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Short Communication

Analysis of the synaptonemal complex of the nine-banded armadillo, *Dasypus novemcinctus*

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Abstract

The synaptonemal complex (SC) of three specimens of the nine-banded armadillo (*Dasypus novemcinctus)* was analyzed. Thirty-two bivalents (2n = 64) were observed, 31 of them being autosomes and one an XY sexual bivalent. Chromosome synapsis processes and nucleolus structure changes were analyzed in zygotene and pachytene cells, allowing a detailed description of the beginning of meiotic prophase in this species. There was complete synapsis of X and Y chromosomes. Some abnormalities in SC were observed in cells during zygotene and at the beginning of pachytene, but not in cells in the middle and late pachytene, suggesting the occurrence of synaptic adjustments in their SC.

INTRODUCTION

The Dasypodidae family has nine genera, and the genus *Dasypus*, six species. The species of this family have a highly conserved phenotype, making its taxonomy problematic (Jorge *et al.*, 1985). Widely distributed, the nine-banded armadillo, *D. novemcinctus*, is found in northeastern Argentina, all of eastern South America, Central America, Mexico, and in the southeastern part of the United States (Storrs *et al.*, 1974). The importance of *D. novemcinctus* as a model for basic research on reproduction and genetics has long been recognized (Storrs *et al.*, 1974).

The *D. novemcinctus* karyotype was described by Beath *et al.* (1962), with 2n = 64 chromosomes, consisting of two large metacentric pairs, four large acrocentric pairs, 14 medium-sized acrocentric pairs, six medium-sized metacentric pairs and five small acrocentric pairs. The X chromosome is a large metacentric and the Y chromosome is the smallest acrocentric. Benirschke *et al.* (1969) and Jorge *et al.* (1985) found several chromosomal polymorphisms in this species in North America.

One of the most important characteristics of the first meiotic prophase is the presence of the synaptonemal complex (SC), a protein structure formed at the beginning of prophase I between the sister chromatids of each bivalent. In the SC, kinetochores are seen as a prominent differentiation of the lateral elements (LEs), while the telomeres, attached to the nuclear envelope, are seen as dark regions in the SC extremities (Moses, 1977). New cytological techniques developed to analyze the SC have allowed detailed studies of chromosome synapsis at meiosis, including the identification of zygotene and pachytene substages (Solari, 1980; Dollin *et al.*, 1989; Greenbaum *et al.*, 1990; Villagomez, 1993). Moreover, SC studies have greatly contributed to karyotypic studies of several species, mainly mammals (Gillies, 1989).

We examined SC in *Dasypus novemcinctus* spermatocytes, in different zygotene and pachytene substages, to determine the structure and behavior of the autosomes, sex chromosomes, and nucleoli.

MATERIAL AND METHODS

Spermatogenic cells were collected from three male nine-banded armadillos, *Dasypus novemcinctus*, about one year old, collected in Botucatu, SP, Brazil. The SC was analyzed by the surface spread technique (Santos, 1993). Spermatocytes were lysed with 0.01% Triton X100, fixed in 4% paraformaldehyde and stained with 50% silver nitrate (Howell and Black, 1980). SC were transferred to 75-mesh electron microscope grids, examined with a Phillips EM301 electron microscope, at 80 kV, and photographed on Kodak-Eastman film. Photographs of 41 cells were taken.

RESULTS AND DISCUSSION

Complete cells of *D. novemcinctus* in zygotene and pachytene stages contained 32 bivalents, 31 autosomes and one XY sex pair (Figure 1a). Previous cytogenetic studies of this species also indicated 2n = 64 chromosomes including the XY sex chromosomes (Beath *et al.*, 1962; Jorge *et al.*, 1985). In the extremities of the LEs, darkly stained telomeric plaques were observed, as has been found for other organisms (Solari, 1989).

