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Chromosome Polarization during Spermatogenesis of Orthoptera*

Polarización cromosómica durante la espermatogénesis de Orthoptera

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The chromosome polarization and the relationship between centrioles and centromeric regions are studied during spermatogenesis of some orthopteran species. The study was carried out using some light and electron microscopy techniques. Centrioles appear polarizing chromosomes during meiotic prophase and a close relationship is observed between centrioles and the sex chromosome during some prophase stages. Microtubules developed apparently from centrioles, are in contact with the outer nuclear envelope at the level of the sex chromosome. Spermatid nuclei shows a chromosome polarization in which all centromeres are disposed towards the nuclear base and each chromosome seems to occupy a definite position in the nucleus.

Spermatogenesis has been analysed in a large number of species, and these reports have demonstrated the variety of morphological patterns that spermatid and sperm show (1). The main part of studies have concerned to the process in which a spermatid becomes a sperm (i.e. spermiogenesis) and many reports have been devoted to the study of structures such as nuclei, Golgi and acrosome formation, mitochondria, etc. (2). On the other hand meiotic primary spermatocytes have been extensively studied in some species with special reference to some nuclear structures (3).

The location of the different cell components during spermatogenesis seems to be poorly studied, but some aspects attract attention of some authors: the position of the centrioles during meiosis as a polarizing element has been commented in early papers (4), later, similar aspects were observed under electron microscopy and information was obtained, in some species, concerning the role of centrioles to maintain a nuclear or chromosomal polarization. Besides, the arrangement of chromosomes in the nuclei of sperms has been analysed in some cases. In grasshopper sperms, Inoue and Sato (5) using polarizing light, and Taylor (6) using autoradiography demonstrates that chromosomes appear in a tandem disposition in the sperm nucleus. During spermiogenesis in the coccid *Steatococcus* Moses and Wilson (7) show by light and electron microscopy that the two chromosomes of the sperm are arranged in a tandem disposition. By several methods different authors show that during amphibian spermiogenesis the chromosomes appear with an orderly disposition in the sperm nuclei (8).

In this paper the organization of some cell components during spermatogenesis is

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studied in some grasshoppers, using some light and electron microscopical techniques.

MATERIAL AND METHODS

Adult males of *Chorthippus bicolor, Oedipoda coerulescens* and *Locusta migratoria* were treated as follows: some testes were fixed in 3:1 ethanol acetic mixture, and after stained with 2o/o acetic orcein as usual. These slides were used as controls of the karyotypes and nuclear characteristics of spermatogenetic cells. Similar samples were stained by means the Bloom and Goodpasteure procedure (9) employing 2% $AgNO_3$ for 24-72 hrs at 50oC. Other testes were fixed in 2% Glutaraldehyde in Sorensen's buffer (pH 7,2) at room temperature; after washing in the buffer samples were postfixed in 2% Osmium tetroxide prepared in the same buffer. Samples were dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate. In some cases thick sections obtained with the Ultratome were observed under phase contrast or under light microscopy stained with Toluidine Blue. In these sections we localize the different stages of spermatogenesis.

An LKB Ultratome and a Philips 300 electron microscope were used.

To observe living cells testes were observed after gradual flattening in the isotonic medium described by Nicklas and Staehly (10).

RESULTS

a) *The morphology of seminiferous tubules in grasshopper testis*

Studies realized at early times of cytology report the evidence of an arranged organization of seminiferous tubules in grasshoppers as well as in other insects (4). These tubules show cells located from the apical to the terminal region, which is associated to the eferent duct. Spermatogonia appears in the apical region, and consecutively to the terminal position are observed the spermatocytes, spermatids and sperms (Fig. 1). On the other hand, cells appear grouped in cysts, which functionally are syncytium in which all cells show synchrony.

Cells can be counted, specially spermatids, and in this form the number of previous divisions can be known (spermatogonial and miotic divisions). The spermatid numbers are commonly 64, 128, 156, nevertheless in some cases numbers of 16, 32 and 521 have been observed in other groups (2). Moreover the elongated form of the seminiferous tubule, which occurs in grasshoppers, permit to know the position of cells in a polarized view.

b) *Organization of primary spermatocytes*

Primary spermatocytes are clearly identifiable by the size and nuclear meiotic characteristics. Cysts containing 64 or 128 spermatocytes are observable in the species analysed. The nuclei show typical meiotic phases when observed under light microscopy, and during pachytene-diplotene the unique sex (X) chromosome appears as a heterochromatic structure associated to the nuclear envelope (Fig. 2). In thick sections observed under phase contrast or stained with Toluidine Blue as well as in *in vivo* preparations, apart from the sex chromosome the nucleolus is clearly seen, appearing as a group of granules. In these cases the position of the nucleolus seems to be related to the sex chromosome in such a form that in *Chorthippus* they appear closely located, but in *Oedipoda* are observed in opposite sides of the nucleus (Figs. 3 and 4).

