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Nuclear changes during spermiogenesis in two chrysomelid beetles

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Abstract

Ultrastructural and cytochemical studies were carried out on sperm nucleus of the beetles, Coelomera lanio and Diabrotica speciosa. Nuclear development involves changes in the shape and in the degree of chromatin condensation, with specific aggregation patterns of DNA-histone complex occurring during this process. Lamellar and paracrystalline arrangements of the nuclear material were observed in the Diabrotica speciosa spermatid and in the Coelomera lanio spermatozoon, respectively. Ethanolic-phosphotungstic acid technique suggest the presence of basic proteins in the nuclear material of spermatids. This reaction disappears during chromatin condensation. Chromatin condensation patterns may reflect specific intranuclear mechanisms and offers protection to the genome during spermatozoon transport to the oocyte.

Keywords: Chromatin condensation; cytochemistry; electron microscopy; nucleus; spermatozoon; chrysomelids

Introduction

The spermatozoon is a highly specialized cell which has many unique properties. The main compartments of a typical insect spermatozoon are the head, containing nucleus and acrosome, and the tail which contains axoneme and mitochondrial derivatives (for reviews, see Phillips, 1970; Baccetti, 1972). Sperm nucleus development is characterized by the change of a spherical to a highly asymmetric configuration and by chromatin conversion from a dispersed to a very condensed state (Tokuyasu, 1974; Fawcett, 1971). It has also been shown that during spermiogenesis the histones complexed to DNA are exchanged for specific arginine-rich basic proteins, the protamines. These proteins are responsible for a high degree of condensation chromatin in the nucleus (McMaster-Kaye and Kaye, 1976; Loir and Lanneau, 1978, 1984; Loir and Courtens, 1979; Mello, 1987; Quagio-Grassiotto and Dolder, 1988). Condensation of the sperm chromatin occurs in specific arrangement, which appear to be characteristic of the differentiation stage and species (Cruz-Landim and Ferreira, 1976: Riess et al., 1978; Werner and Bawa. 1988). In the present study we used electron microscopy and cytochemistry to analyse the morphofunctional nuclear changes during spermiogenesis of two species of beetle.

Material and Methods

The insects used were male adults of (‘orlomera lanio and Diabrotica speciosa collected in Brasilia (Brazil). Coelomera Zanio is a herbivore of Cecropia, and Diubrotiur speciosa is an important leguminous pesl.

Transmission Electron Microscopy

Testes were dissected and fixed overnight at 4°C in a solution containing 4% paraformaldehyde, 2% glutaraldehyde and Lic4 sucrose in 0.1 M cacodylate buffer, pH 7.3. After fixation, the specimens were rinsed in buffer, and post-fixed in 1% osmium tetroxide, followed by block-staining in 0.5% aqueous uranyl acetate. The material was dehydrated in ethanol and embedded in Epon. After sectioning and staining with uranyl acetate and lead citrate the sectmns were examined in a JEOL JEM 100 C Transmission Electron Microscope.

Cytochemistry

The technique of ethanolic-phosphotungstic acid (E-PTA), modified from Bloom and Aghajanian (1968) was used for detection of basic proteins in spermatid and spermatozoon. Testes were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 5 hr at 4”C, dehydrated in an ethanol serie and treated for 24 hr at 4°C with 2% phosphotungstic acid in absolute ethanol; then they were washed in absolute ethanol and embedded in Epon. Thin sections were 441 stained with uranyl acetate and lead citrate or observed unstained.

Results and Discussion

The spermatids of Coelomera lanio and Diabrotica speciosa beetles undergo specific morphofunctional modifications during spermiogenesis. The acrosome and flagellum formation occurs simultaneously with the nuclear transformations, which change in shape and in chromatin condensation degree. These events follow the general pattern described for other Coleoptera (Baccetti and Daccordi, 1988; Burrini et al.. 1988).

