Holocentric chromosomes of arachnids: Presence of kinetochore plates during meiotic divisions

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Abstract

An ultrastructural study of holocentric chromosomes during meiotic division I and, for the first time, on meiotic division II of three arachnids (the scorpion *Tityus bahiensis* and the spiders *Dysdera crocata* and *Segestria florentina)* is presented. While the results obtained in spiders are similar to those obtained in species previously analyzed, *T. bahiensis* is an exception to the rule since it shows kinetochore plates during division I. Furthermore, such plates were observed in the three species during division II.

Introduction

In 1967, Buck published the first ultrastructural study centering attention on holocentric chromosomes during both mitotic and meiotic divisions. He demonstrated that during the mitotic division of *Rhodnius prolixus* there is an extended plate (kinetochore plate, KP) covering most of the surface of each chromosome into which the spindle fibers are inserted. However, metaphase-I bivalents lack KPs and the elements of the spindle come directly in intimate relation with the chromatin during meiotic division I. Similar results were reported in *Oncopeltus fasciatus* by Comings & Okada (1972). The ultrastructural analysis of meiotic division I of *Bombyx mori* (Friedländer & Wahrman, 1970; Holm & Rasmussen, 1980) also agree with these results.

Taking into consideration the ultrastructural differences between mitotic and meiotic chromosomes, Comings & Okada (1972) postulated that the KP was absent during meiosis in order to allow chiasma terminalization. On the other hand, White (1973 p. 490) asserts that the terminalization of chiasmata in this type of chromosome proves that the kinetochore material, if present, is very divided during the diplotene-diakinesis stage, since at the end of prophase I only terminal chiasmata can be observed.

This paper describes the ultrastructure of holocentric chromosomes during the meiotic divisions of three different species of arachnids: *Tityus bahiensis, Dysdera crocata* and *Segestria florentina.* The scorpion *T. bahiensis* was extensively analyzed from the cytological point of view by Piza (1939, 1943, 1944) and Brieger & Graner (1943; for a complete review, see White, 1973). It can be concluded that this species possesses holocentric chromosomes and a male meiosis considered as achiasmatic. *D. crocata* and *S. florentina,* both primitive spiders, were cytologically studied by M. O. Díaz (personal communication). According to this author, both species possess chiasmatic meiosis but *D. crocata* reveals a pre-reductional meiosis, while *S. florentina* shows a post-reductional meiosis. In a previous study, focused on the ultrastructure of sex chromosomes during the spermatogenesis of both spiders, no KPs were observed during division I (Benavente & Wettstein, 1980).

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Material and methods

The testes of adult specimens of *Tityus bahiensis* (obtained from the Instituto Butantán, Brazil), *Dysdera crocata* and *Segestria florentina* (both collected in the Department of Montevideo, Uruguay), were fixed in 1.6% glutaraldehyde for three hours. The fixing liquid was washed off by passing through phosphate buffer (pH 7.2) during an hour and then post-fixed in OsO_4 (1%) for another hour. Once the latter fixing liquid was rinsed off, the testes were dehydrated using an ethanol series (25, 50, 70, 96, 100%). The dehydration was continued with acetone. Durcupan ACM (Araldita, Fluka) was used for embedding. The sections were obtained with a MT2-B Porter-Blum ultramicotome, set on one-hole grids previously covered with Formvar and stained with uranyl acetate (5%) for 24 hours. At the same time, short serial sections were prepared for electron microscopy in accordance with the Wettstein & Sotelo procedure (1967). Thick sections were also obtained (0.2–0.6 μm) as light-microscopical controls, which were stained with toluidine blue (1%) in 1% borax. For the observations of the thin sections a Siemens Elmiskop I (80 KV) was used.

