

Synaptonemal complexes and XY behavior in two species of argentinian armadillos: *Chaetophractus villosus* and *Dasypus hybridus* (Xenarthra, Dasypodidae)

R. B. SCIURANO, M.S. MERANI, JIMENA BUSTOS, AND A.J. SOLARI

Centro de Investigaciones en Reproducción (CIR)- Facultad de Medicina, UBA. Paraguay 2155, (1121) Buenos Aires, Argentina.

Key words: Xenarthra, XY chromosomes, meiosis, synaptonemal complexes

ABSTRACT: Spermatocytes from the two armadillo species, *C. villosus* and *D. hybridus* were studied in microspreads for synaptonemal complexes (SCs) and in thin sections for electron microscopy (EM). The complete SC karyotype generally agrees with previous reports on mitotic chromosomes, except for the sex chromosomes. The X chromosome is submetacentric in both species and the Y is the shortest one in *C. villosus* and the second shortest in *D. hybridus*, and an extremely acrocentric one. A SC is formed along the total length of the Y chromosome, and this SC persists along all the pachytene substages. A single recombination nodule (RN) is located in the region of the SC nearest to the attachment to the nuclear envelope. The lateral element (LE) of the X axis in the SC shows a wavy aspect in most of the SC length distant from the nuclear envelope. Nucleoli are attached to acrocentric or submetacentric bivalents, are visibly double in some cells, and in thin sections show an elaborate nucleolonema. Some differences in the XY are species-specific, as the higher degree of tangling and stronger heteropycnosis in *D. hybridus*. The effective, single crossover of the XY pair is highly localized, despite the permanence of a long tract of SC.

Introduction

Armadillos are included in the family Dasypodidae of the order Xenarthra, which radiated in the past from South America towards the Northern hemisphere. Xenarthrans, and especially armadillos, are interesting as living species related to a rich paleontological registry, which included among other, glyptodonts classically studied in Argentina. Furthermore, their relatively long ancestry and the special embryological features

(polyembryony) in the genus *Dasypus* makes armadillos an interesting material for cytogenetic and genomic studies.

Recently, studies on the DNA of 12 of the 13 living Xenarthra genera (Delsuc *et al.*, 2002) yielded strong evidence that Xenarthra is one of the four major clades of placental mammals, supporting the antiquity and basal position of this order. Furthermore, the suborder Cingulata, which comprises armadillos, has been analysed as regards molecular phylogeny, showing that the genus *Dasypus* and the genus *Chaetophractus* are the most phylogenetically distant (Delsuc *et al.*, 2002), in agreement with the large reported differences in sperm size and structure (Cetica *et al.*, 1993). Thus, an analysis of meiosis centered on species of these two genera may give clues to the whole suborder Cingulata, which in itself is one of the oldest radiations of eutherian mammals.

Address correspondence to: Dr. Alberto J. Solari. Centro de Investigaciones en Reproducción (CIR), Facultad de Medicina, UBA. Paraguay 2155, (1121) Buenos Aires, ARGENTINA.
E-mail: asolari@fmed.uba.ar
Received on May 16, 2005. Accepted on October 17, 2005.

Karyological studies of armadillos are scanty, and most of them have been reviewed by Jorge *et al.* (1985). Beath *et al.* (1962) and Jorge *et al.* (1985) have reported karyotypes of species from the genus *Dasypus*, and a single study of the synaptonemal complexes has been reported on *Dasypus novemcintus* (Scavone *et al.*, 2000).

The present paper is aimed to describe the synaptonemal complex karyotypes, the nucleolar features, and the behaviour of the sex chromosomes in males from two species, *D. hybridus* and *C. villosus*. The behaviour of the XY pair shows that despite a rare permanence of a full synaptonemal complex throughout pachytene, which stretches along the complete Y chromosome, there is a single recombination nodule (crossover) which is located towards the segment nearest to the nuclear envelope. In *D. hybridus*, nucleolar organizers (NORs) are located in a specific bivalent and elaborate nucleolar extensions are formed at early pachytene. The Y chromosome seems to be structurally identical in both species. The kinetochore of the Y is practically attached to the telomere, making the Y an extremely acrocentric chromosome, which is the smallest of the SC complement in *C. villosus*, and one of the two smallest elements in *D. hybridus*.

Materials and Methods

The animals were collected as part of a project on Xenarthran biology (PICTR 74, Foncyt).

For this work, two male specimens of *Chaetophractus villosus*, one collected at Pipinas, Provincia de Buenos Aires, the other collected at Trenel, Provincia de La Pampa, were trapped and carried to the laboratory under adequate care. One male specimen of *Dasypus hybridus* was collected at Pipinas, Provincia de Buenos Aires, and carried as above mentioned. The animals, which were also studied in their anatomy and other biological features, were hemicastrated or killed under anesthesia, the testes removed and divided into several pieces for different procedures. The sacrificed animals were deposited in the mammalian collection of the Museo de la Universidad Nacional de La Plata (UNLP). For general testicular histology the pieces were fixed in Bouin fixative, embedded in paraffin and the sections were stained with hematoxylin-eosin. For fine

