Research Article

An Ultrastructural and Fluorescent Study of the Teratocytes of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) from the Hemocoel of Host Alfalfa Weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)

Kent S. Shelby,[1] Javad Habibi,[2] and Benjamin Puttler[3]

[1] USDA Agricultural Research Service, Biological Control of Insects Research Laboratory, 1503 S. Providence Road, Columbia, MO 65203, USA

[2] Division of Endocrinology, Department of Internal Medicine, Health Sciences Center, University of Missouri, Columbia, MO 65203, USA

[3] Division of Plant Sciences (Entomology), University of Missouri, Columbia, MO 65203, USA

Correspondence should be addressed to Kent S. Shelby; shelbyk@missouri.edu

The braconid wasp *Microctonus aethiopoides* Loan is an idiobiont endoparasitoid of alfalfa weevil adults *Hypera postica* (Gyllenhal). After oviposition and subsequent egg maturation, large trophic cells called teratocytes dissociate from the serosa and are released into the host hemocoel. These teratocytes are present in large numbers and are visible to the naked eye. It is thought that they accumulate host hemocoelic metabolites for later consumption by the parasitoid larvae. We have undertaken a microscopic study of these gargantuan and complex cells at approximately seven months after parasitization. Parasitized adult weevils were dissected into medium and teratocytes were fixed, embedded, and sectioned at 1 μm. Teratocytes were stained with various specific fluorescent dyes for plasma membrane, Golgi, nuclei, lysosomes, mitochondria, and endoplasmic reticulum (ER). The surface of each cell is covered with a dense microvillar layer. Analysis of fluorescent images showed that these cells do not have condensed nuclei. ER was abundant around the nuclear envelope. Lysosomes were positioned around the periphery of the nucleus and the Golgi apparatus was significantly enlarged, being located around the nuclear envelope.

1. Introduction

Microctonus aethiopoides Loan (Hymenoptera: Braconidae) was found to be an effective biological control agent for the adult alfalfa weevil *Hypera postica* (Gyllenhal) [1, 2]. *M. aethiopoides* is an idiobiont parasitoid which prevents further host development after parasitization. Following oviposition into the hemocoel of the adult alfalfa weevil host the *M. aethiopoides* egg hatches, releasing both a first instar parasitoid larva and numerous free floating extraembryonic serosal cells which subsequently develop and differentiate into teratocytes [3]. Teratocytes undergo significant hypertrophic growth within the hemocoel of the host. The presence of these large, white opaque cells has been implicated in the determination of host range of *M. aethiopoides* [4–6].

Teratocytes or teratocyte-like cells have been documented in developmental studies of four hymenopteran families: Braconidae, Ichneumonidae, and Platygastridae [7, 8] and have been reported in a single species of chalcids [9]. Teratocytes are thought to function primarily as trophic cells which assimilate host metabolites for later ingestion by the developing parasitoid larvae [7]. However, teratocytes secrete a number of proteins *in vivo* and *in vitro* which may be responsible for the observed alterations of the host endocrine system [10–15] and suppression of the host's immune response against the parasitoid egg and larvae [16–18]. Despite their importance to successful parasitization by braconid parasitoids and their unique functions in suppression of the host immune system and alterations of host endocrinology, the ultrastructure of

teratocytes has been rarely studied [9, 19–21]. The present study was undertaken to elucidate and to document the ultrastructure of these extremely large and important cells.

2. Materials and Methods

2.1. Insects and Teratocyte Collection. Nondiapausing alfalfa weevils, *Hypera postica* parasitized by *Microctonus aethiopoides,* used in this study were obtained by sweeping adults from an alfalfa field near Boonville, Missouri (Cooper Co.) on April 29, 2002. These weevils were collected in late spring and early summer. The weevils were returned to the laboratory and maintained at 5°C on a bouquet of alfalfa in a 0.275 L ice cream carton with a Petri dish as a lid until May 1, at which time some were initially dissected under a dissecting microscope in a Stender dish cover in distilled water to assess the extent of parasitization. Adult weevils were grasped with forceps and a needle inserted in anus and gently teased to expose the contents of the abdomen. A careful search was made to locate the parasite larvae to confirm parasitization. If the host was parasitized teratocytes floated into the dissection medium. The identity of the parasite larvae as *M. aethiopoides* was confirmed by emergence of adult parasites reared from adult weevils collected from the same site. Dissections from weevils maintained in the same manner but for fixing, staining and sectioning of teratocytes Excel 401 tissue culture medium (Gibco Invitrogen; Carlsbad, CA) was used as dissection medium. Dissections were completed 2 days later (May 3), and again on May 6, May 8, and May 10. Teratocytes were collected from the medium for processing as detailed below.