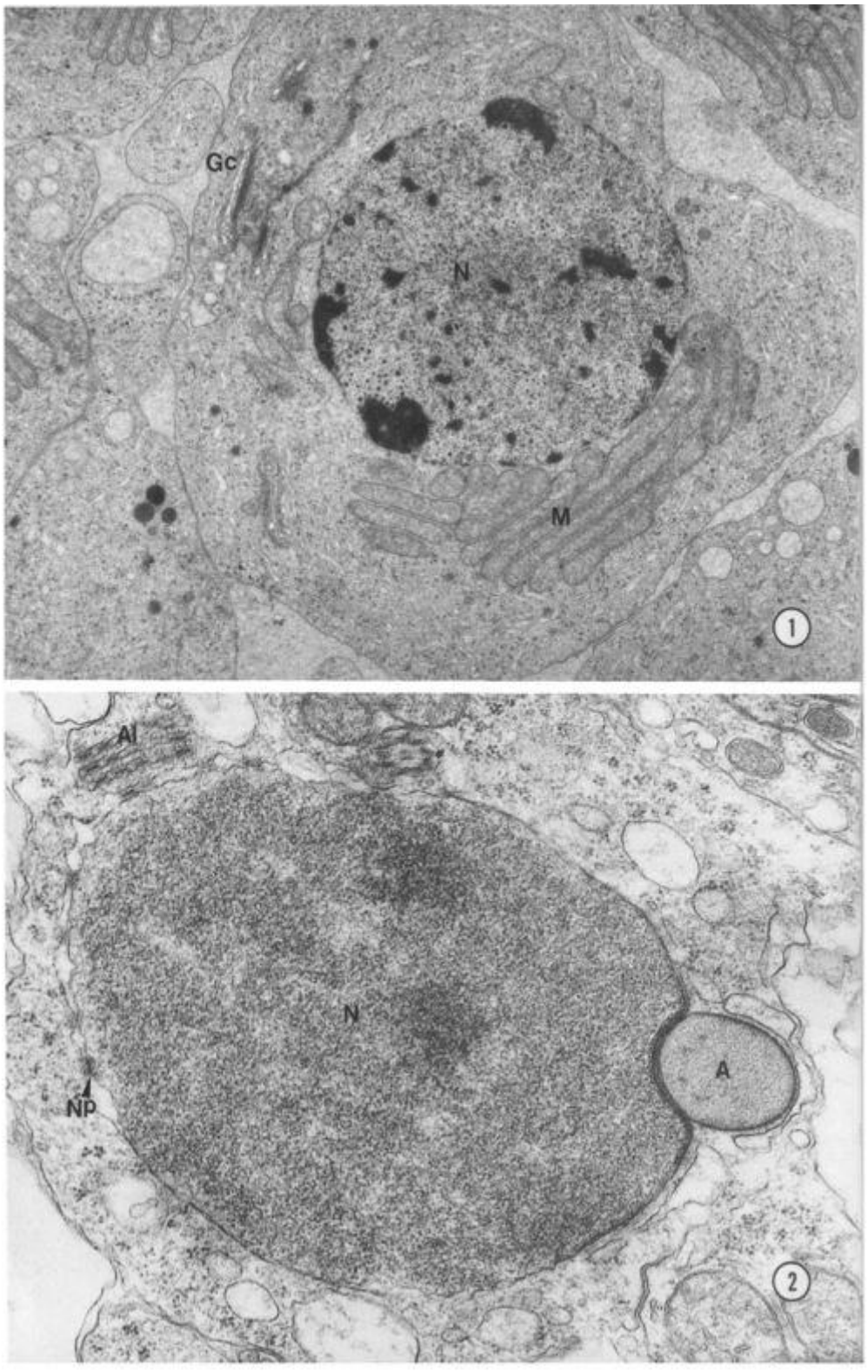
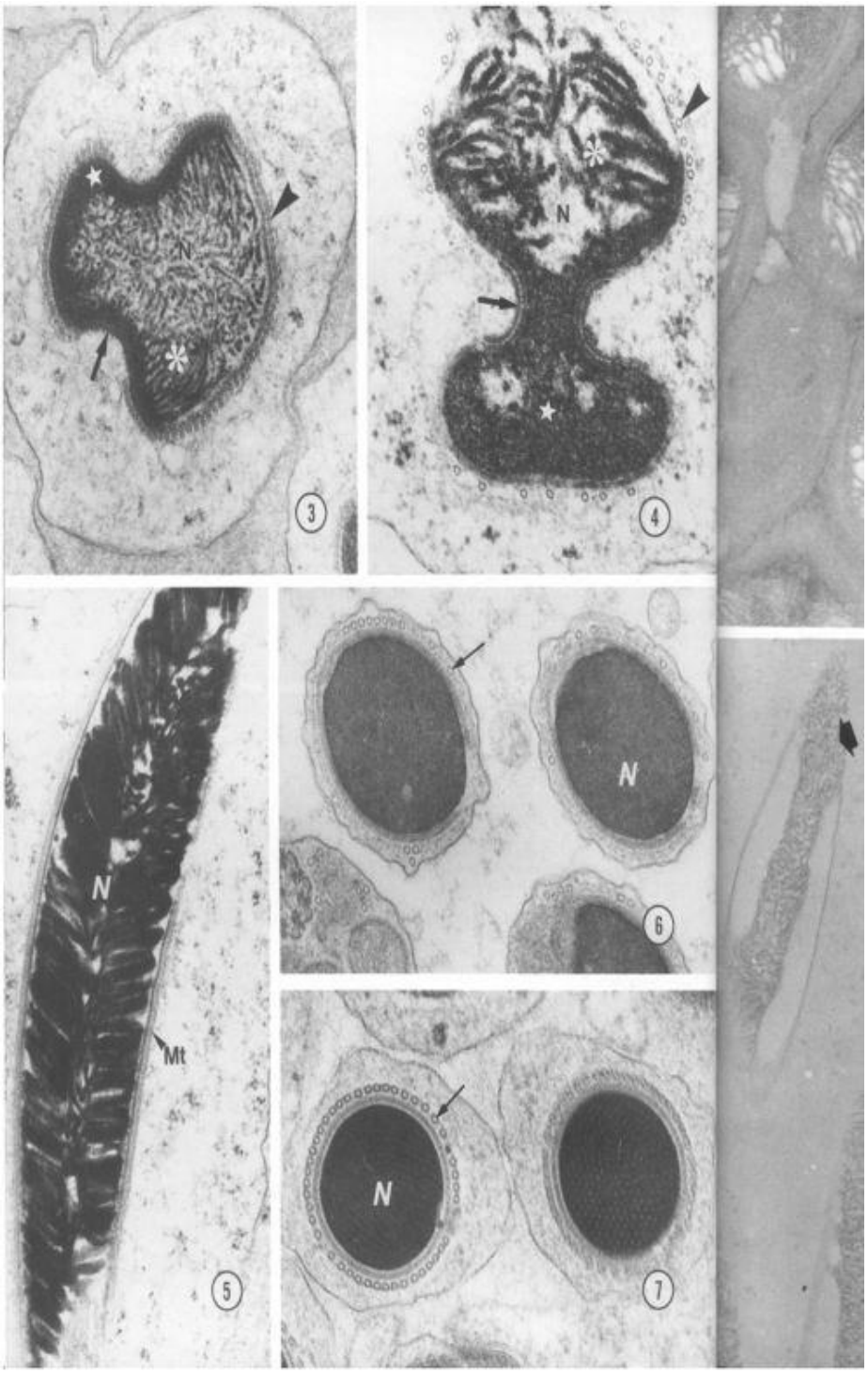