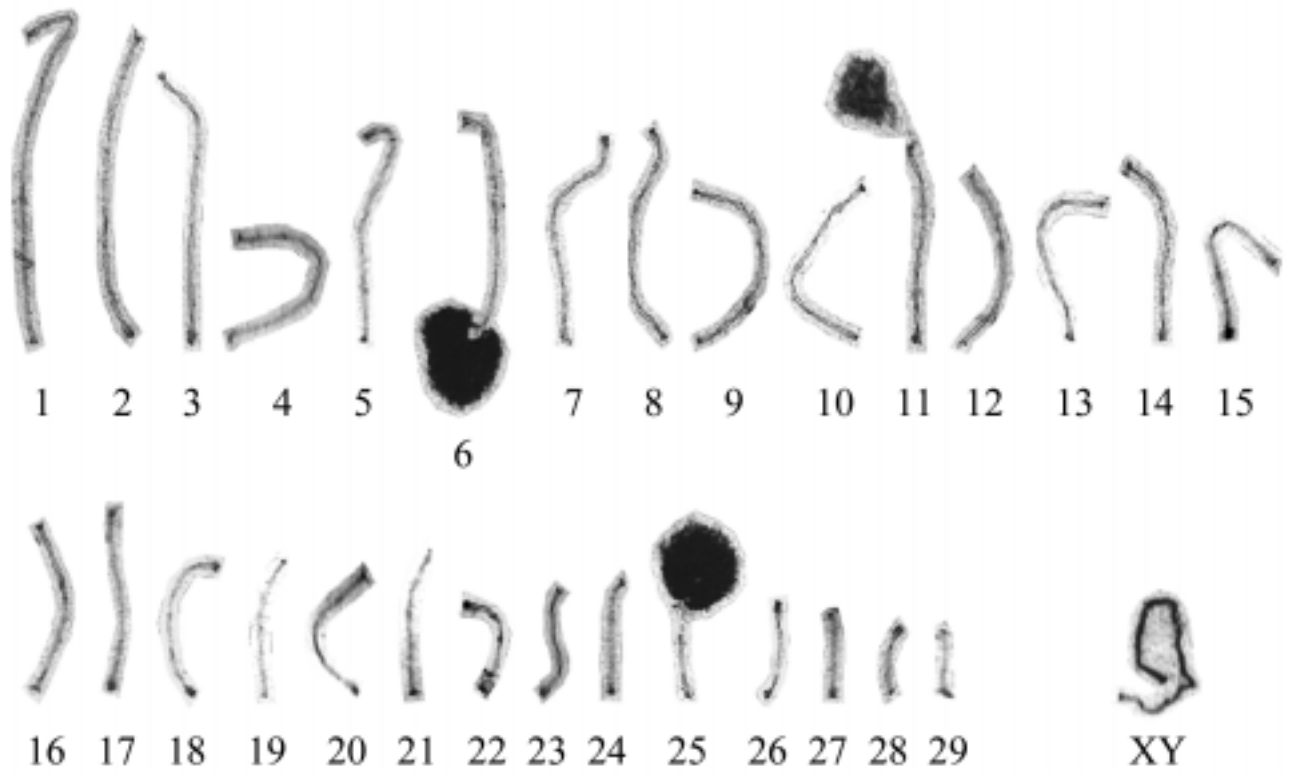
structural cytology, the pieces were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2 and then in OsO₄, embedded in araldite and the thin sections were stained with uranyl acetate and lead citrate. For the specific analysis of synaptonemal complexes (SCs), a variant of the drying-down technique for SCs was used (Solari, 1998). The spermatocytes were stained either with 4% phosphotungstic acid (PTA) in ethanol or with silver nitrate (Howell and Black, 1980). Electron microscopy was done with a Siemens Elmiskop electron microscope, and magnifications were checked with a calibration grid (Pelco International, Redding, Ca., USA). Micrographs were scanned and the digital data were used for measurements with the software Micromesure (Reeves and Tear, 2000).

Results

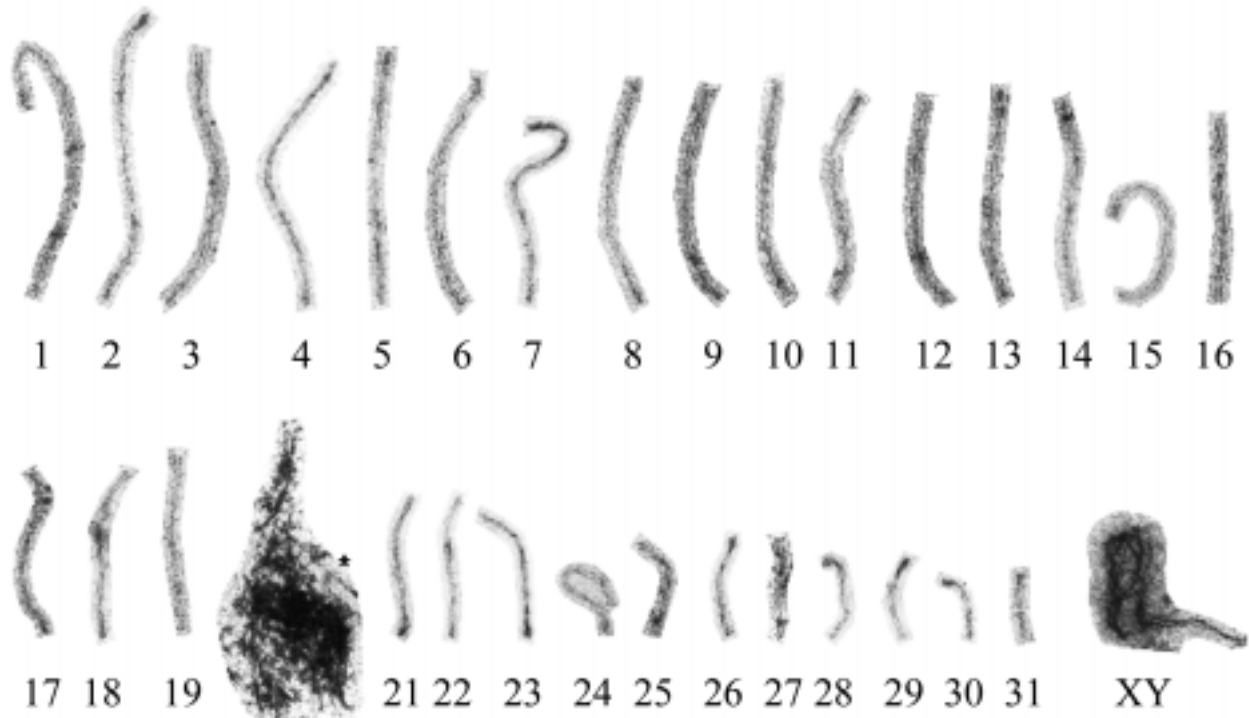
Synaptonemal complex karyotypes and their parallel mitotic karyotypes in C. villosus

The SC karyotype of *C. villosus* shows 29 autosomal SCs and the XY pair (Fig. 1). This SC karyotype corresponds to the already described chromosome number $2n = 60$ (Jorge *et al.*, 1977, 1985). However, the order of the SCs was done according to relative length only, in order to have comparable sets from different cells. The location of centromeres (kinetochores) was observed in PTA-stained specimens, and this procedure revealed 15 autosomal SCs with meta or submetacentric components and 14 autosomal SCs with acrocentric elements. Thus, this classification agrees with that of Jorge *et al.* (1977). However, the identification of individual SCs in silver-stained nuclei, of #11-#13, #14-#16 and #17-#21 was not possible, and thus in these EM preparations there is ambiguity in the identity of some of the SCs. On the other hand, #1 is always identifiable as the longest SC (with sub-medial kinetochore) as well as the two smallest, acrocentric autosomal SCs (#28 and #29). Three nucleoli were observed in the majority of pachytene spermatocytes: in #6, #11 and in #25. The latter is an obvious small submetacentric, which presents its nucleolus in all the cells. More variable are the presence of nucleoli in acrocentric #11 and the submetacentric #6.

