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# The synaptic process in *Locusta migratoria* spermatocytes by synaptonemal complex analysis

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**Summary.** We describe the synaptic process during meiotic prophase in spermatocytes from *Locusta migratoria*, using synaptonemal complex (SC) spreads analyzed by light (LM) and electron (EM) microscopy. At leptotene, a stage of short duration, unpaired axial elements begin to be assembled. Synapsis starts at zygotene, beginning usually at the terminal regions of the bivalents, either at the proximal, centromeric end or at the distal, non-centromeric end; interstitial initiation of synapsis was only occasionally observed in the longer chromosomes. Pairing is asynchronous, and shorter chromosomes are the first to complete synapsis. At pachytene all bivalents are fully synapsed. Diplotene is characterized by the progressive fragmentation of SCs; fragmentation is asynchronous, and affects mainly the longer chromosomes, while the shorter ones maintain their morphology up to late diplotene.

**Key words:** Synaptic process, Synaptonemal complex, *Locusta migratoria*

## Introduction

Synaptonemal complexes (SCs) are proteinaceous structures that provide a physical connection between homologous chromosomes during prophase of the first meiotic division (Loidl, 1990). Their function is largely unknown, but their presence may be necessary to maintain pairing after homology recognition has taken place (Chandley, 1993).

In *Locusta migratoria*, the synaptic process has been studied using serial sections (Moens, 1969) and surface spreading by light and electron microscopy (Counce and Meyer, 1973; Jones et al., 1983; Jones and Croft, 1986). However, these studies were limited to the description of the patterns of meiotic pairing progression (Jones and Croft, 1986).

Since, *Locusta* is often used in basic meiotic studies, as result of the good definition of the components of its bivalents, we analyzed surface spreads of *Locusta migratoria* to describe and quantify the process of SC formation and disintegration during prophase I in this species.

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## Materials and methods

Twenty forty-two-day-old adult male *Locusta migratoria* kept for feed at a constant temperature of 30 °C at the Barcelona Zoo were used in this study. The testes were removed and chopped in an isotonic solution (0.9% NaCl) and the suspension left in a centrifuge tube to allow larger fragments to sediment. The supernatant was changed to another centrifuge tube and the cells washed 1-2 times with isotonic solution. The slides were dipped in 0.5% Formvar solution in chloroform (Felluga and Martinucci, 1976) and dried in the vertical position. One drop of the cell suspension was placed on a sucrose (0.2M) bubble for 1 min and then picked up by touching the surface of the bubble with a Formvar-covered slide. The preparations were fixed with 9% formaldehyde for 10 min and stained with silver nitrate using the technique of Goodpasture and Bloom (1975). Electron microscopy (EM) grids were obtained following the technique of Navarro et al. (1981).

Classification of the SCs follows Croft and Jones (1986) based on the karyotype established by John and Hewitt (1966) that includes three pairs of large chromosomes (L1, L2, L3), five pairs of medium sized chromosomes (M4 to M8) and three pairs of short chromosomes (S9 to S11) plus a single, medium sized X chromosome.

## Results

To characterize the different stages of prophase I, from leptotene to diplotene, we studied a total of 521 spreads; of these, 443 were analyzed by LM and 78 by EM (Table 1).

The frequency of the different stages of prophase I was based on the first 100 cells analyzed by LM. The most frequent stage observed was pachytene (45.9%) followed by zygotene (24.9%), diplotene (19.8%) and leptotene (9.4%).

Leptotene is a short stage, already identified in other species (Guitart et al., 1985) and characterized by the presence of short segments of thin, mostly unpaired lateral elements (Fig. 1a). The axis of the single X chromosome cannot be identified at this stage. The number of nuclei analyzed was 40 by LM and nine by EM.

At zygotene, the lateral elements begin to pair, but the SCs are not fully assembled and they cannot be followed along all their length. Following Jones and Croft (1986) we classified the stage of zygotene into three sub-stages: early zygotene, early-mid zygotene and late zygotene. We analyzed a total of 130 nuclei, 102 by LM and 28 by EM, of which 9 corresponded to early, 58 to early-mid and 63 to late zygotene. The early stages of zygotene were characterized by the presence of fragments of fully assembled SCs of different length, by pre-aligned segments of lateral elements and by single fragments of lateral elements (Fig 1b). In some cases, polarization of telomeres can be observed (Fig. 1c).