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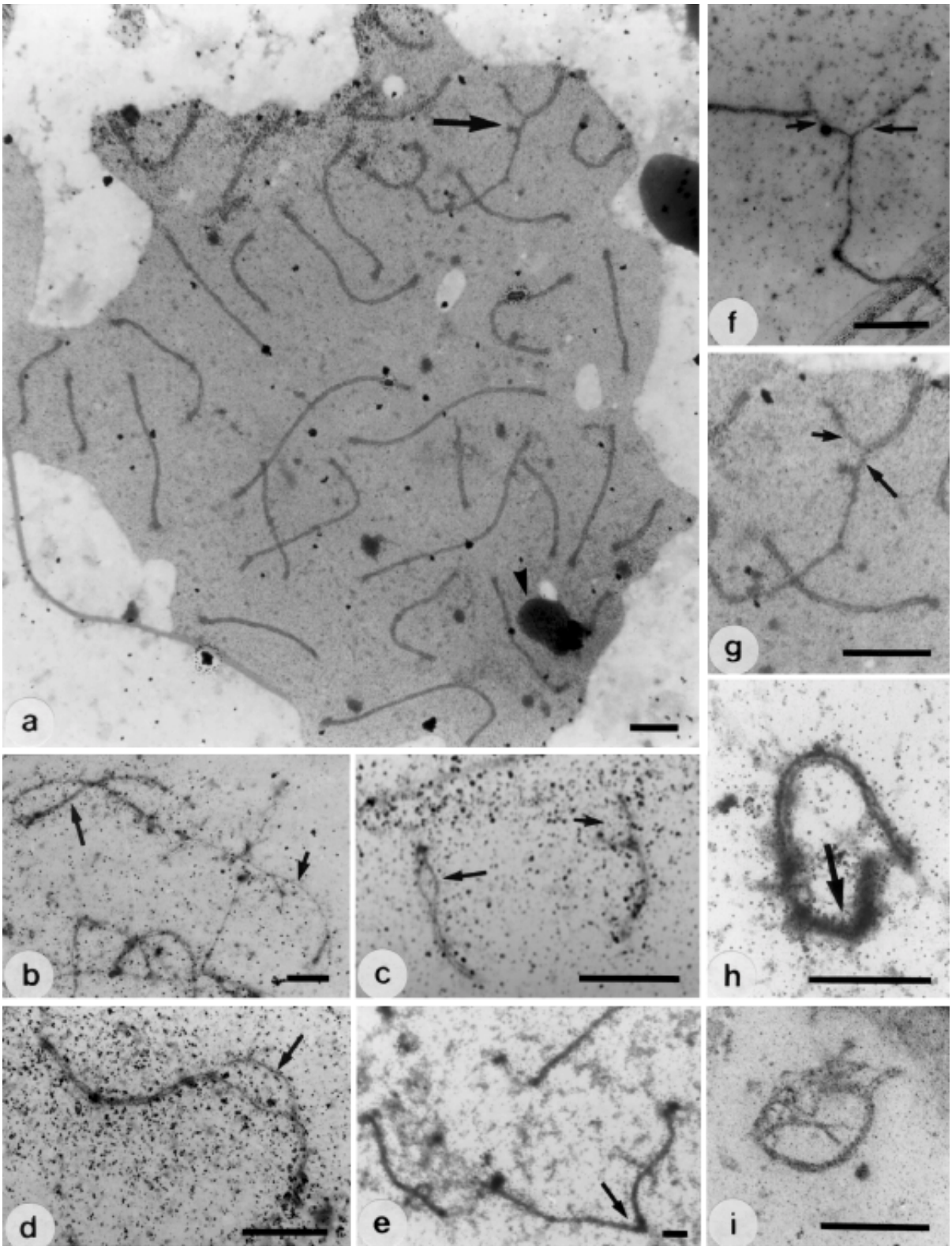

Figure 1 - Synaptonemal complex of *Dasypus novemcinctus*. **a**, Electron micrograph of a early pachytene cell with 31 autosomal bivalents and the XY sexual bivalent (arrow). The arrowhead indicates the nucleolus organizer region; **b**, partial synaptonemal complex showing asynchrony at the beginning of the synapsis process. Large arrow shows a totally paired bivalent and the small arrow points to a bivalent at the beginning of the pairing process; **c**, bivalent with unsynapsed central region (large arrow) and bivalent with only one unsynapsed extremity (small arrow); **d**, bivalent with unsynapsed central region (arrow); **e**, association of two bivalent telomeres (arrow); **f**, late zygotene showing that X (large arrow) and Y (small arrow) chromosome lateral elements remain close but not synapsed; **g**, early pachytene showing the beginning of pairing of the sexual chromosomes; the large arrow shows the X chromosome and the small arrow shows the Y chromosome; **h**, medium pachytene showing total pairing of the sexual chromosomes. The unsynapsed portion becomes heteropycnotic (arrow); **i**, late pachytene showing sexual vesicle. Scale = 2 µm.

