

## PERITROPHIC MATRIX OF THE FORMOSAN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

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The peritrophic matrix in many insects is continuously being synthesized in the mesenteron and excreted with the fecal matter (Wigglesworth 1972; Richards & Richards 1977; Tellam 1996; Lehane 1997). Peritrophic matrices are classified into 2 types according to the way they are synthesized in the mesenteron. Type I is made of concentric lamellae loosely attached to one another and synthesized by the epithelial cells through the length of the mesenteron, while type II is a single uniform layer synthesized by a group of cells in the anterior limit of the mesenteron (Wigglesworth 1972; Tellam 1996; Lehane 1997). The peritrophic matrix is most commonly made of  $\gamma$ -chitin, which is considerably more flexible than  $\alpha$ -chitin while in the presence of water (Herburn 1985). Wigglesworth (1972) mentions that termites may possess type II peritrophic matrices, but this has not been confirmed. The objective of this study was to determine the type of peritrophic matrix present in *Coptotermes formosanus* Shiraki.

Formosan subterranean termites were collected from 2 different localities (City Park and Gretna) around the New Orleans metropolitan area. All the termites were brought to the laboratory in plastic containers. An infested pine log and carton nest were separately transferred to a 75.7-L plastic trash container, containing 10 L of topsoil: sand mixture at 1:1 ratio and 3 L distilled water. Pieces of wood (*Pinus taeda*, *Liquidambar styraciflua*, and *Carya illinoensis*) were added as a source of food. The containers were maintained in the dark at  $27 \pm 3^\circ\text{C}$  for 7 d to allow the termites to settle down. Then, termites were harvested by placing pieces of wet cardboard inside of containers for 8 h. Termites were carefully removed from the cardboard pieces by gentle manual shaking.

Twenty termite workers were randomly selected and manually placed in a fixative solution of 2% glutaraldehyde, 2% paraformaldehyde, 2% acrolein, and 1.5% dimethyl sulfoxide in 0.1 M sodium cacodylate buffer (pH 7.4) (Kalt & Tandler 1971, modified). To facilitate penetration of the fixative into the termite bodies, the selected termites were decapitated under a stereo microscope and a small cut at the tip of the abdomen was made.

Fixed termite abdomens were rinsed 3 times in 0.1 M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide, and embedded in Araldite/Epon epoxy resin (Araldite 502-EMbed 812) as

described by Mollenhauer (1964). Abdomens were cut longitudinally producing 1- $\mu\text{m}$  thick sections with an Ultracut E microtome. Five individuals were selected for sectioning based on the quality of fixation. Section series for each termite were placed on glass microscope slides, allowed to dry on a hot plate for at least 5 min, and labeled.

Sections were stained with a modification of Humphrey and Pittman's (1974) methylene blue, azure II, and basic fuchsin staining technique. This technique requires 2 stain solutions. The blue stain was prepared by mixing 0.13 g methylene blue, 0.02 g azure II, 10 ml glycerol, 10 ml methyl alcohol and 80 ml distilled water. The mixture was stirred and filtered. The red stain was prepared by mixing 0.2 g basic fuchsin in 100 ml distilled water, and diluted 1:4 in distilled water after stirring and filtering. The staining procedure was as follows: (1) The slide was flooded with blue stain for 15-60 seconds, (2) then 4-6 drops of 1% NaOH solution were added and spread over the slide by tilting it for 10 seconds, (3) slides were washed in running water and dried on a hot plate at  $80^\circ\text{C}$ , (4) the red stain was added for 15-30 seconds to slides on the hot plate, and (5) slides were finally rinsed with running water and dried.

Sections were examined with an optical compound microscope (Leica DMLB, Leica Microsystems, Germany) and photographed with a Leica MPS 60 micro photographic system. Color slides were produced on Kodak Elitechrome 160T. Slides were digitalized at 2,000 dpi with a high resolution scanner (Minolta Dimage Scan Multi, Konica Minolta, Japan).

Tissue coloration in sections was sufficiently consistent to differentiate basic tissues. The mesenterial epithelial cells appeared dark-blue to purple (Fig. 1A, MGE), microvilli appeared red (Fig. 1B, MV) and peritrophic matrix pink (Fig. 1A & B, PTM). Microvilli coloration was distinctive enough to make them easily identifiable. The peritrophic matrix was located next to the mass of microvilli (Fig. 1B). Fat body cells appeared pink to red in color (Fig. 1A, FBC).

The peritrophic matrix of *C. formosanus* appears to be synthesized around the invagination (Fig. 1A, FCI) of the stomodaeum into the mesenteron (Fig. 1A, MSE). A group of large pink-colored cells located around the invagination portion of the stomodaeum (Fig 1A & 1B, SC) shows