Observations

Tityus bahiensis

Testis configuration

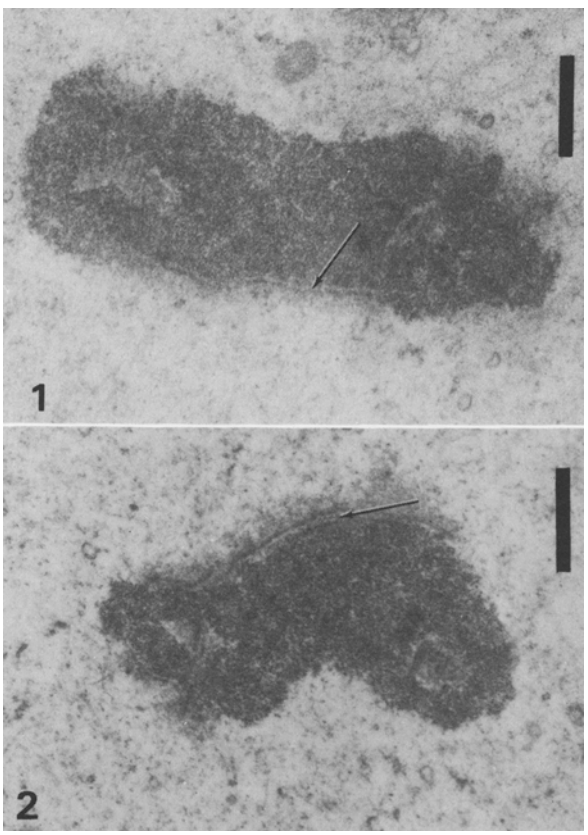
Inside the testicular tube, cells at different stages of spermatogenesis are distributed in a certain orderly manner. The earlier stages are close to the periphery and the most advanced ones near the center. The cells form small synchronous cysts, especially during earlier stages. The low percentage of cells observed in leptotene-pachytene suggests its short duration. In general, the pattern of organization of the testis of *T. bahiensis* is less orderly than the one described for primitive spiders (Benavente & Wettstein, 1980).

Meiotic divisions

Division I: In contrast to gonial divisions, metaphase-I or anaphase-I cells are located far from the tube wall forming small cysts. Occasionally they may also be found isolated. Metaphase-I bivalents are grouped in one or more masses and in most cases it is impossible to determine their exact delimitation.

Contrary to the previously studied species, metaphase-I chromosomes reveal extensive KPs, which partially cover the surface of the side facing the pole (Figs. 1 and 2). It has been impossible to find out the exact length of the plates due to the fact that the chromosomes are fused with each other. The KP is associated to the chromosome by a fibrillar material of lower density. In certain well-oriented sections this material is arranged in such a way that it suggests the existence of a delicate thin plate between the chromatin and the KP (Fig. 2).

Division II: The cells undergoing division II form small cysts near the center of the testicular tube. They are found intermingled with other stages of



Figs. 1–2. Tityus bahiensis: (1) Division I. The arrow points out an extensive KP in relation with a chromosome cluster. Bar: 1 μm.; – (2) Division I. The fibrillar elements that establish relationship between the KP and the chromatin of a chromosome cluster are evident in properly oriented sections as a thin lamina (arrow). Bar: 1 μm.

spermatogenesis, especially with spermatids in various stages of maturity. They are clearly distinguished from other divisions due to their smaller size. As during division I, the chromosomes form masses and no precise delimitation between them can be determined. These masses are covered by KPs on the surfaces that face the poles (Fig. 3).

Dysdera crocata and Segestria florentina

Testis configuration

Testes possess a tubular form in both species. The different stages of spermatogenesis are observed as synchronous cysts. A low frequency of cysts in the leptotene-pachytene stages is observed. On the other hand, a great number of cells in diffuse stage are present. Both facts have been described in detail in a previous paper (Benavente & Wettstein, 1980).

Meiotic divisions

Division I: In both species, division-I cells form small cysts inside the testicular tube, generally midway between the capsule and the center, in contrast to gonial divisions which are located close to the testicular wall. The chromosomes that form a metaphase-I plate are associated (Fig. 4; see also Figs. 7 and 9 in Benavente & Wettstein, 1980) in such a way that it is impossible to recognize their limits. In the case of *S. florentina* (Fig. 4) a mass of different morphology corresponding to the sex chromosomes (X_1 and X_2) is distinguishable. Chromosomes of both species show no KPs during division I. The division-I cysts of *D. crocata* were serially sectioned. The reconstructions did not show either morphologically differentiated locations or regular distribution of the microtubule insertion points.