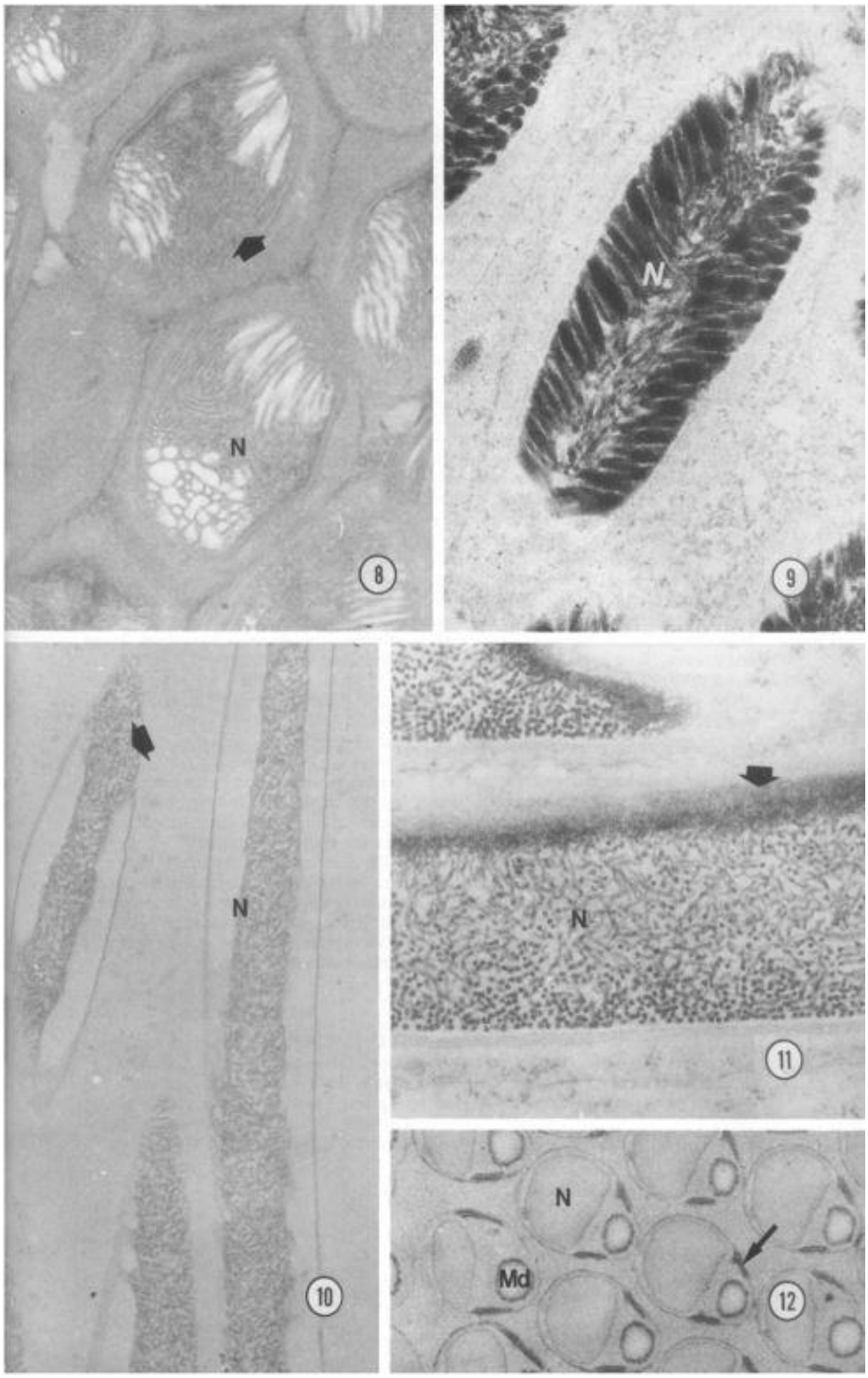