By early-mid zygotene, the lateral elements of the SCs are almost complete, and some of the bivalents showed full or partial synapsis (Fig. 1d). In some cases, part of the axis of the X chromosome could be identified.

At late zygotene, most SCs were fully assembled (Fig 1e) although some regions are still unpaired. The X chromosome axis was very tenuous and difficult to identify.

To characterize the synaptic process, we analyzed the SCs that could be followed from end to end in good quality, early-mid and late zygotenes. In the 51 early-mid zygotene nuclei we detected 442 pairing-initiation regions, with a mean of 8.7 per cell (range from 6 to 16). Of these, 57.3% (253/442) corresponded to the distal, non-centromeric end of the bivalents and 35.5% (157/442) to the proximal, centromeric end, identifiable by its thickened kinetochore region (Fig. 1c-e). Only 7.2% (32/442) pairing-initiation points were interstitial, and corresponded to the longer bivalents.

In the 51 early early-mid zygotene nuclei analyzed, we identified 112 fully paired SCs. Of these, 66.1% (74/112) corresponded to the S group and 43.9% (38/112) to the M and L groups.

In the 58 nuclei at late zygotene we found 31 bivalents that had not completed pairing. All of them corresponded to the larger bivalents. All S group bivalents were fully paired at this stage. At pachytene, we analyzed 239 nuclei (205 by LM and 34 by EM). In most of them, the 11 autosomal bivalents were fully paired (Fig 1f). The axis of the X chromosome was always difficult to distinguish due to its light staining. We observed terminal associations in 33.1% (Fig. 2a) and interlockings and heterosynapses (Figs. 2b and c) in 0.4% of the cells; these abnormalities resulted in asynapsis of some bivalent regions.

At diplotene, the number of nuclei analyzed was 96 by LM and seven by EM. This stage is characterized by the fragmentation of the SCs and, based on the number of fragments, the nuclei were classified as early (1-10 fragments approximately; all SCs, even those fragmented, identifiable), mid ( more than 10 fragments, only the SCs of group S remained unfragmented and could be identified) and late diplotene (numerous fragments, all SCs fragmented, except for one or two SCs of group S). The frequency of each of these sub-stages was 55.3% (57/103), 25.3% (26/103) and 19.4% (20/103), respectively.

Fragmentation of SCs at diplotene was progressive, and may be accompanied by the desynapsis of the lateral elements in longer SCs. In early diplotene, fragmentation affected mainly the longer bivalents (Fig.