FIGURES 1 and 2. SC karyotypes of *C. villosus* (Fig. 1) and *D. hybridus* (Fig. 2). The three nucleoli of *C. villosus* are attached to their NOR-bearing SCs. In Fig. 2, the single SC bearing the nucleolus of *D. hybridus* is marked with an asterisk.



1



2

SC karyotype of *D. hybridus*

The SC karyotype of *D. hybridus* agrees with that reported for mitotic chromosomes, which is generally described as $2n=64$. In fact, we find 31 autosomal SCs plus the XY pair, corresponding to a chromosome set of $2n=64$ (Fig. 2). As no PTA-stained specimens for autosomes were studied in this species, we cannot comment on the relationship with mitotic karyotype morphology. On the other hand, a single SC was always observed attached to the single, large and filamentous nucleolus observed in pachytene nuclei. This is a small SC, longer than the Y axis and the axis of the smallest acrocentric SC. This NOR-containing SC could correspond to #22 in the related species *D. novemcinctus*, which has been classified by Barroso and Seunanz (1991) as submetacentric in that species.

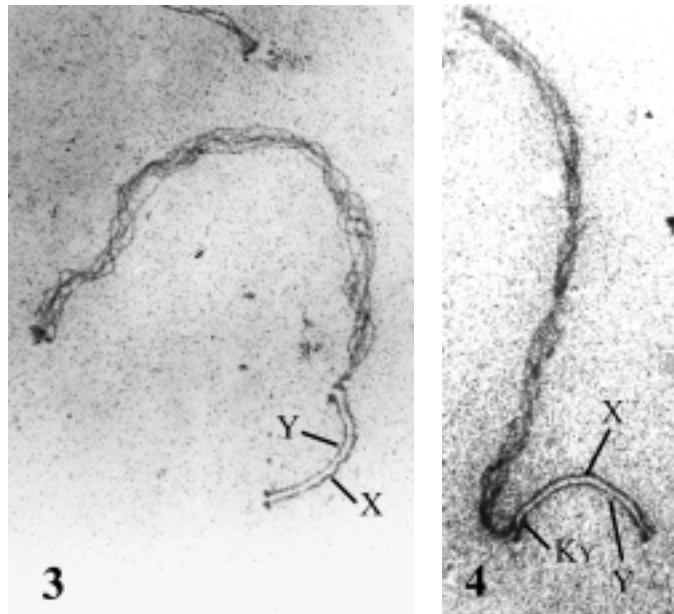
It should be remarked that in *D. hybridus* the smallest acrocentric is shorter than the Y axis.

General features of the XY pairs in both species

The general appearance of the XY pair is very similar in both species. It forms a conspicuously heteropycnotic body at advanced pachytene stages and it shows two unequal axes (X axis and Y axis) in earlier stages (Figs. 3 and 4). The absolute length of the Y axis in *C. villosus* is $4.0 \mu\text{m} \pm 0.8 \mu\text{m}$ ($n=11$). In *D. hybridus* the Y axis is $4.1 \mu\text{m} \pm 0.6 \mu\text{m}$ ($n=8$).