2.2. Light Microscopy. Teratocytes were immediately fixed in 3% paraformaldehyde in HEPES wash buffer for 10 min in a gentle vacuum and 2 hrs at room temperature for fluorescent studies. For light microscopy and TEM studies, the teratocytes were placed in fixative solution (2.5% glutaraldehyde/2% paraformaldehyde, 70 mM HEPES, and pH 7.4) for 3 hrs. Then, the teratocytes were dehydrated in an ethanol series, infiltrated, and embedded in methacrylate for fluorescent studies and in Epon/Spurr's resin for light microscopy. Semithin sections of the teratocytes were stained with 0.5% acid fuchsin/0.5% toluidine blue in ultrapure water. The slides were air-dried at room temperature, mounted with Permount, and viewed with an Olympus (Melville, NY) or Nikon (Melville, NY) microscope. Images were captured using ImagePro (Media Cybernetic, Silver Spring, MD) and Spot (Diagnostic Instruments, Inc.) software and further edited using Adobe Photoshop 6.0 software.

2.3. Fluorescence Microscopy. Using a modification of the technique of Baskin et al. [22–24] teratocytes were encased in a sandwich of Formvar supported on a copper wire loop. A copper wire loop (36 ga) was made and flattened between two flat pieces of steel. Small rectangular films of 0.25% of Formvar in ethylene chloride were floated on water and the loop plunged into the middle of rectangle so that a film of Formvar surrounded the wire. A number of loops were made

in advance. The loops covered with a film of Formvar were placed on a drop of water on a piece of clean glass separately. The fixed teratocytes were gently placed on the center of the Formvar surface. This assembly was coated with another layer of Formvar, sandwiching teratocytes between two layers of Formvar.

Teratocytes sandwiched between layers of Formvar were dehydrated in an ethanol series at −20°C for 30 minutes for each step and then infiltrated with methacrylate (80% butyl methacrylate, 20% methyl methacrylate, and 0.5% benzoin ethyl ether; Aldrich) and 10 mM DTT. Teratocytes were embedded in fresh methacrylate mix in BEEM capsules and the plastic was polymerized under UV light in a cold room (4°C) overnight. Blocks were sectioned at 1 μm using a Reichert Cut S ultramicrotome (Leica, Wien, Austria). Serial sections of teratocytes were placed on drops of 5% ammonium hydroxide on silane-coated slides. The sections were annealed onto the slide by gentle heating on a slide warmer and the slides were stored at 4°C for further staining. Entire 1 μm sections of eight teratocytes were deplasticized with acetone for 10 minutes, washed with HEPES buffer for 30 minutes, and cleared with 0.1% Tween 20 in PBS for 15 minutes. Nonspecific binding was minimized by incubation in blocking buffer (5% BSA, 1% nonfat dry milk, 1% gelatin, and 0.01% sodium azide).

Organelle specific stains purchased from Molecular Probes (Eugene, OR) were incubated with semithin sections according to manufacturer's recommendations (http://www.probes.com). Initial range finding experiments with fluorescent dyes, over several orders of magnitude, allowed us to derive an optimal concentration for each fluorescent dye. The sections of two cells were stained with 500 nM of DiI (a plasma membrane specific stain) in HEPES wash buffer for 90 min. Then the sections were washed with the same buffer for 30 min and were counterstained with 500 nM TO-Pro3 (a DNA specific stain) for 90 min. Sections of two other teratocytes were stained with 2 μM of NBDC$_6$ (a Golgi apparatus specific stain) for 90 min and counterstained with 100 nM of Lysotracker (a lysozyme specific stain) for 90 min. The sections of two more teratocytes were stained with 250 nM of Mitotracker (a mitochondrial specific stain) for 90 min and counterstained with 500 nM of DioC$_6$ (an endoplasmic reticulum specific stain) for 90 min. Slides were mounted with Moviol and were imaged by confocal microscopy.