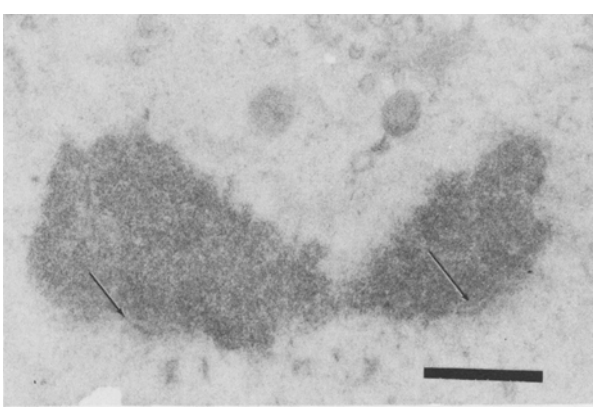


Fig. 3. Tityus bahiensis. Extensive KPs (arrows) are also present during the second meiotic division. Bar: 1 μm.

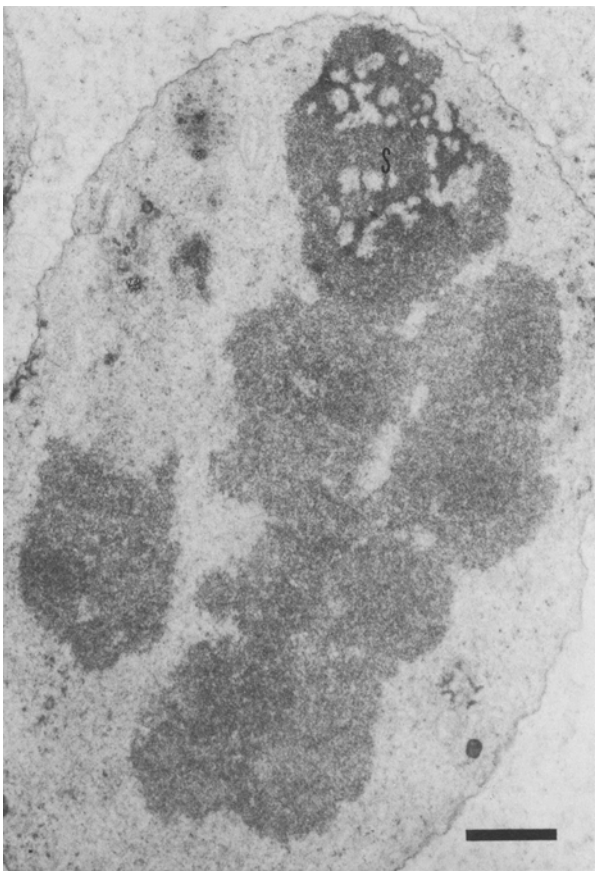


Fig. 4. Segestria florentina. Division I. Absence of KPs is evident. The chromatin mass of different morphology (S) has been identified as the sex chromosomes (X_1 and X_2). Bar: 1.5 μm.

Division II: In both species divisions II form small cysts near the center of the testicular tube. As in divisions II of *T. bahiensis* KPs were observed associated with a single mass of chromosomes (Fig. 5). Unlike division I, no sex chromosomes could be identified in *S. florentina.*

Discussion

The ultrastructure of holocentric chromosomes has been studied in few species: *Rhodnius prolixus* (Buck, 1967), *Bombyx mori* (Friedländer & Wahrman, 1970; Holm & Rasmussen, 1980), *Oncopeltus fasciatus* (Comings & Okada, 1972), *Dysdercus*