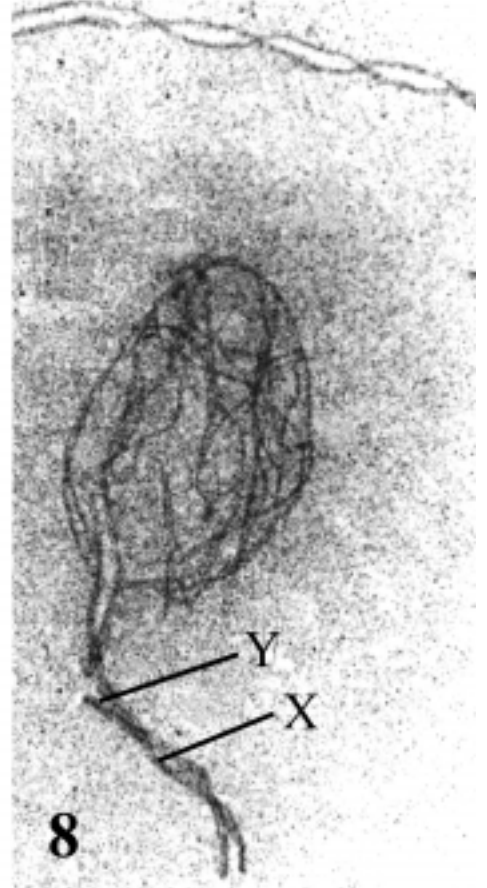
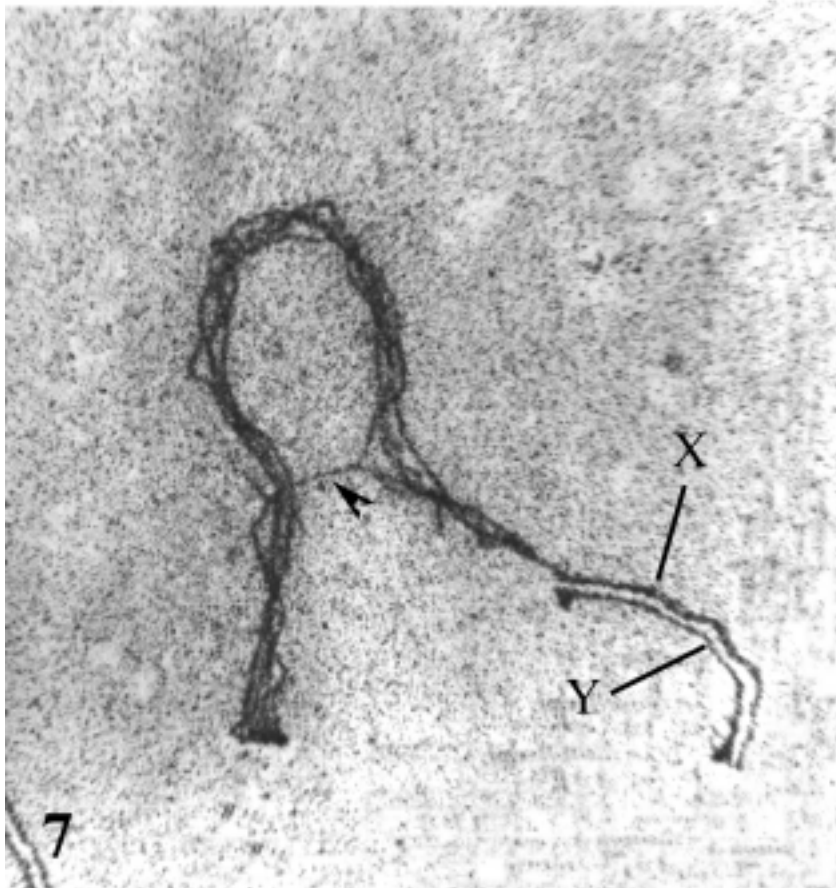
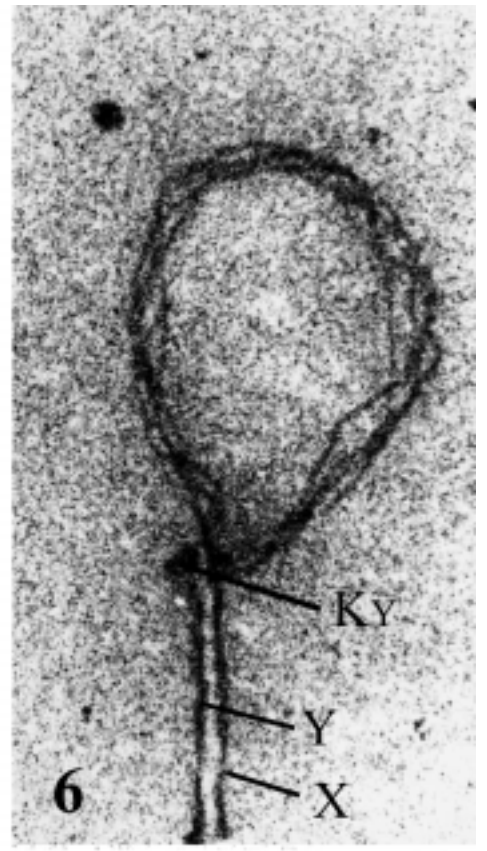
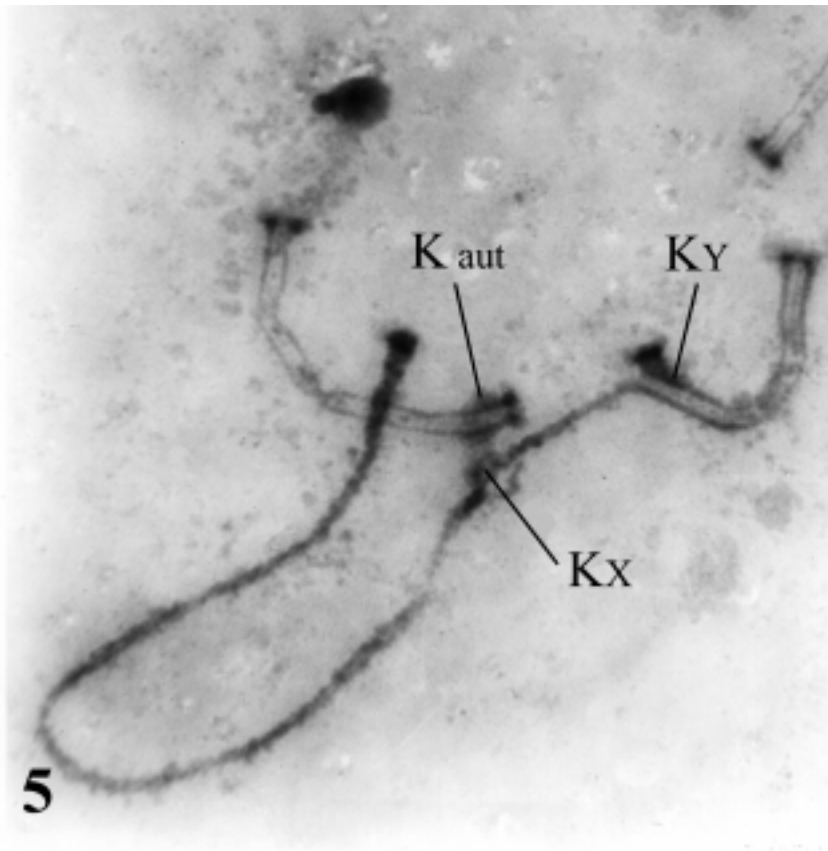
Relative lengths and centromeric indexes (CIs) of the X and Y axes

The length ratio between the X axis and the Y axis



FIGURES 3 and 4. Electron micrographs of silver-stained XY pairs of *C. villosus* (Fig. 3) and *D. hybridus* (Fig. 4) at early-mid-pachytene. The X-axis of *C. villosus* shows waviness or irregularities (arrowhead) in the upper part of the SC, and it splits into 4 strands in the differential region (arrow). The Y axis of *D. hybridus* shows the kinetochore (KY) as a dense protrusion before its ending. X 9,000.

