ISSN- 0102-9010

ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES ON DIAPAUSE SPERMIOGENESIS IN PHYTOPHAGOUS BUGS (HEMIPTERA: PENTATOMIDAE)*

Adrienne de Paiva Fernandes[1,2,3], Fernanda Lopes Peixoto[1] and Sônia Nair Báo[3]

[1]University Center of Brasília - (UniCEUB), Faculty of Heatlh Sciences (FACS), 70970-075, Brasília, DF, Brazil,
[2] Department of Cell Biology, Institute of Biology, State University of Campinas (UNICAMP), C.P. 6109, 13083-970, Campinas, SP, Brazil,
[3] Laboratory of Electron Microscopy, Department of Cell Biology, Institute of Biology, University of Brasília (UnB), Brasília, DF, Brazil.

ABSTRACT

Diapause is a genetically controlled life phase in which the biochemical and behavioral adjustaments that occur in advance are followed by a refractory period of suppressed development. In this study, we investigated whether spermiogenesis in phytophagous bugs continues in adult diapause. The morphology of spermiogenesis during this phase was also examined. During diapause, the testes of phytophagous insects contained vesicles similar to residual bodies. These vesicles showed acid phosphatase activity, which suggested that they were active lysosomes. In addition, the nucleus of spermatids showed an apoptotic pattern with fragmented chromatin. These results indicate that spermiogenesis is discontinued during adult diapause in these bugs, and that apoptotic and phagocytic events may be involved.

Key words: Acid phosphatase, diapause, *Edessa meditabunda*, *Nezara viridula*, spermiogenesis

INTRODUCTION

Phytophagous stink bugs (Hemiptera, Pentatomidae) are the main pests of economically important crops throughout the world [16]. Despite the vast amount of information regarding pest species and the mechanisms of controling them, the potential of these species to damage crop production remains high. More knowledge about the basic biology and ecology of most heteropteran pests is needed, as are new, efficient alternatives of biological control. In this context, a study of the reproductive biology of these insects may provide new approaches to their control.

Numerous reports have described the structure and ultrastructure of hemipteran spermatozoa and spermiogenesis [1,2,5,7-11]. However, little information is available about reproductive biology of these insects during adult diapause [6,15,18].

Correspondence to: Dr. Sônia Nair Báo
Departamento de Biologia Celular, Instituto de Biologia, Universidade de Brasília, CEP 70919-970, Brasília, DF, Brasil. Tel: (55) (61) 307-2424, Fax: (55) (61) 347-6533, E-mail: snbao@unb.br

* Part of this work was presented at the XVIII Congress of the Brazilian Society of Microscopy and Microanalysis.

