chromosome of *Carollia perspicillata*, with $2n$=20/21 XY_1Y_2system (parcial view of the cell). a) two stainings in the females and b) one staining in the males.

lents (Figs. 3a and 3b). In metaphase II, cells with *n*=10 containing the sexual chromosome X (Fig. 3c) and with *n*=11 were encountered, containing the sexual chromosomes Y_1 and Y_2 (Fig. 3d). Analysis of the synaptonemic complex (SC) in the pachytenes of *C. perspicillata* shows the NOR staned by silver nitrate in the sex vesicle, located in the X chromosome, as in the mitotic cells described above. One may observe the more accentuated condensation of the sex vesicle in relation to the autosomal bivalents, staining the entire sexual body (Fig. 3e).

Rhinophylla pumilio – This species presented 2*n*=34 and a simple system of chromosomal sexual determination of the XX/XY type.

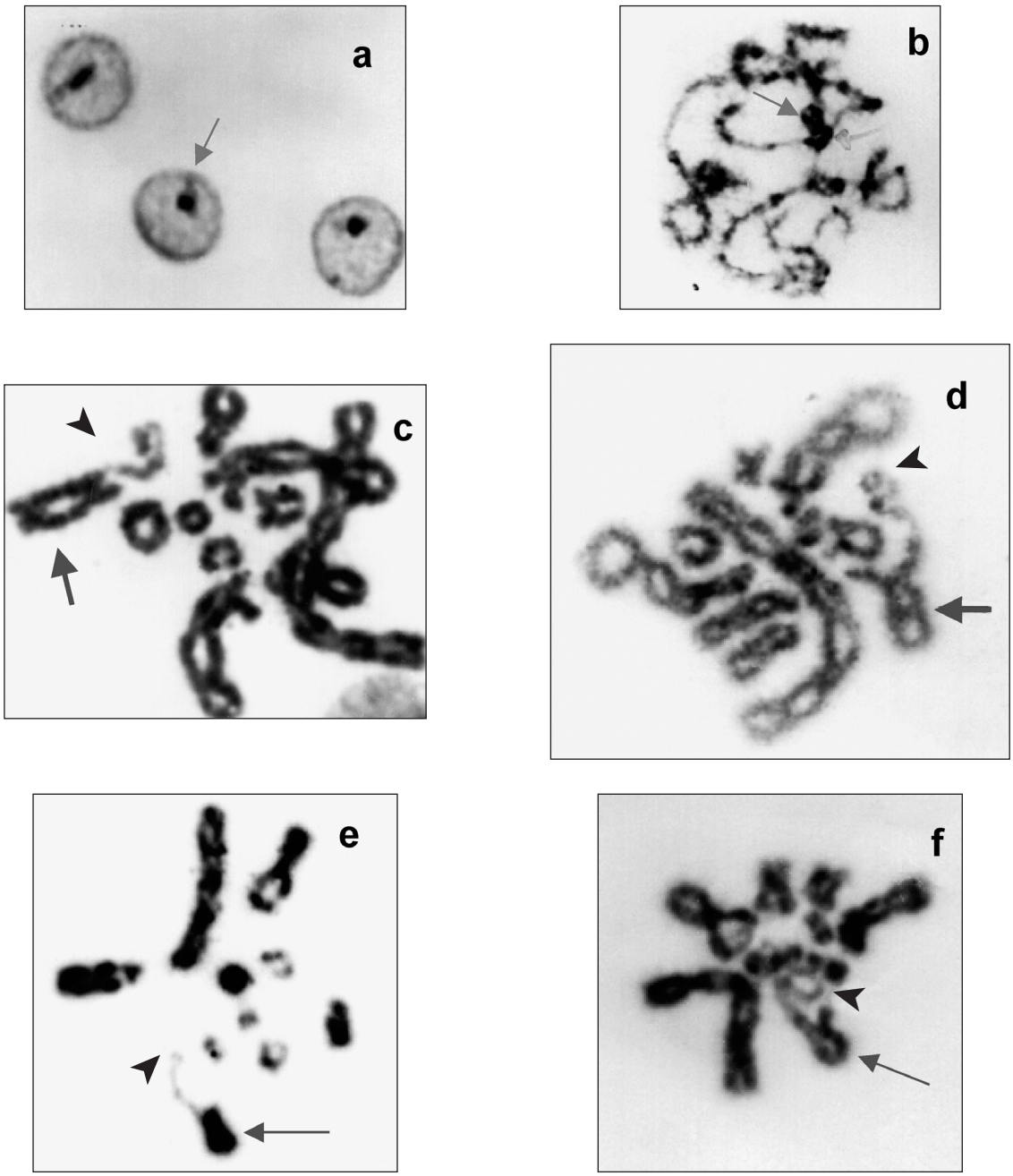

Fig. 2 – Conventional meiosis of *C. perspicillata*, with XY_1Y_2system. a) and b) the arrows indicate typically condensed SV in the initial stages of meiosis. c) and d), e) and f) cells in diplotene with 9 autosomic bivalents presenting chiasmas in all their extension, and 1 sexual trivalent in which the arrows shows at least three chiasmas between Xq distal-Y_2 and the arrowheads indicate point to point pairing between Xp-Y_1.

In the meiotic analysis, the typical sex vesicle in pachytenic cells of mammals was observed, with a sequential behavior, showing a progressive opening of the sex vesicle. As in the other species, in *Rhynophylla pumilio* it was also possible to observe the presence of a sex vesicle in