FIGURES 5-8. Electron micrographs of the XY pairs of *C. villosus* (Figs. 5 and 7) and *D. hybridus* (Figs. 6 and 8). In Fig. 5, PTA staining allows the detection of the grey protuberances of the autosomal kinetochores (Kaut) and those of the X axis (Kx) and the Y axis (KY). The kinetochore of the Y is almost terminal in both *C. villosus* (Fig. 5) and in *D. hybridus* (Fig. 6). Figs. 7 and 8 correspond to late pachytene. While in *C. villosus* there is a bridge in the differential part of the X-axis (arrowhead), in *D. hybridus* there is a net-like array. Figs. 5-6, X 12,000. Figs 7-8, X 9,000.



is : $X/Y = 4.5$, $n=5$ in *C. villosus*, in early pachytene. This ratio is $X/Y = 4.3$, $n=3$ in *D. hybridus*, also in early pachytene.

The X axis can be measured only at early pachytene, as in later substages it forms a net-like, filamentous array in which it is not possible to perform measurements.

The general appearance and length of the Y axis are nearly identical in both species. In PTA-stained pachytene spermatocytes of *C. villosus*, the kinetochores of both axes are clearly established: the Y axis is extremely acrocentric ($CI = 0.1$) and the X axis is submetacentric ($CI = 0.25$) (Fig. 5).

This was a surprising fact, as the X axis of *C. villosus* has been described as clearly acrocentric (Jorge *et al.*, 1977). However, the centromere location in the X of *C. villosus* shows a clearly defined short arm, which is the one that pairs with the acrocentric Y (Fig. 5).

In *D. hybridus* the X is submetacentric and the Y, as in *C. villosus*, is extremely acrocentric and one of the two smallest of the set (Figs. 6 and 2).

Length of SC and variation with time

One of the striking peculiarities of the XY pair in both *C. villosus* and *D. hybridus* is the constancy of its length. This length is $SC(XY) = 3.7 \mu\text{m} \pm 0.8 \mu\text{m}$ (*C. villosus*, $n=15$) and $4.1 \mu\text{m} \pm 0.6 \mu\text{m}$ (*D. hybridus*, $n=8$).

The length of the SC, which covers practically all the Y axis except its proximal end, is retained up to the more advanced pachytene substages (Figs. 7 and 8).

On the other hand, a few XY pairs at a very early stage (as shown by the total absence of axial differentiations in the X axis and incomplete synapsis in autosomes), showed the proximal part of the Y axis in unpaired condition. At very early pachytene or late zygotene, synapsis between the X and Y axes may be incomplete, as 6% of the Y length may be unpaired at these very early stages.

About half of the observed XY pairs showed either twisting or irregularities in one LE of the XY SC. The only affected axis in all cases was the X axis (Figs. 3 and 9). These irregularities correspond to the segment of the SC which is more distant from the nuclear envelope.

Changes in the differential segment of the X axis in each species

In *C. villosus* the most striking differentiation of the differential segment of the X axis is its splitting into a number of strands, which is generally 4, (varying between 2 and 5). These strands seem to be branches and anastomoses of the original axis, which follow the same general direction. Some of these branches form bridges, which extend between the arms of the loop formed by the split X axis (Fig. 7).

There are some species-specific features in this differential segment, as the net-like array is more complex in *D. hybridus*, and the degree of heteropycnosis, as shown by the density of the surrounding chromatin, is much higher in *D. hybridus* when compared to *C. villosus* (Fig. 10).

Localization of recombination nodules (RNs, crossovers) in the XY pair

There is a single recombination nodule in the SC of the XY pair, which is located in a short segment near the attachment site of the SC with the nuclear envelope (Figs. 9 and 11a).