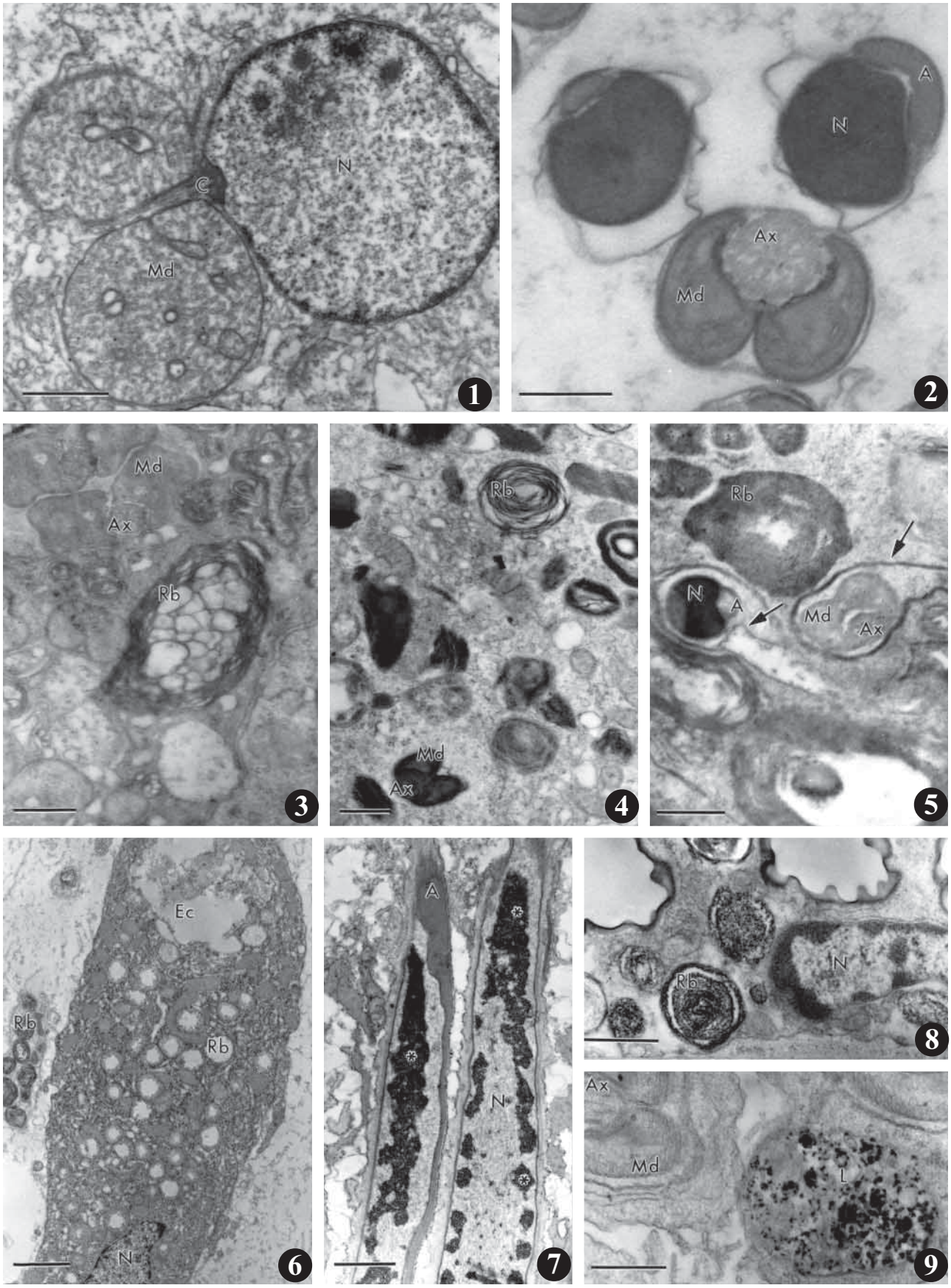
In this study, we investigated whether spermiogenesis continues during adult diapause. The morphological characteristics of spermiogenesis in *Edessa meditabunda* and *Nezara viridula* during this phase were also examined.

MATERIAL AND METHODS

The insects studied were diapausic adult males of the phytophagous bugs *Edessa meditabunda* and *Nezara viridula* (Hemiptera, Pentatomidae) obtained from a laboratory colony reared at the National Center of Genetic Resources (CENARGEN), Brasília, Brazil.

Transmission electron microscopy

The testes were fixed for 4 h at 4ºC in a mixture of 2.5% glutaraldehyde, 4% paraformaldehyde, 5 mM $CaCl_2$ and 3% sucrose in 0.1 M sodium cacodylate buffer, pH 7.3. After fixation, the specimens were rinsed in the same buffer and postfixed in 1% osmium tetroxide containing 0.8% potassium ferricyanide and 5 mM $CaCl_2$ in sodium cacodylate buffer. The material was dehydrated in a graded series of acetone (30-100%) and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate.


C
N
Md
1
A
N
Ax
Md
2
Md
Ax
Rb
3
Rb
Md
Ax
4
Rb
N
A
Md
Ax
5
Ec
Rb
Rb
N
6
A
N
7
Rb
N
8
Ax
Md
L
9

Enzyme cytochemistry

The testes were dissected and briefly fixed for 15 min at 4ºC in 1% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.2. After fixation, the specimens were washed with buffer and incubated for 1 h at 37ºC in 0.1 M Tris-maleate buffer, pH 5.0, containing 7 mM cytidine-5´-monophosphate, 2 mM cerium chloride and 5% sucrose [17]. The substrate was omitted in the controls.

After incubation, the specimens were washed with sodium cacodylate buffer and fixed again for 3 h at 4ºC in a solution containing 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. The specimens were then washed in plain buffer and postfixed in a solution containing 1% osmium tetroxide, 0.8% potassium ferricyanide and 5 mM calcium chloride in 0.1 M sodium cacodylate buffer. After dehydration in acetone, the tissues were embedded in Spurr's resin and thin sections were cut and stained with uranyl acetate and lead citrate prior to examination in a Jeol 100C transmission electron microscope.