Fig. 1. Transverse section through the nuclear region of Coelomeru [anio spermatids. Electron-dense areas of chromatin are present in the nucleus (N). Gc: Golgi complex: M: mrtochondria. X6700. Fig. 2. Transverse section through the nuclear region of Diabrotica spvciosa spermatid. A: acrosome: Al: annulate lamellae: N: nucleus; Np: nuclear pores. x34,000.



Figs 3, 4. Transverse sections of nuclear region of Coelomera lanio (Fig. 3) and Diabrotica speciosn (Fig. 4) spermatids. The nucleus (N) shows condensed chromatin, near to the nuclear envelope (star), and fibrillar chromatin, in the central region (asterisk). Numerous microtubules (arrowhead) surround the nucleus. An amorphous and electron-dense material is observed in the nuclear envelope region (arrow). Fig. 3 X32.000; Fig. 4 x46.000 . Fig. 5. Longitudinal section of nuclear region of Diubrotrca speciosu spermatid. N: nucleus: Mt: microtubules. ~26,000. Fig. 6. Transverse section through the nuclear region of Diubrotrca speciosa spermatids. Microtubules (arrow) surround the nucleus (N) which represent highly condensed chromatin. X39.000. Fig. 7. Transverse section of nuclear region of Coelomera [anid spermatids. Numerous microtubules (arrow) surround the nucleus (N). A paracrystalline aspect is ohserved in nuclear material. X55,000.