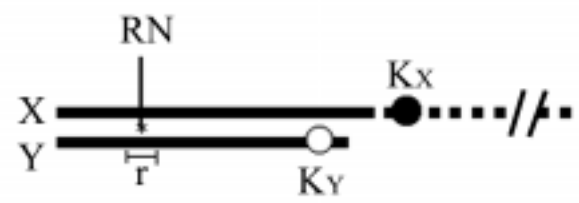
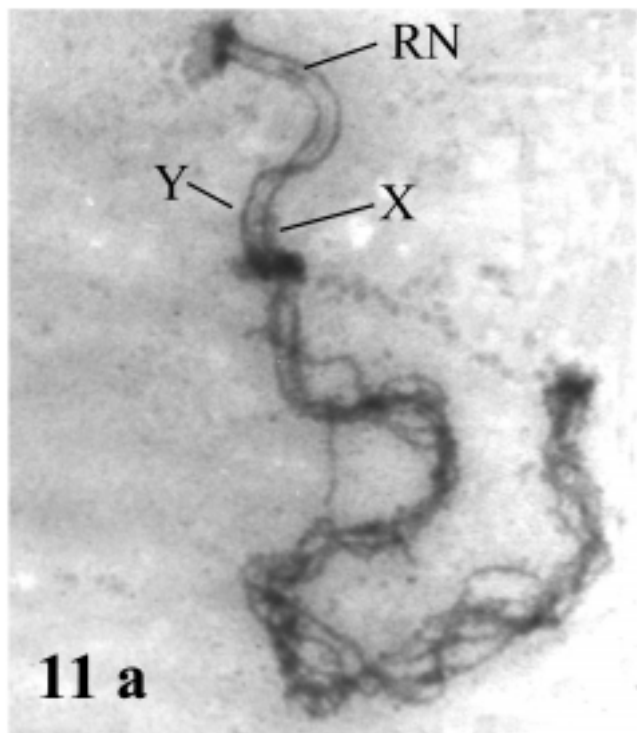
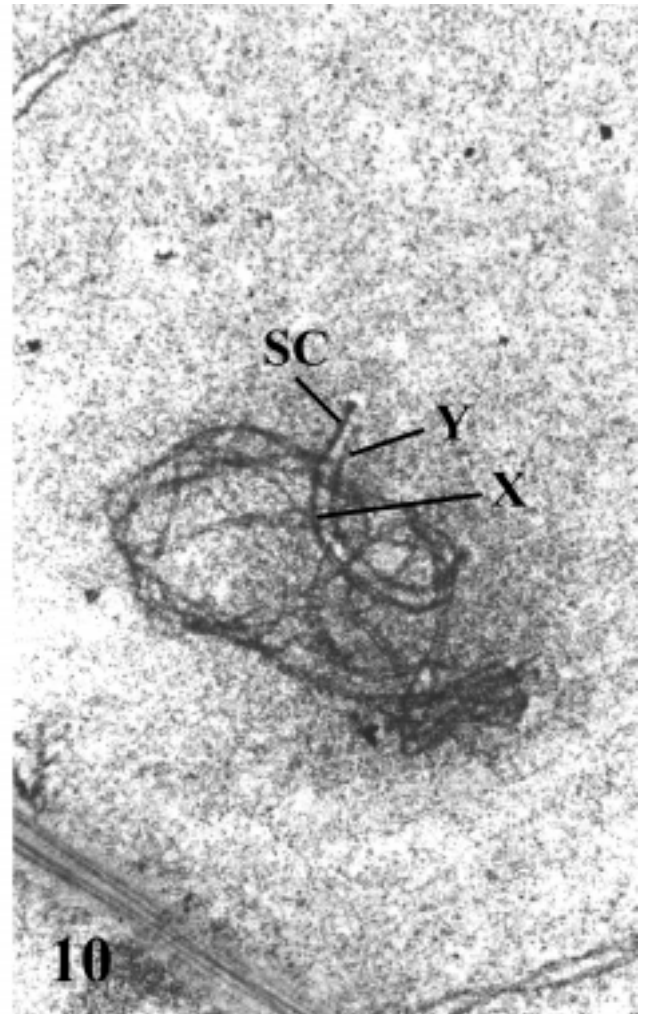
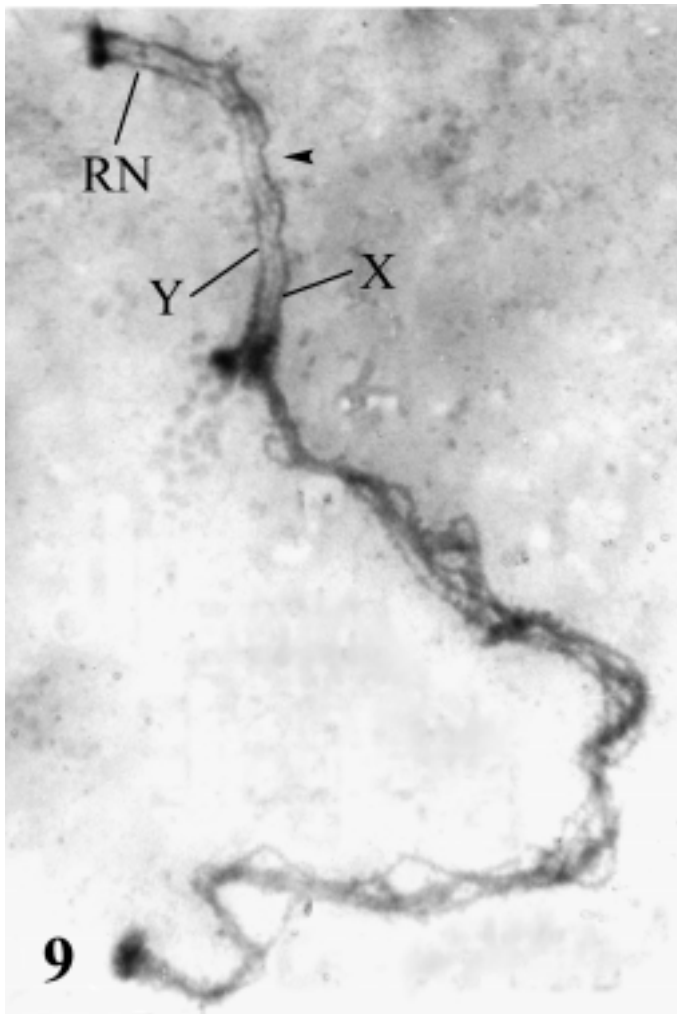
The mean location of the RN is $1.2 \mu\text{m} \pm 0.3$ ($n = 8$) from the nuclear envelope, and its range is $1.6 \mu\text{m}$ to $0.82 \mu\text{m}$ (Fig. 11b). This restriction of RN location has been found to agree with the location of a single crossover as shown by MLH1 immunolocalization (results not shown). Furthermore, the SC segment harboring the RN is always devoid of any of the irregularities or waviness above described (Figs. 3 and 9).

Thin sections

In thin sections, the XY body appears as a densely stained body attached to the nuclear envelope (Fig. 12a). This body shows an evenly spaced and compact chromatin, and sections of single axes or pieces of SC near the nuclear envelope.

In sections of *D. hybridus*, a large nucleolus is distinguished because of many long and curved expansions

FIGURES 9-11. Permanency of the SC in the XY pair and location of the crossover (recombination nodule, RN). In Figs. 9 and 11a, the RN is located on the central region of the SC, near its upper end and before the waviness of the X axis (arrowhead in fig. 9). Fig. 10 shows the permanency of the full SC at very late pachytene in *D. hybridus* (silver staining). In Figure 11b the drawing at scale shows the average location of the RN (asterisk) and its narrow range (segment, below) as regards the extremely acrocentric Y of *C. villosus*. Figs. 9-11a, X 9,000.



11 b

which can fuse to each other and have a finely granular structure (Fig. 12b).

Discussion

Validation of karyological data of Dasypodidae

Our results are in general agreement with the karyotypes described by Jorge *et al.* (1977) for *C. villosus* and *D. hybridus*. However, while these authors have claimed that the X chromosome of *C. villosus* is acrocentric and that it has “a prominent paracentromeric C+ band” (Jorge *et al.*, 1977), we have shown that the X chromosome of *C. villosus* is submetacentric, with a clearly defined short arm which is the one that pairs with the acrocentric Y chromosome. The X axis of *D. hybridus* is also submetacentric. Thus, the X chromosome from both species studied here are very similar in morphology, and the Y chromosomes are also very similar in size, in the CI and in their behaviour (see **Results**). Our results also show that while the Y chromosome is the smallest element in *C. villosus*, it is not the smallest one in *D. hybridus*. This fact points to a lack of coherence in previous reports (Jorge *et al.*, 1977, 1985) that state that there are no differences between the karyotypes of *D. hybridus* and that of *D. novemcinctus*, although in karyotypes from male specimens the latter species shows a longer Y chromosome. A re-examination of the karyotypes of males in largest samples of specimens seems to be needed to define this problem.