D. novemcinctus spermatocytes were classified into two substages of zygotene and three substages of pachytene by examination of autosome and sex chromosome LEs according to criteria proposed by Greenbaum *et al.* (1990). Early and late zygotene processes were identified according to the autosomal synapsis complexity. The early, middle and late substages of pachytene were identified by the degree of LE shortening, sexual vesicle condensation and nucleolus dispersion.

Synapsis is asynchronous in *D. novemcinctus*. Totally paired bivalents were observed simultaneously with other bivalents at the beginning of the pairing process (Figure 1b). Autosome synapsis begins either at one telomere and continues linearly towards the opposite telomere or begins at both telomeres and progresses towards the centromere (Figure 1c). These observations can be explained by the occurrence of one- and two-armed chromosomes (Beath *et al.*, 1962). *D. novemcinctus* autosome behavior resembles that observed in other mammalian species with one- and two-armed chromosomes (Moses, 1977; Gillies, 1989).

In some centromeric regions there was a longer delay in LE synapsis (Figure 1d), possibly due to the presence of heterochromatic segments, which have been observed in chromosomes of several armadillo species (Jorge *et al.*, 1985). Asynapsed areas at early pachytene has been observed in some heterochromatic human chromosome segments (Solari, 1980) and more recently in other mammalian species (Koykul and Basrur, 1995; Fagundes and Yonenaga-Yassuda, 1996).

Nucleoli were identified in chromosomes 2 and 24. Association of nucleoli with LEs was frequently seen during zygotene and early pachytene (Figure 1a). This association disappeared during the middle and late pachytene. The sequence of nucleolus fragmentation was similar to that described for other mammals (Solari, 1989). Secondary association of the nucleolus with the XY bivalent, as seen in humans and mice (Solari, 1989), was not observed.

In late zygotene unpaired sex chromosomes were in close proximity (Figure 1f). Synapsis of these chromosomes started during early pachytene in the long arm extremity of X chromosome (Figure 1g). This is also observed in cattle (Switonski *et al.*, 1990), mink (Koykul and Basrur, 1995) and mice (Fagundes and Yonenaga-Yassuda, 1996). During middle pachytene, the homologous segment and the pseudoautosomal portion of the sex chromosome were almost totally synapsed. The remainder of the X chromosome was totally heteropycnotic (Figure 1h). Synapsis of the sex chromosomes was completed during late pachytene, as indicated by a marked thickening of the unsynapsed X segment and formation of the sexual vesicle (Figure 1i). Synapsis of the sexual chromosomes occurred in a such way that the X and Y chromosomes became entirely paired (Figure 1h). Complete X and Y chromosome synapsis is uncommon but has been observed in mammalian species such as mice (Joseph and Chandley, 1984), cats (Gillies and Cowan, 1985) and mink (Koykul and Basrur, 1995).

Several chromosome pairing failures were observed in the zygotene and pachytene cells, e.g., telomere associations (Figure 1e), univalents, pairing delays and interlockings. The frequency of these cell anomalies declined from late zygotene to late pachytene. This could be because synaptic adjustment reduces pairing failures, as has been proposed for several mammalian species (Ashley *et al.*, 1981; Moses and Poorman, 1981; Gabriel-Robez *et al.*, 1986; Dollin *et al.*, 1991).

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RESUMO

Foi analisado o complexo sinaptonêmico (SC) de três espécimens de tatu galinha (*Dasypus novemcinctus)*. Foi observada a ocorrência de 32 bivalentes (2n = 64), 31 dos quais correspondiam aos autossomos e um ao bivalente sexual XY. Os processos de sinapse cromossômica e transformação na estrutura do nucléolo foram analisados em células em zigóteno e paquíteno, permitindo uma detalhada descrição do início da prófase meiótica dessa espécie. Uma característica importante observada foi o completo emparelhamento dos cromossomos X e Y. Algumas anormalidades do SC foram observadas em células em zigóteno e no início de paquíteno, porém essas anormalidades não foram observadas em células no meio e fim de paquíteno, sugerindo a ocorrência de ajustamentos sinápticos no SC.

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