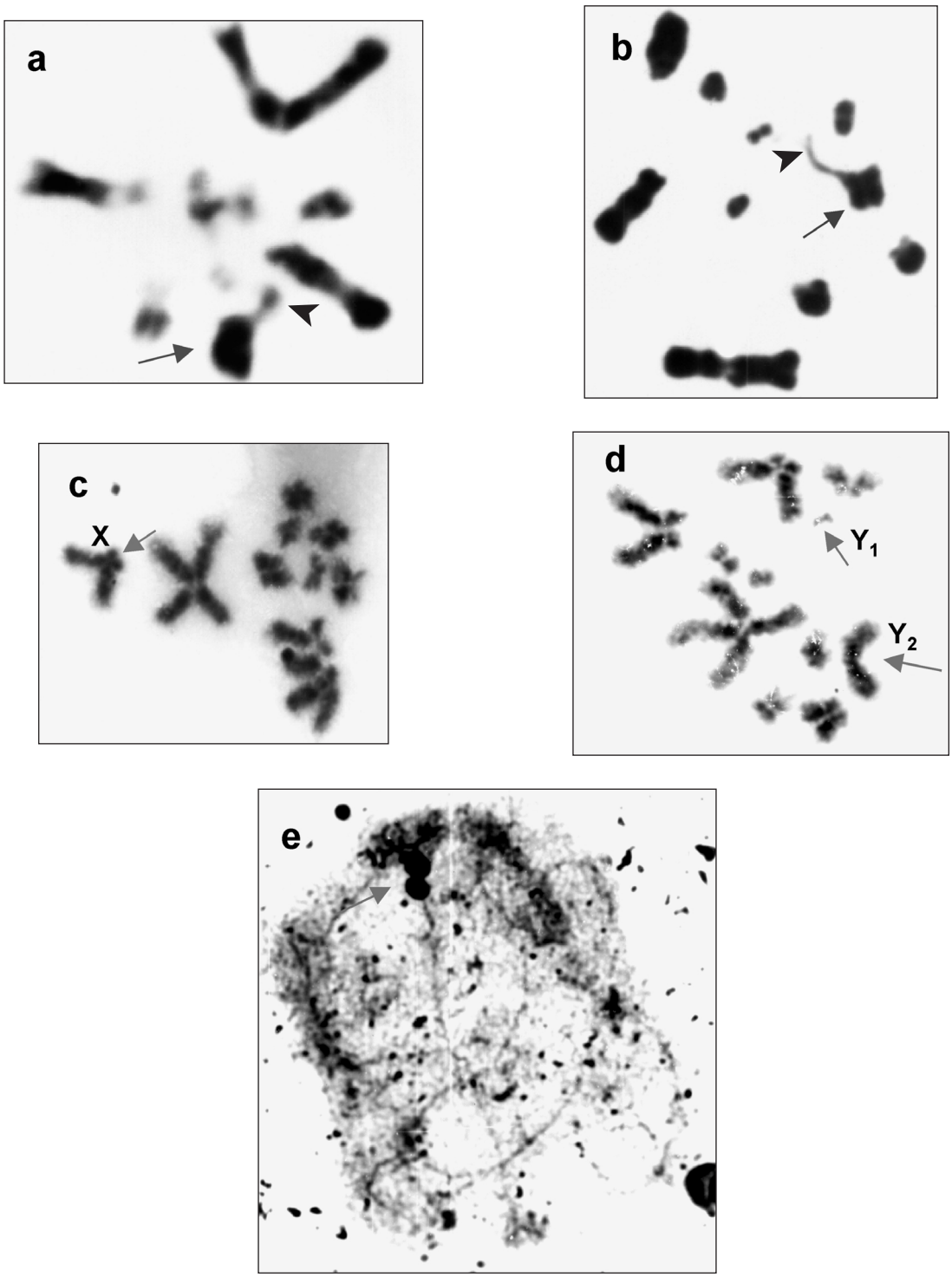

Fig. 3 – Conventional meiosis and synaptonemic complex of *C. perspicillata*, with XY_1Y_2 system. a) and b) cells in metaphase I with 9 autosomic bivalents and 1 sexual trivalent; the arrowheads indicate point to point pairing between Y_1-X and the arrows indicate pairing between Y_2-X similar to the autosomics. c) MII, with n=10 (X) and d) MII with n=11 (Y_1Y_2). e) the arrow indicates NOR staining in the SV.

the progressive substages of prophase I, such as an initial pachytene with a more evident condensation (Fig. 4a), intermediary pachytene in which the vesicle already begins one opening (Fig. 4b), and delayed pachytene, with the sex vesicle open, in the form of a ring (Fig. 4c). In diplotene, with autosomal bivalents presenting chiasmas along the chromosomic axes, precocious condensation of the sexual bivalent in relation to the autosomes (Fig. 4d) was observed. In diacinesis, 16 bivalents were found, 15 being autosomal and 1 sexual XY (Fig. 4e).