Under the electron microscope a relationship can be frequently established between the sex chromatin and the centrioles. The chromatin of the X chromosome appears as dense chromatin associated to the nuclear envelope. Close to this region a pair of centrioles appear (Fig. 6). This relationship is observable from preleptotene stages to pachytene, and was analysed specially in *Chorthippus*. In relation to centrioles and the outer face of the nuclear envelope a great amount of microtubules of about 25 nm in diameter appears. These microtubules, which apparently are originated from the centrioles, contact with the nuclear envelope (Fig. 6). When spermatocytes stained with

silver are observed under light microscopy, several dark points are seen localized in a region of the nucleus (Fig. 5). These points seems to represents kinetochores, because when they are observed in metaphase chromosomes appear in the centromeric region as two points.

c) *Organization of spermatids*

Early spermatids stained with acetic orcein show the X chromosome only in half of the cells (Fig. 7 and 8). When this chromosome appears is dark and deeply stained and it is associated to the nuclear envelope by one end. When the spermatid develops the nuclei is an elongated and flattened structure. Sex chromosome appears in these cases associated to one end of the nucleus, just in the nuclear base and oriented to one side of the nuclear envelope (Fig. 8). When silver staining is used it is observed that dark stained areas appear in the nuclear base (Fig. 9), which seems to represent kinetochores, or at least centromeric regions. On the other hand nucleoli appear in all cases in the center of the nucleus of young spermatids (Fig. 10), as was reported by Fonzo (11) in spermatids of *Oedipoda.* (Fig. 11).

DISCUSSION

a) *The close relationship between centrioles and the nucleus*

A close relationship is observed in all cases between centrioles and the nucleus during some periods of spermatogenesis. This relationship is established between the centriole itself and the nuclear envelope, as occurs in spermatids, as well as between centriolar microtubules and nuclear envelope as occurs in spermatocytes. During interphase the relationships between microtubules and nuclear envelope are poorly known (12) and this type of relationship has been demonstrated in stages which preceeds the nuclear envelope disruption, prior to mitotic metaphase (13). By this reason microtubules in relation to the nucleus, such as occurs in primary spermatocytes, are difficult to understand in view of its function. Nevertheless it can be speculated if they have a possible role in the polarization of the nuclear material. In some cases suggestions are made about the prealignement of chromosomes in stages previous to meiotic pairing (14-15-16). In this aspect the role of centrioles would be a polarizing action upon the chromosomes. In support of this idea is that a similar action of centrioles seems to occur during spermiogenesis in some species. The nuclear condensation, which involves the condensation and sometimes the lamellation of chromatin, starts in the region close to the centrioles and after it extends to all the nuclear regions (1, 17).

b) *The position of chromosomes during spermatogenesis*

As comented in the Introduction some papers have been published about the organization of chromosomes during spermatogenesis. Apart from the cases of meiotic chromosomes, the organization of the nucleus in sperms was studied in invertebrates and vertebrates using different methods, polarizing light, radioactive precursors, and some Giemsa banding techniques. The silver method seems not to be used previously with this purpose, and we suggested that it can be usefully employed in these and related aspects. The dark points stained with silver seem to represent kinetochores or some material of the centromeres. The number of these stained regions coincides with the haploid genome when counted in spermatids or pachytene spermatocytes, and besides in meta or anaphase chromosomes similar dark points appear in the region of kinetochores. Recent studies show that kinetochores seem to be different in chemical constitution to the rest of the chromosome (18). In this form the chromosomes seem to be clearly polarized during spermiogenesis. And the position of some chromosomes, like the X chromosome would indicate that each chromosome appear with a definite position in the spermatid nucleus. The role

of this particular arrangement would be related to the head shape of the sperm, but more conclusions about this seems to be speculative.

The polarization of nuclear material during some stages of spermatogenesis seems to be clear, but more studies are required for to know if this polarization is maintained during all the process of spermatogenesis.