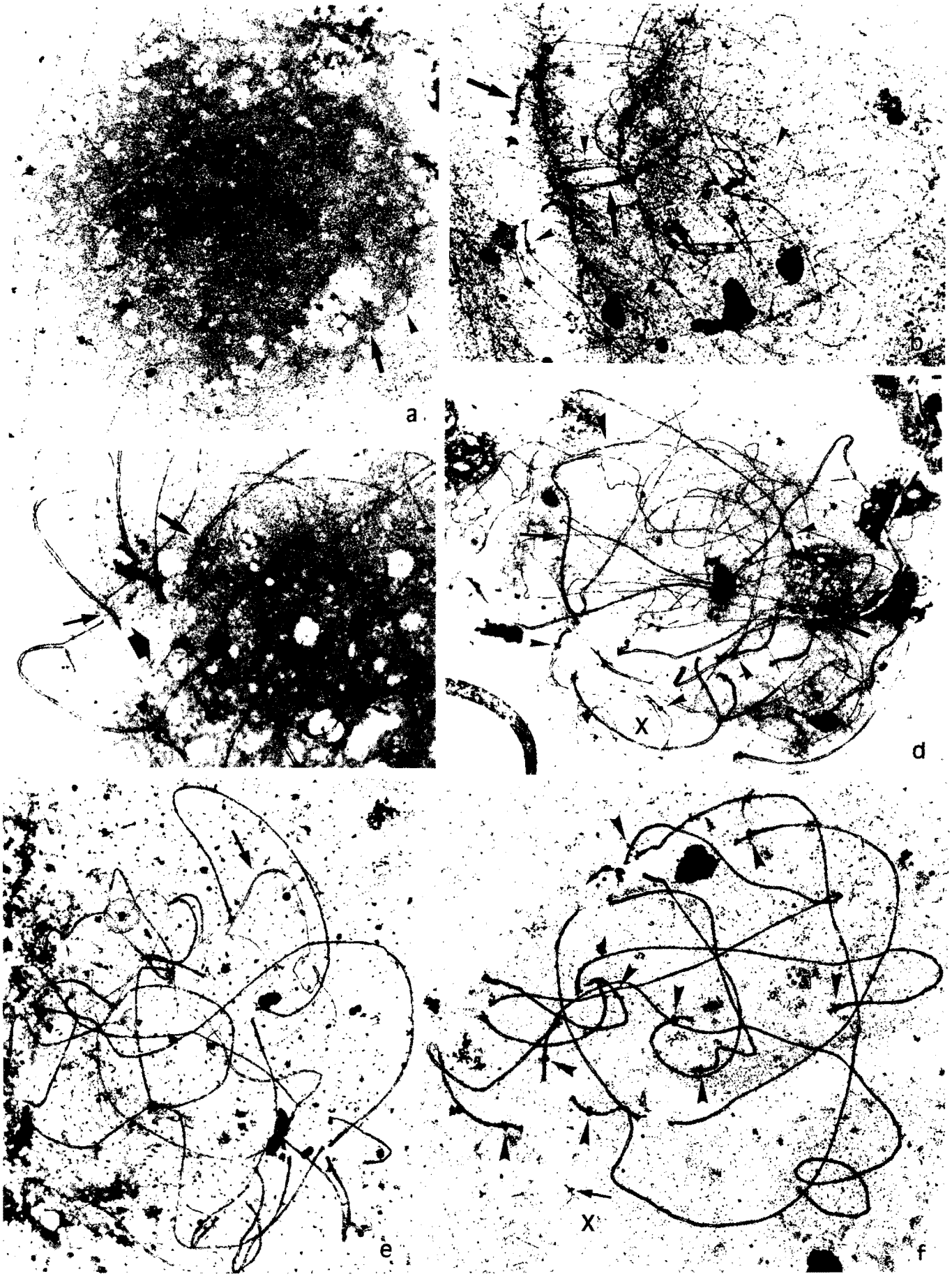
**Table 1.** Stages of Prophase I analyzed by LM and EM .

| PROPHASE I STAGE | No. OF CELLS ANALYSED BY | |
|---|---|---|
| | LM | EM |
| Leptotene | 40 | 9 |
| Zygotene | 102 | 28 |
| Pachytene | 205 | 34 |
| Diplotene | 96 | 7 |
| TOTAL | 443 | 78 |

**Table 2.** Percentage and localization of the pairing-initiation points (PI) detected in 51 early-mid zygotene cells.

| PI LOCALISATION | No. OF PI | (%) |
|---|---|---|
| Proximal SCs | 157 | (35.5) |
| Interstitial SCs | 32 | (7.2) |
| Distal SCs | 253 | (57.3) |
| TOTAL | 442 | (100.0) |

**Fig. 1.** Electron micrographs of leptotene-pachytene nuclei of male *Locusta migratoria*. **a.** Leptotene with short segments of paired (arrow) and unpaired (arrowheads) lateral elements. **b.** Early zygotene with fully paired SC segments (arrows) and presynaptic alignment of LEs (arrowheads). **c.** Detail of an early-mid zygotene nucleus showing polarization of LEs (large arrow), aligned LEs (arrowhead), paired proximal segments (large arrow) showing a centromere (c) and paired distal segments (small arrow). **d.** Early-mid zygotene with fully synapsed bivalents (large arrow) and bivalents partially synapsed at their terminal (small arrow) or interstitial (large arrowhead) regions. Some kinetochores are visible (small arrowheads), including that of the X chromosome (X). **e.** Late zygotene; most SCs are fully paired, but some of the longer ones (arrow) still show unpaired regions. **f.** Pachytene with 11 fully synapsed autosomal bivalents and the axis of the X chromosome (X). Kinetochores (arrowheads) are identifiable, even in the X chromosome (arrow). a, d-f, EM, x 2.500; b, EM, x 4,500; c, EM, x 4,000


a
b
d
X
e
f
X

3a,b). Desynapsis was observed in 15 interstitial regions (68.2%) and in 7 terminal regions (31.8%).