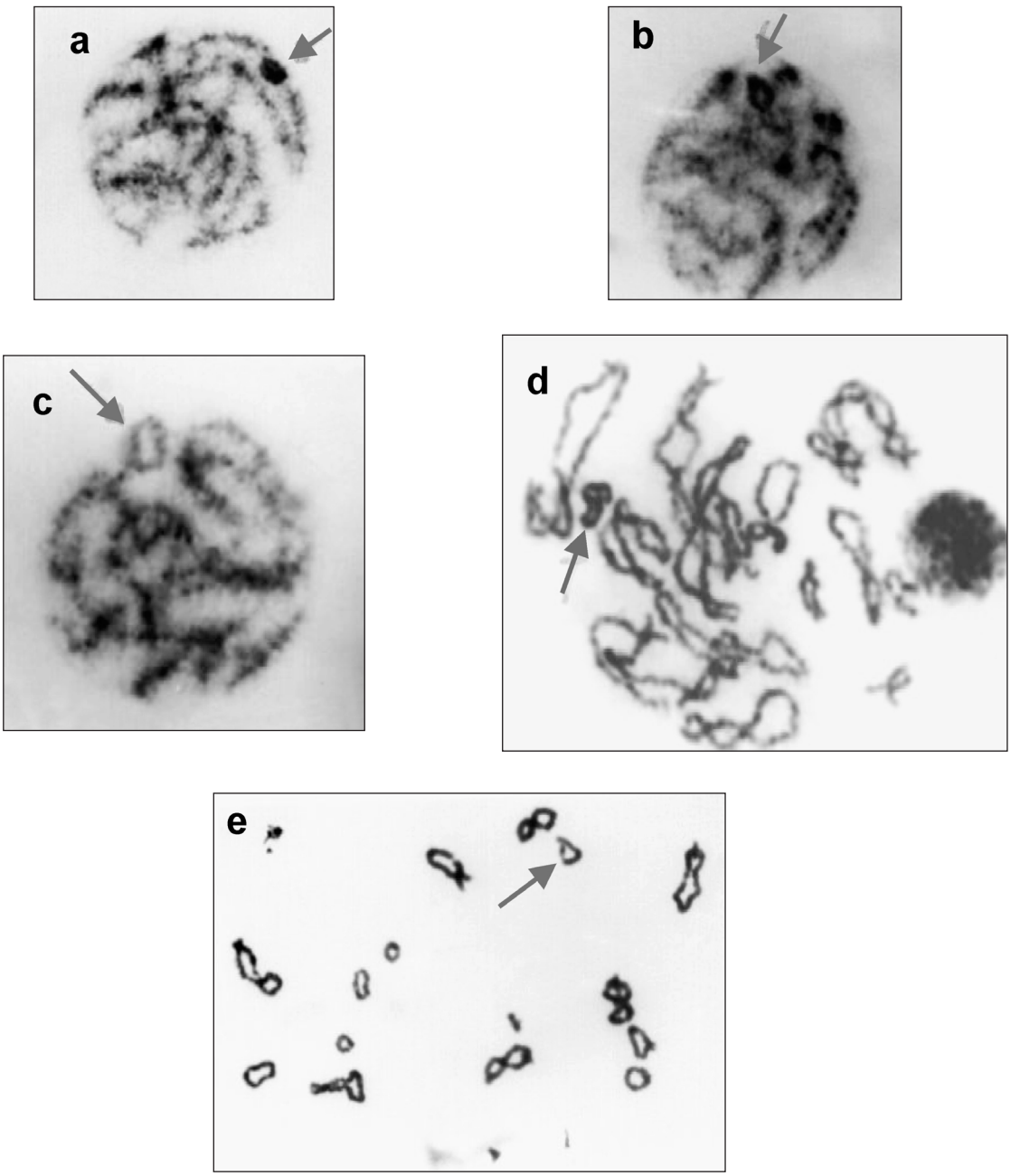

Fig. 4 – Conventional meiosis of *Rhinophylla pumilio*, with simple XY system. a) in initial pachytene the arrow shows SV with more evident condensation. b) in intermediary pachytene the SV begins an opening (arrow). c) in delayed pachytene the arrow indicates an open SV in the form of a ring. d) in diplotene the arrow shows the sexual bivalent with precocious condensation in relation to the autosomic bivalents. e) in diacinesis the arrow indicates the sexual bivalent.

DISCUSSION

Rhynophylla pumilio and *Carollia perspicillata*, despite being in the same subfamily have different sexual systems: simple XX/XY and multiple of type XY_1Y_2, respectively. The XY_1Y_2 system in males of *C. perspicillata* originated after the translocation of one autosome to the X. Thus, the autosomal homologue begins to act as sexual, calling Y_2 and Y_1 is the real one of the system. The occurrence of multiple sexual systems in independent lineages within the family Phyllostomidae, involves different autosomes in translocation to the X. This indicates that the sexual systems arose more than once during the evolutionary history of the family.

STOCK (1975), compared the karyotypes of *C. castanea* with *C. perspicillata.* They observed homology in the morphology of all the chromosomes among the two species. It was possible to identify the ancestral autosome of *C. castanea*, which was translocated to *C. perspicillata*, which suggests an autosome-X fusion, leading to the formation of the multiple XY_1Y_2system, in *C. perspicillata*.

Nucleolar Organizer Region – Analysis of the distribution of the NORs in *C. perspicillata* showed little variability, which was found only in the interstitial region of the long arm of X (Fig. 1). Staining was not observed in the Y_2 homologue to autosome-X, indicating the staining of the nucleolar organizer region only in the two original X chromosomes of the *C. perspicillata* females. According to FAGUNDES (1993), in the majority of the cells analyzed in female rodents, only one of the X chromosomes presented a positive staining in the NORs, although in a few metaphases the two X were marked. These data confirm the hypothesis of the incomplete inactivation of one of the X chromosomes of the female mammals, in which some regions escape this process, and remain active during the entire cellular cycle. According to YONENAGA *et al.* (1983), the two Xs may be genic activity at the same time.

According to GOODPASTURE and BLOOM (1975), there is one NOR in the telomere of the long arm of Y in *C. castanea*. Thus, it is probable that there was a loss of this NOR during evolution, leading to a disparity of genic activity (DNAr) in males of *C. perspicillata* with only one staining in X.