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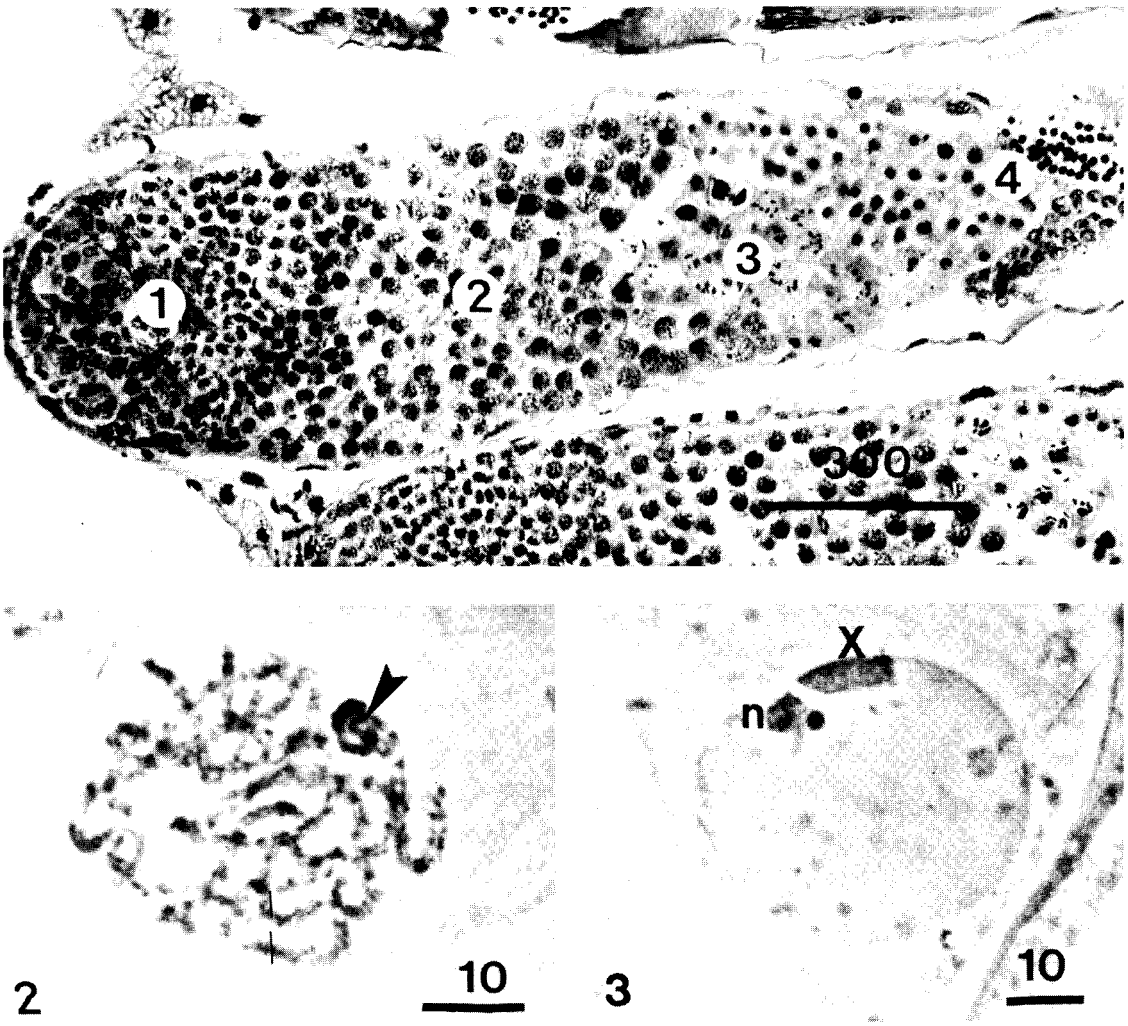

Fig. 1. Thick section from a block embedded with Epon and stained with Toluidine Blue. At left appear the apical portion of the tubule containing spermatogonia (1) in different stages. Spermatocytes can be observed during meiotic prophase stage (2) as well as during 1st Meiotic metaphase (3). Spermatids appear disposed in the terminal portion of the tubule (4). From *Ch. bicolor.*

Fig. 2. Pachytene stained with acetic Orcein. Autosomal bivalents and the heteropicnotic X chromosome (arrow) are observed. From *L. migratoria.*

Fig. 3. Thick section from a block of *Ch. bicolor* observed under phase contrast microscopy. The Nucleolus (n) appears close to the X chromosome (X).