By mid diplotene, the number of fragments increased. Shorter bivalents were the last to show fragmentation (Fig. 3c). At late diplotene, most SCs were fragmented; only the shorter ones maintained their morphology up to late diplotene (Fig 3d).

## Discussion

In *Locusta migratoria*, as well as in other species, the leptotene stage is of short duration and difficult to identify. The number of fragments of lateral elements is always much higher than the number of bivalents (Moens, 1969) and the axis of the X chromosome cannot be identified. Progression of synapsis at zygotene takes place after pairing-initiation at the terminal ends of the bivalents, while the interstitial regions do not assemble until later in this stage (Moens, 1973; Jones and Croft, 1986). The behaviour of the axis of the X chromosome is difficult to analyze, because of its light staining. The axis is usually found at the periphery of the nucleus (Moens, 1969).

The mean number of pairing-initiation regions observed by us was 8.7 per nucleus cell (range from 6 to 16), which is lower than the mean number of chiasmata per cell (16.1) (unpublished data). This is in contrast with the earlier data of Croft and Jones (1986) who detected a mean number of pairing initiation regions (25.8) in *Locusta migratoria* higher than the mean number of chiasmata (range from 14-17 per cell) described by different authors (reviewed by Tease and Jones, 1978). This difference may result from the different stages of zygotene studied, because in early zygotene this number is lower, and it increases with progression of the stage.


**Fig. 2.** Details of three electron micrographs of pachytene nuclei of male *Locusta migratoria* showing: **a.** Association of a fully synapsed bivalent and a lateral element, inducing asynapsis (arrow). **b.** Interlocking of a SC with one of its own lateral elements, preventing synapsis; **c.** Heterosynapsis (arrow) between a fully paired SC and a lateral element, inducing asynapsis. a, c, EM, x 4,000; b, EM, x 4,200

The beginning of synapsis is terminal in most cases (92.8% of cases), and it starts at the ends of the bivalents, mainly at their distal, non-centromeric ends. These observations agree with the data reported by others authors in the same species (Moens, 1969; Counce and Meyer, 1973; Jones and Croft, 1986) and in other Orthoptera (Fletcher, 1977; Solari and Counce, 1977). The presence of interstitial pairing-initiation regions is infrequent (7.2%), but has also been described by Jones and Croft (1986) in the longer bivalents. The presence of interstitial pairing regions is frequent in species with very long chromosomes, such as *Lilium* (Holm, 1977) or *Zea mays* (Gillies, 1975) or in cells with very elongated chromosomes, such as human oocytes (Bojko, 1983; García et al., 1987).

According to some authors (Moens, 1973) pairing in the heterochromatic regions of the chromosomes of *Locusta* is delayed, while according to others (Jones and Croft, 1986) pairing-initiation is not influenced by this characteristic. In our study, we found a tendency of the centromeric region of the bivalents to participate less frequently in pairing-initiation than the euchromatic end, thus suggesting a possible delay induced by heterochromatin, as already proposed in other species.

In our study, synapsis was asynchronous and the smaller bivalents were the first to complete it, even by late zygotene. Our observations are similar to those of Counce and Meyer (1973) and Jones and Croft (1986) in *Locusta*.

In other Orthoptera (Solari and Counce, 1977; Fletcher, 1979) and even in humans (Rasmussen and Holm, 1980), small chromosomes are the first to complete synapsis. According to Rasmussen and Holm (1980) this could result, apart from their size, from the fact that their telomeres can move in the nuclear membrane more easily and faster than those of longer bivalents.

Finally, we describe for the first time the characteristics of diplotene in *Locusta* male cells. The diplotene stage, is characterized by the fragmentation and desynapsis of the bivalents, similar to the disintegration process observed in human (Vidal et al., 1984) and Chinese hamster (Moses, 1977) spermatocytes. However, in other species, such as in mouse spermatocytes (Solari, 1970; Guitart et al., 1985; Grao et al., 1989), diplotene is characterized by desynapsis, and the structure of the lateral elements is maintained until the end of the stage.