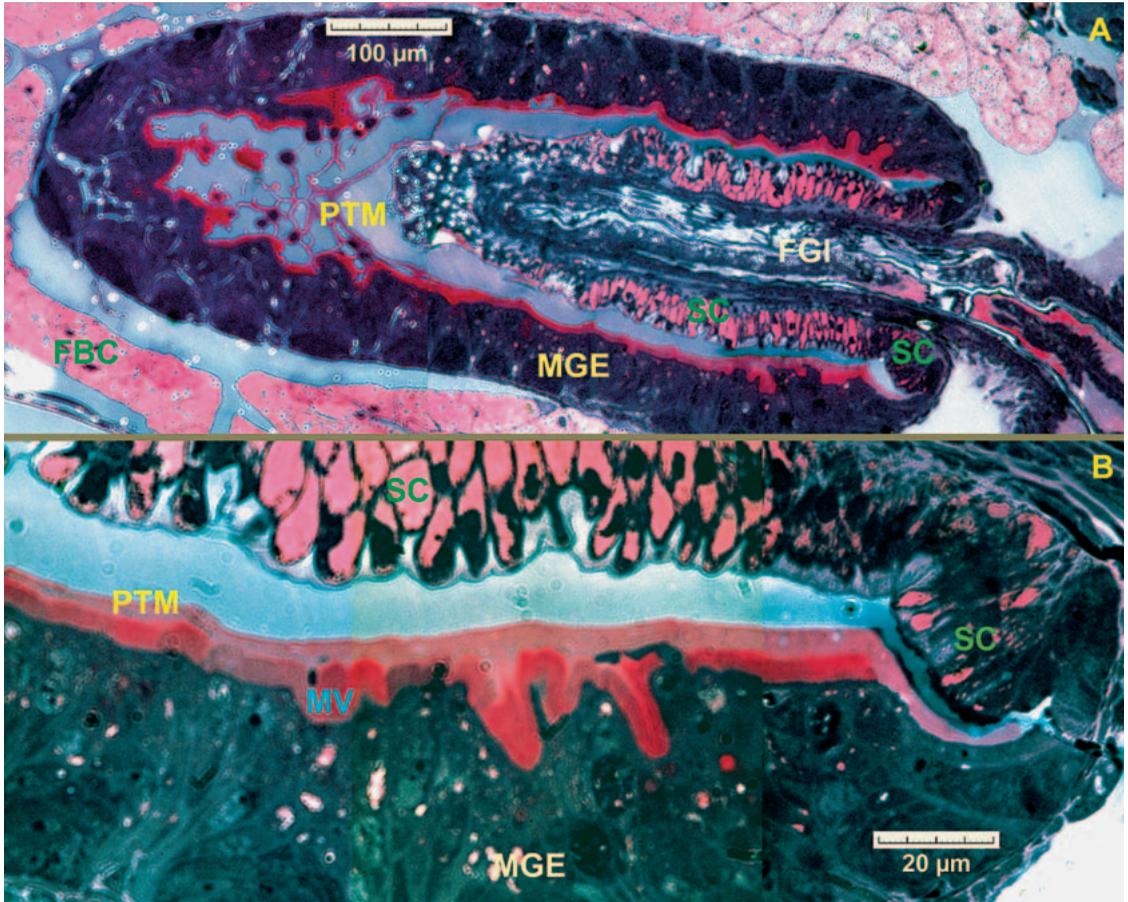


Fig. 1. Longitudinal section of foregut and midgut junction of *C. formosanus* workers. (A) Lower magnification showing the foregut invagination (FGI) into the midgut and midgut epithellium (MGE). (B) Higher magnification of foregut invagination showing secretory cells (SC) and peritrophic matrix (PTM). A is a composite of 2 pictures taken with a 10 × ocular and 20 × objective; B is a composite of 3 pictures taken with a 10 × ocular and 100 × objective immersed in oil.

a clear substance that migrates to the lower part of the invagination area. These cells appear to be secretory in nature producing a clear substance that is easily distinguishable (Fig. 1B). At the base of the invagination, the peritrophic matrix (PTM) seems to originate from the clear substance produced by the pink secretory cells and then covers the inner part of the mesenteron (Fig. 1B).

Our observations showed the presence of structures in the anterior part of the mesenteron resembling those described by Wigglesworth (1972) for type II peritrophic matrix (Fig. 1). Also the structure of the peritrophic matrix observed in workers of *C. formosanus* is a continuous layer instead of independent lamellae as described by Wigglesworth (1972). Based on these two characteristics, we describe the peritrophic matrix of *C. formosanus* workers as a type II.

The rate of type II peritrophic matrix production varies from 1 to 10 mm/h in different insect species (Waterhouse 1954). These production rates make type II peritrophic matrices particularly vulnerable to the action of chitin synthesis inhibitors.

#### SUMMARY

The peritrophic matrix of workers of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) was studied from stained histological sections. Termites were decapitated, fixed in a mixture of paraformaldehyde-glutaraldehyde, embedded in epoxy, sectioned at 1 µm thickness, and stained. Our observations showed the presence of structures in the anterior part of the mesenteron resembling those described for a type II peritrophic matrix.

## REFERENCES CITED

- HERBURN, H. R. 1985. The integument, pp. 139-183 *In* M. S. Blum [ed.], *Fundamentals of Insect Physiology*. John Wiley and Sons, New York.
- HUMPHREY, C. D., AND F. E. PITTMAN. 1974. A simple methylene blue-azure II-basic fuchsin stain for epoxy-embedded tissue sections. *Stain Technol.* 49: 9-14.
- KALT, M. R., AND B. TANDLER. 1971. A study of fixation of early amphibian embryos for electron microscopy. *J. Ultrastruct. Res.* 36: 633.
- LEHANE, M. J. 1997. Peritrophic matrix structure and function. *Annu. Rev. Entomol.* 42: 525-550.
- MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* 39: 111-114.
- RICHARDS, A. G., AND P. A. RICHARDS. 1977. The peritrophic membrane of insects. *Annu. Rev. Entomol.* 22: 219-240.
- TELLAM, R. L. 1996. The peritrophic matrix, pp. 86-114 *In* M. J. Lehane and P. F. Billingsley [eds.], *Biology of the Insect Midgut*. Chapman & Hall, London, UK.
- WATERHOUSE, D. F. 1954. The rate of production of the peritrophic membrane in some insects. *Aust. J. Biol. Sci.* 7: 59-72.
- WIGGLESWORTH, V. B. 1972. *The Principles of Insect Physiology*, 7th Edition. Chapman & Hall, London.