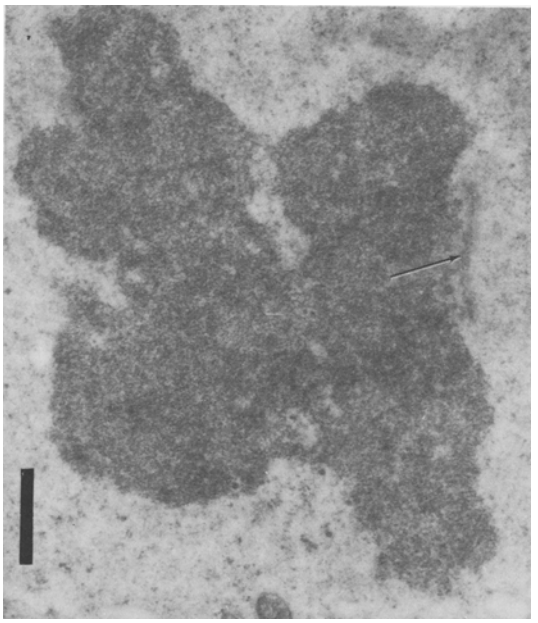


Fig. 5. Dysdera crocata. Division II. Arrow: KP. Bar: 1 μm.

intermediatus (Ruthmann & Permantier, 1973), *Dysdera crocata* and *Segestria florentina* (Benavente & Wettstein, 1980). Information on both mitotic and meiotic chromosomes is available in three species (*R. prolixus, O. fasciatus* and *D. intermediatus*). The data on meiotic chromosomes correspond to division I, except for a micrograph of a metaphase II of *O. fasciatus* published by Comings & Okada as collateral information.

Although the information available on each particular species is in general fragmentary, the existence of KPs during mitosis and their corresponding absence during the first meiotic division seems to be the general rule.

KPs have been interpreted as the ultrastructural expression of the diffuse kinetochore due to the morphological similarities they present with localized kinetochores. Although this evidence is not conclusive, it constitutes a valid hypothesis. However, as movement of holocentric chromosomes during meiosis I usually occurs in the absence of KPs further studies will be necessary for a definitive evaluation of these facts.

To explain the ultrastructural differences observed between mitotic and meiotic divisions, Comings & Okada (1972) postulated that a KP firmly attached to the chromosome and extended lengthwise on it, would make chiasmata terminalization impossible from the mechanical point of view, during diplotene-diakinesis.

This hypothesis is based on some postulates which I think are controversial: (a) the kinetochore material would be assembled as early as diplotene in species with monocentric chromosomes; (b) it is classically considered that chiasmata shift from an interstitial position to a terminal one.

Three-dimensional reconstructions of nuclei at the diplotene stage of the mouse (Solari, 1970), the rat (Wettstein, personal communication) and the mole cricket *Scaptericus borrelli* (Sotelo *et al.*, 1973) do not support the first postulate. Furthermore, ultrastructural analysis of the meiotic prophase of a species considered to be achiasmatic with holocentric chromosomes *(T. bahiensis)* does not show organized kinetochore material during any of the meiotic prophase stages (Benavente & Wettstein, unpublished results).

The studies performed by Tease (1978) and Tease & Jones (1978) present evidence against chiasmata terminalization in an orthopteran. Using the Brd-U substitution procedure, these authors demonstrated that in all monochiasmatic bivalents the crossing-over point coincided with the chiasma. Furthermore, Solari (1979) was able to show that the location of the chiasmata at diplotene and metaphase I agrees with the location of the recombination nodules at pachytene in the hemipteran *Triatoma infestans.*

The information available on the three species herewith presented is summarized in Table 1. Re-

Table 1. Summary of the information available on the three species.

Species	Type of meiosis	Chiasmata	Kinetochore plates		
			Mitosis	Div. I	Div. II
Tityus bahiensis	unknown	–	+*	+	+
Dysdera crocata	pre-red.	+	+*	–	+
Segestria florentina	post-red.	+	?	–	+

* Benavente, unpublished

garding *T. bahiensis,* Piza (1939, 1943, 1944) postulated the existence of dicentric chromosomes. On the other hand, Brieger & Graner (1943; for review, see White, 1973 p. 419) concluded that *T. bahiensis* possesses holocentric chromosomes. The ultrastructural results presented here support this second conclusion. According to Díaz (personal communication), *D. crocata* shows a pre-reductional meiosis while *S. florentina* shows a post-reductional one. No correlation was found between this fact and the ultrastructural organization of the kinetochore in both species. Finally, it must be stressed that this is the first time that presence of KPs during division I *(T. bahiensis)* and division II has been reported.

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