Significance of permanent total synapsis of the Y chromosome and differentiations in the unpaired segment of the X chromosome

In a previous report on a different species (*D. novemcinctus*) Scavone *et al.* (2000) have reported a complete synapsis between the X and Y chromosomes, and that the long arm of the X chromosome is the one that pairs with the small acrocentric Y.

Our results show that complete XY synapsis is shared with *D. hybridus* and *C. villosus*, two phylogenetically distant species of Cingulata, and thus this kind of full pairing may be characteristic of the family Dasipodidae. However, as shown in **Results**, in *C. villosus* it is the short arm of the submetacentric X chro-

mosome the one that pairs with the Y axis and thus the specificity of pairing in the species *D. novemcinctus* remains to be tested. Despite some contrary claims (Scavone *et al.*, 2000), full synapsis between the XY pair is transitory, to say the best, in most reported mammalian species (Solari, 1993). The permanent, total synapsis between the Y and the corresponding region of the X chromosome is actually seen only in the presently reported Xenarthran species and in the rodent *Galea musteloides* (Solari *et al.*, 1993) in which there is also a peculiar dissociation of the SC in later pachytene substages.

On the other hand, the X-axis differentiations (splittings, mainly in *C. villosus* and net-like anastomosing, mainly in *D. hybridus*) are found in varying degrees of intensity, among many other mammalian species.

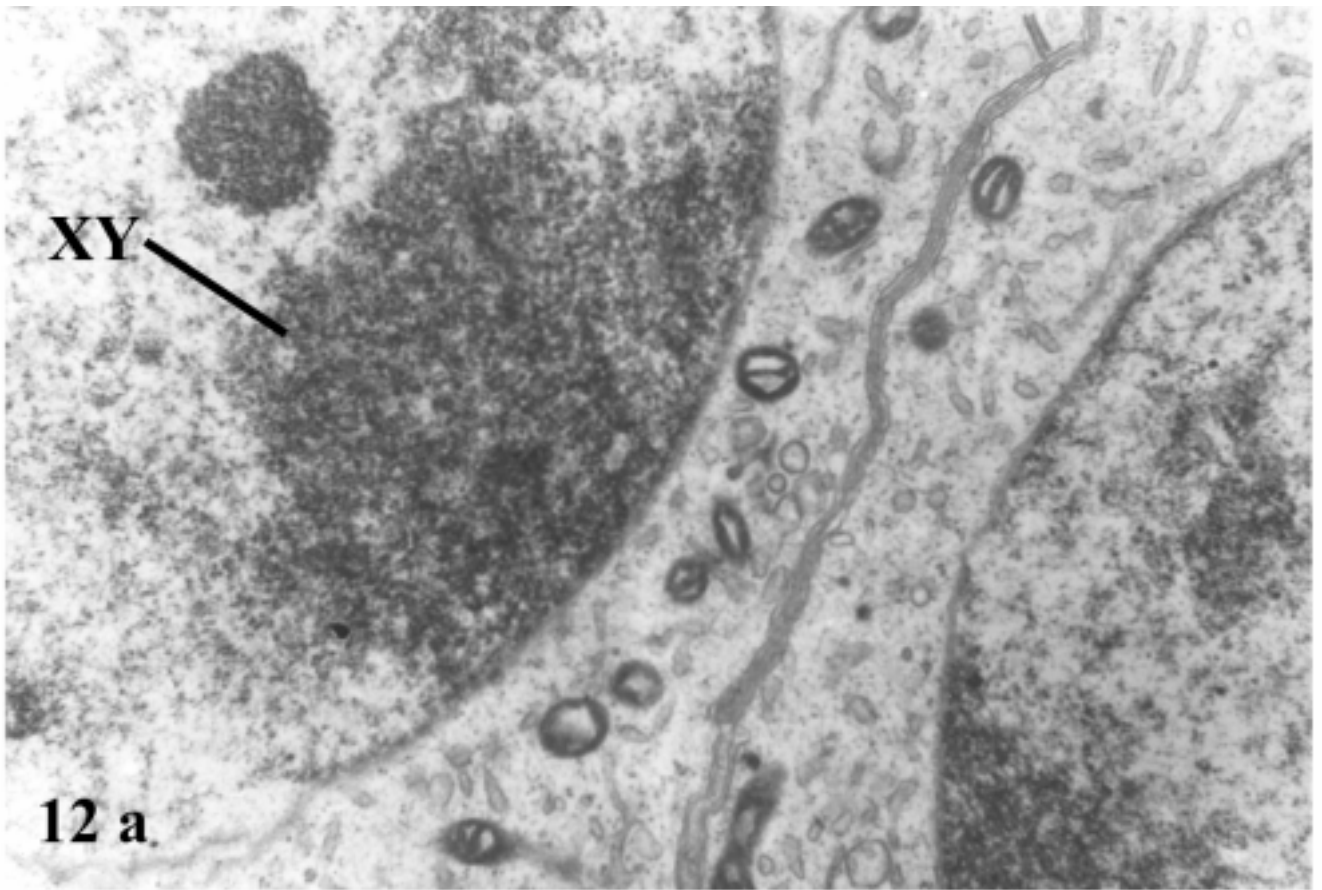
Presence of a pseudoautosomal region in the XY pair

The present results show that there is a localized region for the presence of recombination nodules in the XY pair of *C. villosus* (also tested with MLH1 foci). The recombinational region, commonly known as *pseudoautosomal region* of the XY pair of mammals (Solari, 1980, 1993) is here restricted to the distal 30% of the Y axis (see **Results**), near the nuclear envelope. Thus, if this narrow localization of recombination is also present in the related species *D. novemcinctus*, the end-to-end attachment of the X and Y chromosomes at first meiotic metaphase described by Barroso and Seuanez (1991) would be explained as the result of a terminal chiasma.