Figs g-12. Thin sections of E-PTA treated spermatids and spermatozoa Figs 8. 9. Sections of Diabroticu specrosa spermatids Fig. 8. The nucleus (N) shows reaction only in regions where chromatin condensation appcx incomplete (arrow). Sections without stains. x 19.000. Fig. 9. The nuclear material (N) displays an increase of contrast in sections stained with uranyl acetate and lead citrate. x 19,000. Figs l&12. Sections of Coelomera lanio spermatids and spermatozoa. Fig. 10. The nucleus (N) shows reaction only in regions where chromatin condcnsatmn appear incomplete (arrow). Sections without stains, x22,000. Fig. Il. The nuclear material(N) appear highly condensed near the nuclear envelope (arroti). Sections stained with uranyl acetate and lead citrate. X38,tK)O. Fig. 12. Transversal section of nucleus (N). Electron-dense plate (arrow) shows positive reaction. Md: mitochondrial derivatives. Sections without stains. X33.000.

The alteration in the nucleus begin with the change from a spherical to a triangular shape with depressions alongside, finally becoming elongate and lance-like. Numerous alterations were also observed in the chromatin.

During the early spermatid phase, the nucleus resembles that of somatic cells with electron-opaque chromatin (Fig. 1). Subsequently, there was a gradual condensation of this chromatin, with an increase of its electron density (Fig. 2). As differentiation follows, the nucleus begins to acquire a triangular configuration and chromatin fibrillar with a distinct aspect were observed. In the nuclear material two relatively dense regions could be distinguished: one near to the nuclear envelope, showing homogeneously condensed chromatin attached to it, and the other in the nucleus central region, showing the chromatin with a fibrillar aspect (Figs 3, 4). Structural changes of nuclear development have been described for cricket (Cruz-Landim and Ferreira, 1976; Kierszenbaum and Tres, 1978), fruit-fly (Tokuyasu, 1974; Quagio-Grassiotto and Dolder, 1988), and scorpion (Riess et al., 1977; Werner and Bawa, 1988).

Our observations have demonstrated the peculiar organization of the nuclear material during spermatid differentiation of Diabrotica speciosa. The fibrillar chromatin is organized in the lamellae throughout the nucleus, from the periphery towards the center (Fig. 5). This arrangement disappears in the mature sperm cell (Fig. 6), suggesting that chromatin organization patterns may be characteristic of the spermatid differentiation stage. A paracrystalline arrangement of nuclear material observed in spermatozoa of Coelomera lanio (Fig. 7) has not been reported elsewhere. Previous studies carried out on cricket spermatids have shown a sticklike chromatin arrangement (Cruz-Landim and Ferreira, 1976) while the nucleus of scorpion spermatozoa present a lamellar arrangement of the chromatin (Riess et al., 1977; Werner and Bawa, 1988), indicating the existence of a variety of chromatin arrangements.

Electron-dense reaction product revealed by the ethanolic-phosphotungstic acid technique (E-PTA) was observed in the nucleus of the spermatids, indicating the presence of basic protein. The reaction occurs only in regions where chromatin condensation appears incomplete (Figs 8, 10). This result is similar to those described in nucleus spermatids of the cricket (Kierszenbaum and Tres, 1978) and fruit-fly (Quagio-Grassiotto and Dolder, 1988). Sections stained with uranyl and lead staining showed an increase of chromatin contrast during the process of condensation (Figs 9, 11). E-PTA reaction in the nuclear material reduced with the chromatin condensation increase (Fig. 12). This may reflect the substitution of histones for specific basic proteins in the chromatin, which permits a higher degree of condensation and therefore blocks the interaction of basic proteins-E-PTA. Electrondense

plates, localized in the implantation region of the flagellum, were also positive for basic protein reaction.

During the beetles' nuclear transformations, structures such as microtubules and cytoplasmic membranes have been found surrounding the nucleus (Figs 3-7). This has been similarly reported for several other insects (Kessel, 1966; Schrankel and Schwalm, 1974; Tokuyasu, 1974). The presence of microtubules and cytoplasmic membranes in spermatids with this precise orientation with respect to the nucleus, suggest that they may be involved with nuclear elongation, since these structures disappear after this event is completed. The different chromatin arrangement patterns may reflect specific intranuclear mechanisms. This peculiar pattern of condensation observed in sperm chromatin may also offer sufficient protection to the genome during its transportation to the oocyte.

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