RESULTS

The general structure of spermiogenesis in *Edessa meditabunda* and *Nezara viridula* based on transmission electron microscopy has been described in detail elsewhere [7,9]. During normal spermiogenesis, the spermatids undergo a series of modifications that result in the formation of highly differentiated spermatozoa which, after capacitation, are able to fertilize oocytes.

During adult diapause, spermiogenesis begins normally, with the spermatids undergoing typical modifications, including nuclear elongation and tail formation (Fig. 1), that culminate with the formation of complete spermatozoa containing a head-piece (with an acrosome and nucleus) and tail (with an axoneme and two mitochondrial derivatives) (Fig. 2).

In adult diapause, the cytoplasm of cystic cells contained conspicuous vesicles similar to residual bodies (Figs. 3 and 4). Another type of vesicle contained fragments of spermatozoa (Fig. 5). A further characteristic of spermiogenesis in adult diapause in these insects was the presence of cysts bearing only a few or no spermatids but showing several residual bodies (Fig. 6). Although some spermatids became spermatozoa, many of them did not complete development and their nuclei apparently underwent apoptosis, with a characteristic fragmentation of their chromatin (Fig. 7).

A cytochemical test for acid phosphatase demostrated the presence of an electron dense precipitate in the residual bodies scattered throughout the cystic cell cytoplasm (Fig. 8), as well as in active lysosomes surrounding early spermatids (Fig. 9).

DISCUSSION

Spermiogenesis involves the structural and physiological transformation of organelles to more adapted forms at the time of fertilization. These changes have been described for *E. meditabunda* [9] and *N. viridula* [7] during the normal development.

Diapause is a genetically controlled life phase in which the biochemical and behaviour adjustments that occur in advance are followed by a refractory period of suppressed development. In temperate regions, diapause is associated with survival during winter, when the normal growth is not possible. In the tropics, diapause may enhance survival during periods of drought which are characterized by low humidity and a limited food

---

Figure 1. Section through *E. meditabunda* diapausic testes showing the inicial development of spermatids. Centriole (C); mitochondrial derivative (Md); nucleus (N). X 18 200. Bar: 1 µm.
Figure 2. Transverse section through *E. meditabunda* diapausic testes. Note the completely formed spermatozoa. Acrosome (A); axoneme (Ax); mitochondrial derivatives (Md); nucleus (N). X 99 000. Bar: 1 µm.
Figures 3-5. Sections through diapausic *E. meditabunda* (3 and 5) and *E. meditabunda* (4) cystic cell cytoplasm. The diapausic cystic cell cytoplasm contains residual bodies (Rb). Some vesicles contain fragments of spermatozoa (arrows). Acrosome (A); axoneme (Ax); mitochondrial derivatives (Md); nucleus (N); residual bodies (Rb). X 26 000; X 20 800 and X 28 600, for figures 3, 4 and 5, respectively. Bars: 0.5 µm.
Figure 6. Section through an *E. meditabunda* cystic cell, showing an empty cist (Ec) and conspicuous residual bodies (Rb). Nucleus (N). X 5 980. Bar: 2 µm.
Figure 7. Spermatids of *E. meditabunda* showing fragmented chromatin (asterisks) similar to an apoptotic pattern. Acrosome (A); nucleus (N). X 13 000. Bar: 1 µm.
Figures 8 and 9. Acid phosphatase reaction. Section through a cystic cell of *E. meditabunda* showing the reaction product located on the residual bodies (Rb) and active lysosomes (L). Axoneme (Ax); mitochondrial derivatives (Md); nucleus (N). X 32 000. Bars: 0.5 µm.

supply [3]. Insects may overcome such adverse conditions by entering periods of dormancy and reproductive inactivity, either through diapause or quiescence.

During larval and pupal diapause, spermiogenesis ceases as a result of a new endocrine balance in each developmental phase [4]. By the end of diapause, the endocrine balance is reestablished and spermiogenesis proceeds again [12-14].

The abnormal development of spermatids during adult diapause in *E. meditabunda* and *N. viridula* and the presence of several conspicuous residual bodies in cysts cells suggests that the germ cells are eliminated during the develpment. The presence of reaction product for acid phosphatase within these residual bodies indicates that they could be active lysosomes. Moreover, the presence of vesicles containing fragments of spermatozoa may indicate that the germ cells are phagocytosed and subsequently digested by active lysosomes.