According to HSU *et al.* (1968), the original NOR may have been located in the terminal portion of the long arm of X in *C. castanea*, thus, after fusion with one autosome, NOR becomes interstitial (Xq) in *C. perspicillata*; however, GOODPASTURE and BLOOM (1975), observed NOR staining in the distal segment of the short arm of X rearranged in *C. castanea*. The data obtained in this paper agree with the staining of terminal Xp NOR of the ancestral species *C. castanea* observed by GOODPASTURE and BLOOM (1975), suggesting an alternative hypothesis in the evolution of X in *C. perspicillata*, in which the autosome was translocated to the short arm of X. After this rearrangement the Xp de *C. castanea* begins to be Xq, originating the interstitial Xq NOR of *C. perspicillata.*

Meiosis – In *Rhinophylla pumilio* with simple XY sexual system, in the pachytenic cells this pair formed a typically condensed sex vesicle with precocious behavior in relation to the autosomes, as in the majority of the eutherian mammals. In *C. perspicillata*, with a multiple system, one may note a formation of typical SV in the pachytenes, and, beginning in the more advanced stages, such as diplotene and diacinesis, the sexual trivalent (XY_1Y_2) presents differences in behaviors of the axes of the original XYs in relation to the translocated autosome. Based on visualization of the formation of at least three chiasmas in the autosome-X pairing (Fig. 2d), one may prove genic activity in this region of the sexual trivalent, as well as in the autosomal bivalents, with chiasmas at various chromosomic points.

It is known that the autosome translocated to the X of *C. perspicillata* is separated by a secondary constriction, in which the NOR, of the original X axis is located. From this data, one may propose a hypothesis similar to the one applied for groups of mammals that present autosome-sexual rearrangements, in which the original segment of X is separated by a heterochromatic block of the autosomal segment. This block probably impedes the diffusion of inactivation to the autosomal segment of X, during meiosis (PATHAK *et al.* 1973; STOCK 1975; KASAHARA and DUTRILLAUX 1983; DELOBEL *et al.* 1998; NORONHA *et al.*

2001). The same hypothesis applies to the NOR of *C. perspicillata*, which impedes the spread of inactivation and maintains the sexual trivalent (XY_1Y_2) in two independent parts during meiosis, as attested by genic activity in the region of autosomal pairing and condensation in the sexual segment.

In *C. perspicillata*, instead of heterochromatin, there is possibly a nucleolar organizer region, which blocks the diffusion of inactivation to the autosome, impeding its progression to the segments of the translocated autosomes, allowing the production of viable gametes.

According to DELOBEL *et al.* (1998), the pulling of the autosomes to within the SV make them hypercondensed, as happens with the sexual chromosomes. This explain the degeneration of the spermatocyts, suggesting autosomal inactivation through a diffusion effect of inactivation. At no moment we detect degeneration of the spermatocyts of *C. perspicillata* but instead the presence of metaphase II with differences in the haploid number, justified by the segregation of the two Ys to the pole opposite X, assuring the production of balanced gametes. In *C. perspicillata*, the autosome translocated to X continues its original genic activity, without interfering in the function of sexual determination.

Synaptonemic complex – Analysis of the behavior of the sexual axes through the SC in *C. perspicillata* was not as instructive as conventional meiosis, due to NOR staining in X, making the delineation of chromosomic axes difficult. In any case, the differentiation of the condensation of the sex vesicle related to the autosomes was observed. The NORs were observed in initial pachytene, linked to the axis of the X chromosome. In this fashion, this chromosome was easily recognized in the sex vesicle of *C. perspicillata*. According to PATTON and GARDNER (1971), the NORs began to degrade with the progress of pachytene, disconnecting from the chromosomic axes and appearing as points scattered throughout the cell. Our data confirms the initial phase of pachytene observed in figure 3e, in which the NOR stays intact in the X chromosome, corroborating the mitotic results, in which only in sexual chromosome (X) is found staining nucleolar organizer region.

Acknowledgements – The authors are grateful to CAPES, CNPq, UFPA, Brazil, for financial support. We also thank the students and technicians from Laboratório de Citogenética-UFPA, Emilio Goeldi Museum and Evandro Chagas Institute who contributed to this research, especially to the Chiroptera Team (Nelson, Luis and Adailton).

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Received November 5, 2002; accepted January 30, 2003