Evolutionary significance of the behaviour of sex chromosomes in Dasypodidae

Among mammals, there are three different patterns of behaviour of the meiotic sex chromosomes in the male sex (Solari, 1993). In the most primitive ones, the subclass Prototheria (monotremes), represented by only three living species (platypus and the echidnas) there is no XY body, and there is a complex sex-chromosome system including an X, a presumptive Y and 8 additional chromosomes that are associated by reciprocal translocations (Grutzner *et al.*, 2004). On the other hand, the marsupials (metatherians) have an XY system, and

FIGURES 12a-12b. Thin sections of spermatocytes from *D. hybridus*. In 12a the XY body (XY) formed by the XY pair is shown as a mass of tightly packed chromatin attached to the nuclear envelope. In 12b one of the long, arm-like expansions of the nucleolus is marked by an arrow. X 18,000.



an XY body in spermatocytes, but they lack a SC between the X and Y (Solari and Bianchi, 1975; Solari, 1993) and the X and Y axes are associated by a special “dense plate” (Solari and Bianchi, 1975) which has been shown to contain the protein SCP3 of the lateral elements of the SC (Page *et al.*, 2003).

Among eutherian mammals the rule (with some exceptions, reviewed in Solari, 1993) is that there is an XY pair that forms an XY body in spermatocytes, and that has a single recombination nodule (crossover) which is highly localized near the nuclear envelope (Solari, 1993).

The present results add a new taxon to those already known to have recombination restriction in their heteromorphic sex chromosomes (Solari, 1993). Given the basal position of Xenarthra in the phylogenetic tree of eutherian mammals (see **Introduction**), it is challenging to assume if some of the features of the XY pair in Cingulata are primitive: that is, if full synapsis of an extremely acrocentric Y, in which the distal region is the only one in which gene recombination occurs, has some selective value, for instance, the inhibition of the proximal Y to engage in non-homologous recombinational events, that could give rise to a multiple sex-chromosome system. It is left for wider explorations of Xenarthran meiosis a conclusion on this matter. The localization of suitable markers, like the Sry gene and some of the markers in the conserved X chromosome would also be interesting for such phylogenetic analysis. The interest in the basic genetics of these species is furthered by the recognition that armadillos are natural hosts of *Trypanosoma cruzi* type II, the prevalent agent of Chagas disease (Yeo *et al.*, 2005).

Acknowledgements

This work was supported by a grant from FONCYT (PICTR 00074) and from Conicet (2173 and 2174) and UBACYT (M008 and M051). MSM and AJS are members of the Carrera del Investigador (Conicet). The helpful assistance of Dra. M.I. Pigozzi with MLH1 immunolocalization, and that of Lic. M. I. Rahn and C. Deparci in the preparations is gratefully thanked.

References

- Barroso CML, Seuanes H (1991). Chromosome studies in *Dasyopus*, *Euphractus* and *Cabassous* genera (Edentata: Dasypodidae). *Cytobios* 68: 179-196.
- Beath MM, Benirschke K, Brownhill LE (1962). The chromosomes of the nine-banded armadillo, *Dasyopus novemcinctus*. *Chromosoma* 13: 27-38.
- Cetica P, Sassaroli L, Merani MS, Solari A (1993). Comparative spermatology in Dasypodidae (*Priodontes maximus*, *Chaetophractus villosus* & *Dasyopus hybridus*). *Biocell* 18: 89-103.
- Delsuc F, Scally M, Madsen O, Stanhope MJ, de Jong WW, Catzeflis FM, Springer MS, Douzery EJP (2002). Molecular phylogeny of living Xenarthrans and the impact of character and taxon sampling on the placental tree rooting. *Mol Biol Evol* 19: 1656-1671.
- Grutzner F, Rens W, Tsend-Ayush E, El-Mogharbel N, O'Brien PC, Jones RC, Ferguson-Smith MA, Marshall Graves JA (2004). In the platypus a meiotic chain of ten chromosomes shares genes with the bird Z and mammal X chromosomes. *Nature* 432: 913-917.
- Howell WM, Black DA (1980). Controlled silver staining of nucleolus organizing regions with a protective colloidal developer: A one-step method. *Experientia* 36: 1014-1015.
- Jorge W, Meritt DA, Benirschke K (1977). Chromosome studies in Edentata. *Cytobios* 18:157-172.
- Jorge W, Orsi-Souza AT, Best R (1985). The somatic chromosomes of Xenarthra. In: The evolution and ecology of armadillos, sloths and vermilinguas, G. G. Montgomery (ed.). Smithsonian Institution Press, Washington.
- Page J, Berrios S, Rufas JS, Parra MT, Suja JA, Heyting C, Fernandez-Donoso R (2003). The pairing of the X and Y chromosomes during meiotic prophase in the marsupial species *Thylamys elegans* is maintained by a dense plate developed from their axial elements. *J Cell Sci* 116:551-560.
- Reeves A, Tear J (2000). Micromesure for Windows, version 3.3. Free program distributed by the authors through Internet. Link: <http://www.colostate.edu/Depts/Biology/Micromesure>
- Scavone MDP, Oliveira C, Bagagli E, Foresti F (2000). Analysis of the synaptonemal complex of the nine-banded armadillo, *Dasyopus novemcinctus*. *Genet Mol Biol* 23: 613-616.
- Solari AJ (1980). Synaptonemal complexes and associated structures in microspread human spermatocytes. *Chromosoma* 81: 315-337.
- Solari AJ (1993). Sex chromosomes and sex determination in vertebrates. pp 109-135. CRC Press, Boca Ratón.
- Solari AJ (1998). Structural analysis of meiotic chromosomes and synaptonemal complexes in higher vertebrates. In: Berrios M, ed., Nuclear structure and function. *Methods in Cell Biol.* vol 53, pp 235-256. Academic Press, San Diego.
- Solari AJ, Bianchi NO (1975). The synaptic behaviour of the X and Y chromosomes in the marsupial *Monodelphis dimidiata*. *Chromosoma* 52: 11- 25.
- Solari AJ, Merani MS, Burgos MH (1993). Dissociation of the synaptonemal complex in the XY body of *Galea musteloides* (Rodentia, Caviidae). *Biocell* 17: 25-37.
- Yeo M, Acosta N, Llewellyn M, Sanchez H, Adamson S, Miles GAJ, Lopez E, Gonzalez N, Patterson JS, Gaunt MW, Arias AR, Miles MA (2005). Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillo hosts of *Trypanosoma cruzi* II, including hybrids. *Int J Parasitol*, 35: 225-233.