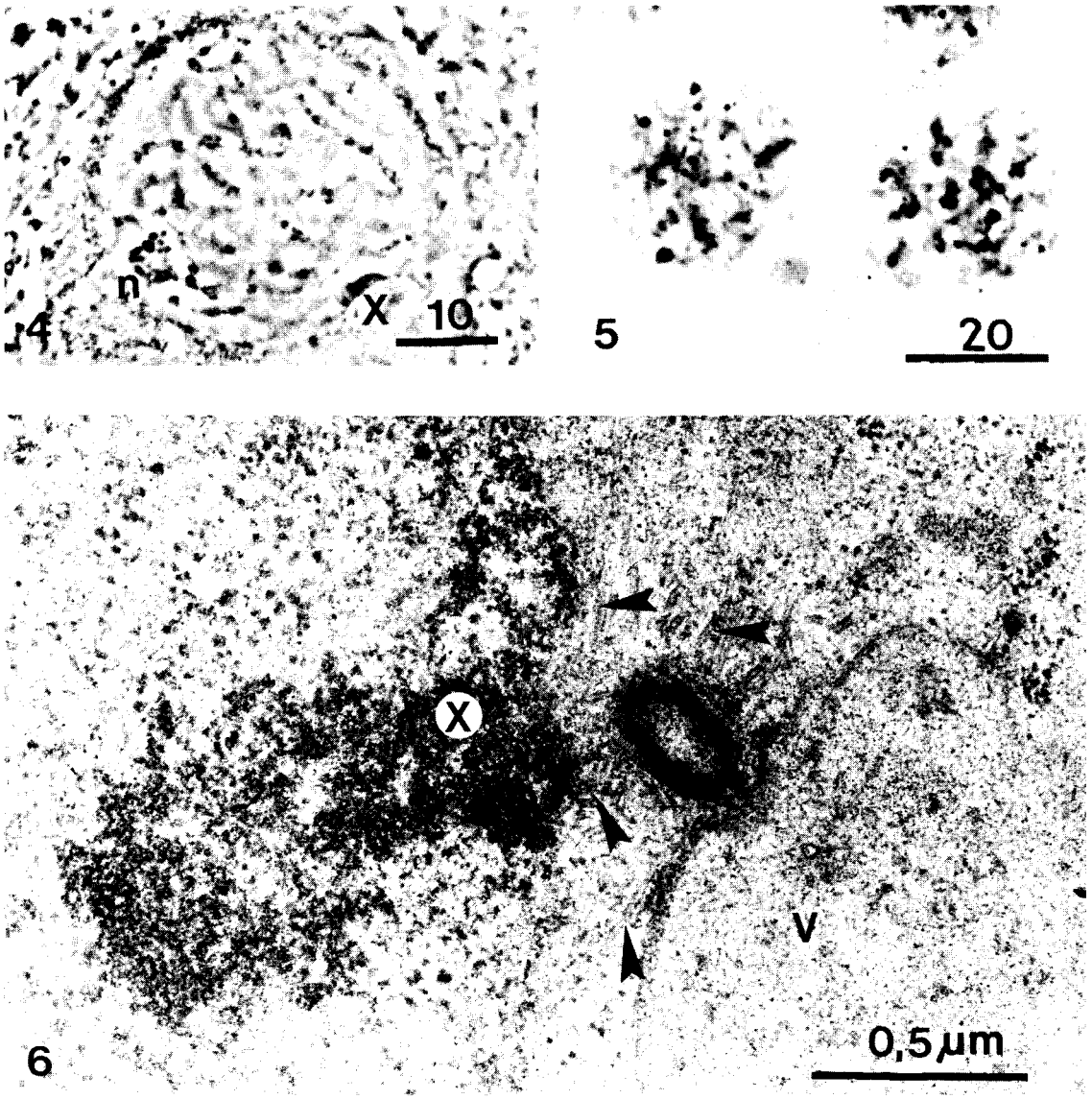

Fig. 4: In vivo observation of a pachytene spermatocyte of *O. coerulescens.* The pachytene bivalents are observed in the nucleus, and the nucleolus (n) appears in a opposite side of the X chromosome (X).

Fig. 5. Primary spermatocyte of *L. migratoria,* after silver method, several dark points appear in the nuclei.

* Bars in all pictures are represented in microns.

Fig. 6. Electron micrograph of a primary spermatocyte of *Ch. bicolor* during pre-pachytene stage. The centriole appears associated to a vesicle (V). A great quantity of microtubules (arrows) connect the centriole with the nucleus. Dense chromatin in this nuclear area represent the X chromosome (X).