Another characteristic observed here was the apoptotic pattern of spermatid nuclei which contained fragmented chromatin. This finding suggests that apoptosis may occur during adult diapause and may involve lysosomal activity in order to interrupt spermiogenesis during this phase.

ACKNOWLEDGMENTS

The authors thank Dr. M. Borges and Mr. H. Moreira dos Santos for supplying the insects. This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

1. Afzelius BA, Dallai R, Lindskog P (1985) Spermatozoa of saldid bugs (Insecta, Hemiptera, Leptopodomorpha). *J. Ultrastruct. Res.* **90,** 304-312.
2. Báo SN, de Souza, W (1994) Structural specialization in the flagellum of the spermatozoon of the bloodsucking bug (*Rhodinius prolixus*; Hemiptera, Reduviidae). *Tissue Cell* **26,** 299-308.
3. Chapman RF (1998) The endocrine organs and hormones. In: *The Insects Structure and Function.* 4th ed. Cambridge University Press: Cambridge.
4. Chippendale GM, Alexander BR (1973) Spermatogenesis in the south-wester corn borer, *Diabrotica grandiosella*. 2. Resumption in diapause larvae. *Ann. Entomol. Soc. Am.* **66,** 747-752.
5. Dallai R, Afzelius BA (1980) Characteristics of the sperm structure in Heteroptera (Hemiptera, Insecta). *J. Morphol.* **164,** 301-309.
6. De Wilde J (1954) Aspects of diapause in adult insects with special regard to the Colorado beetle, *Leptinotarsa decemlineata*. *Arch.Neerl.Zool*. **10,** 375-385.
7. Fernandes AP, Báo SN (1998) Spermiogenesis in phytophagous bugs (Hemiptera, Pentatomidae): an ultrastructural study. *J. Submicrosc. Cytol. Pathol.* **30,** 485-493.
8. Fernandes AP, Báo SN (1999) Ultrastructural localization of enzymatic activity during spermiogenesis in two phytophagous bugs (Hemiptera: Pentatomidae). *Tissue Cell* **31,** 349-356.
9. Fernandes AP, Báo SN (2001) Ultrastructural studies on spermiogenesis and spermatozoa of insect pests (Hemiptera: Pentatomidae). In: Sarma, R. (ed). *Current Research Advances in Agriculture & Entomology* pp.13-26. World Wide Research Network, Kerala, India.
10. Fernandes AP, Curi G, Báo SN (1998) Contribution of the Golgi complex-endoplasmic reticulum system during spermiogenesis in three species of phytophagous bugs (Hemiptera: Pentatomidae). *Int. J. Insect Morphol. Embryol.* **27**, 235-240.
11. Fernandes AP, Curi G, França FGR, Báo SN (2001) Nuclear changes and acrosome formation during spermiogenesis in *Euchistus heros* (Hemiptera: Pentatomidae). *Tissue Cell* **33,** 286-293.
12. Friedlander M (1982) Juvenile hormone and regulation of dichotomous spermatogenesis during the larval diapause of codlin moth. *J. Insect Physiol.* **28,** 1009-1012.
13. Friedlander M (1997) Control of the eupyrene-apyrene sperm dimorphism in lepidoptera. *J. Insect Physiol.* **43**, 1085-1092.
14. Friedlander M, Reynolds SE (1992) Intratesticular ecdusteroid titers and the arrest of sperm production during pupal diapause in the tobacco hornworm *Manduca sexta*. *J. Insect Physiol.* **38,** 693-703.
15. Friedlander M, Scholtz CH (1993) Two different pattern of interrupted spermatogenesis in winter diapause and summer quescence in the desert beetle, *Omorgus freyi*. *J. Morphol.* **218,** 347-358.
16. Panizzi AR (1997) Wild hosts of pentatomids: ecological significance and role in their pest status on crops. *Annu. Rev. Entomol.* **42,** 99-122
17. Pino RM, Pino LC, Bankston PW (1981) The relationship between the Golgi apparatus, GERL, and lysosomes of fetal rat liver Kupffer cell examined by ultrastructural phosphatase cytochemistry. *J. Histochem. Cytochem.* **29**, 1061-1070.
18. Tran B, Huignard J (1992) Interactions between photoperiod and food affect the termination of reproductive diapause in *Bruchus rufimanus* (Boh) (Coleoptera, Bruchidae). *J. Insect Physiol.* **38,** 633-642.

Received: October 17, 2001
Accepted: January 30, 2002