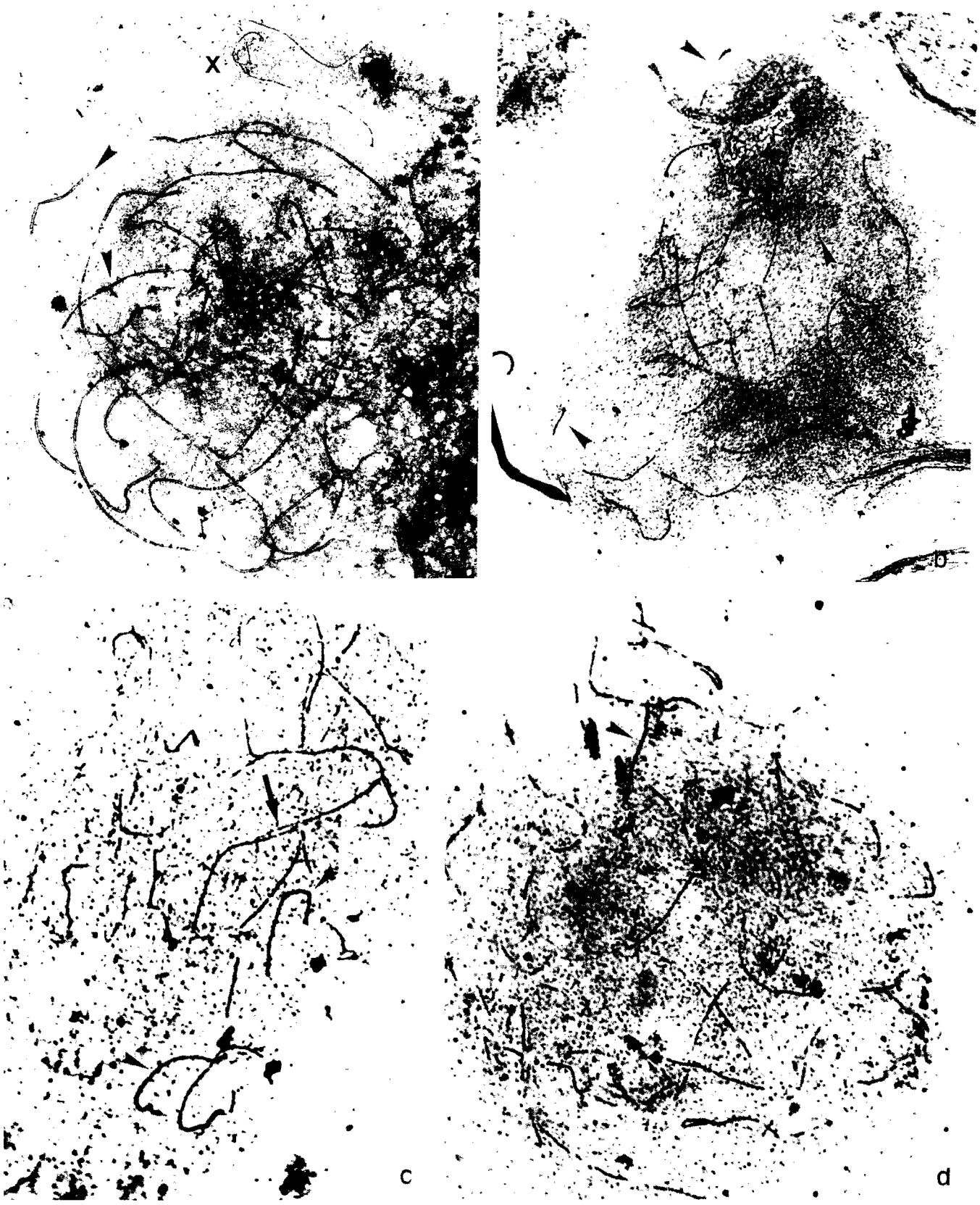



**Fig. 3.** Diplotene nuclei of *Locusta migratoria*. **a.** EM image showing fragmentation of some SCs (arrowheads). **b.** LM image showing a more intense fragmentation of SCs (arrowheads). **c.** LM image of a mid diplotene showing intense fragmentation and desynapsis of lateral elements (arrow). Two small bivalents are still intact (arrowheads). **d.** LM image of a late diplotene with most SCs fragmented; one small bivalent is still intact intact (arrowhead). a, EM, x 2,500; b-d, LM, x 1,600

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