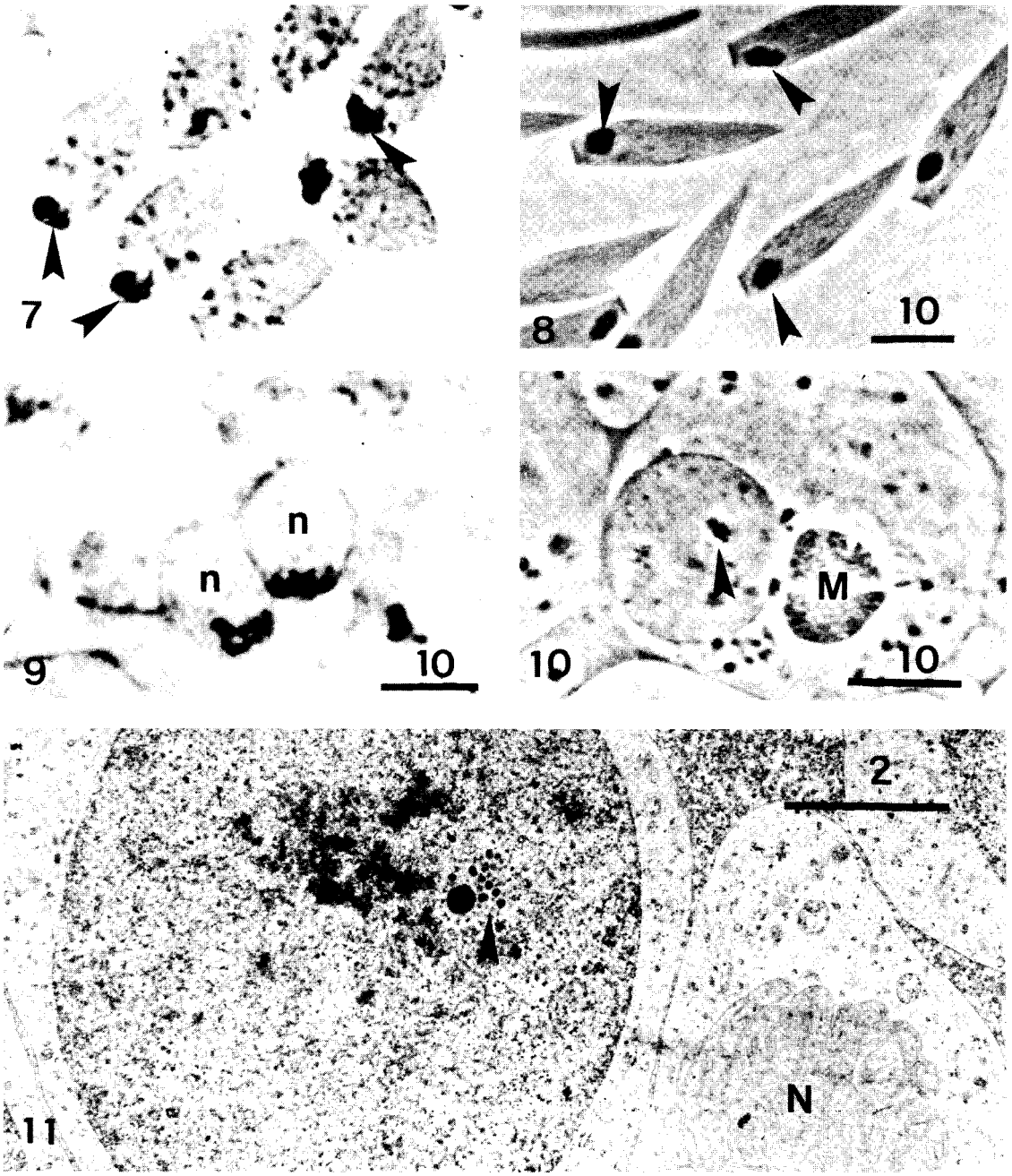

Fig. 7. Spermatids of *Ch. bicolor* stained with acetic Orcein The X chromosome appears as a dense chromatin mass in the nuclear base (arrows).

Fig. 8. Elongated spermatids in which the X chromosome appears in one side of the nuclear base (arrows).

Fig. 9. Spermatids from *L. migratoria,* stained with silver. Dark dense points appear in the nuclear base, but the rest of the nucleus is unstained. n: Nucleus.

Fig. 10 Living spermatid of *O. coerulescens* observed under phase contrast microscopy. The nucleolus is observed in the center of the nucleus (arrow). M: Mitochondrial derivatives surrounding the flagellum.

Fig. 11. Electronmicrograph of a spermatid of *O. coerulescens.* The nucleolus (Arrow) appears in the center of the nucleus and is formed by rounded dense masses. N: Nebenkern (mitochondrial derivatives) from other spermatid.

* All Bars in these figures are represented in Microns.