2.4. Transmission Electron Microscopy (TEM). Teratocytes encased between two Formvar layers were fixed in 2% glutaraldehyde/2% paraformaldehyde in 0.1 M cacodylate buffer. After fixation, the samples were rinsed three times in buffer and then postfixed in 1% OsO$_4$ in the same buffer. Samples were then rinsed three times for 20 min each with ultrapure water. Tertiary fixation was done in 1% aqueous uranyl acetate, followed by three rinses of 20 min each in ultrapure water. The samples were dehydrated in an ethanol series and infiltrated with Epon/Spurr's resin, and the resin was then polymerized at 55°C for two days after which the blocks were stored in a desiccator until sectioning. Ultrathin sections were
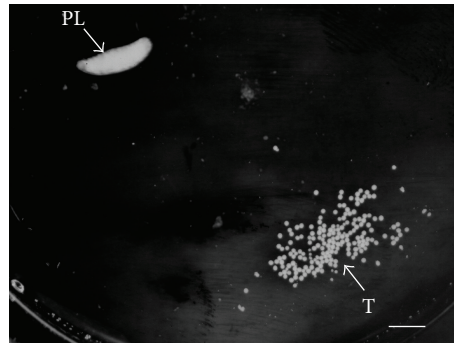
FIGURE 1: Bright-field image of *M. aethiopoides* larva and teratocytes collected from a single host adult *H. postica* hemocoel at approximately seven months after parasitization showing hypertrophied *M. aethiopoides* teratocytes. PL: parasitoid larvae, T: teratocytes, scale bar = 1 mm.
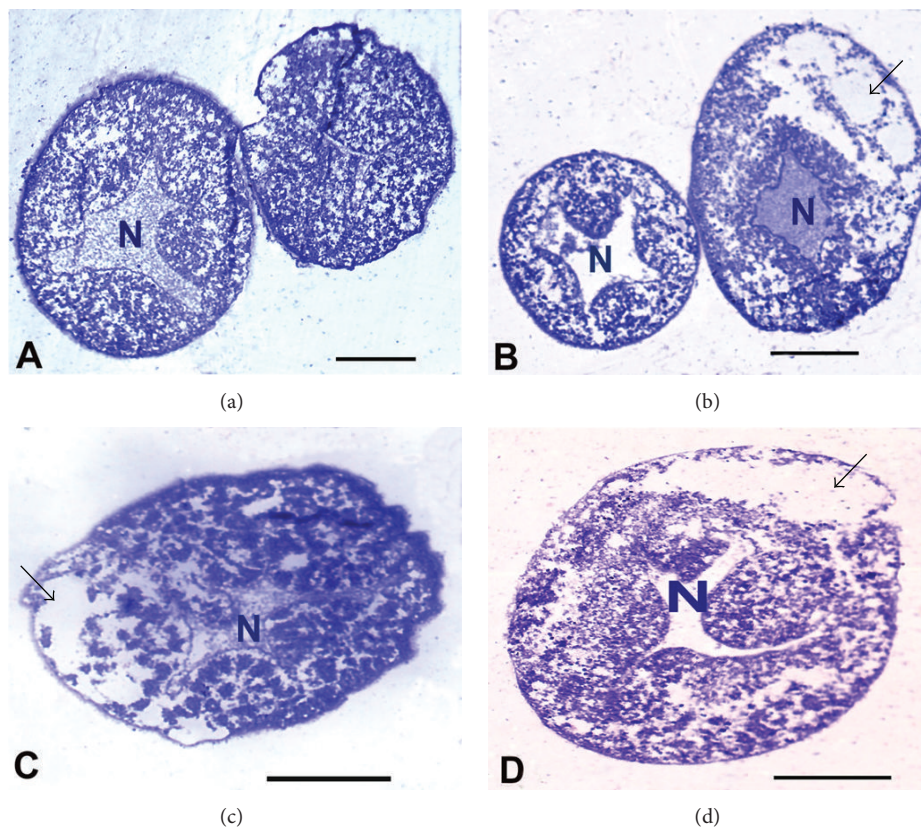

(a)

(b)

(c)

(d)

FIGURE 2: Bright-field images of different 1 μm (semithin) sections from different areas of teratocytes of *M. aethiopoides* were stained with 0.5% toluidine blue and counterstained with 0.5% acid fuchsin showing amorphous nuclei. N: nuclei. Large unstained areas (arrow). Scale bar = 50 μm.

cut with a Reichert Ultracut S ultramicrotome (Leica, Wien, Austria). Standard TEM procedures were applied to ultra-thin sections of the teratocytes on copper grids.

3. Results

Teratocytes collected from the hemolymph of field-collected adult alfalfa weevils at approximately seven months after parasitization were examined by bright-field microscopy. When parasitized weevils were dissected under saline or tissue culture medium numerous teratocytes were easily visualized. No attempt was made to definitively determine the mean number of teratocytes per host. Teratocytes were extremely large hypertrophied, opaque buoyant spherical cells, visible to the naked eye (Figure 1). Since only a single developmental stage was available for study, earlier stages of teratocyte development, release, and growth were not observed and are not presented in this study. At the time of collection, all teratocytes appeared to have attained maximal diameter. A cytological investigation of teratocyte internal structures
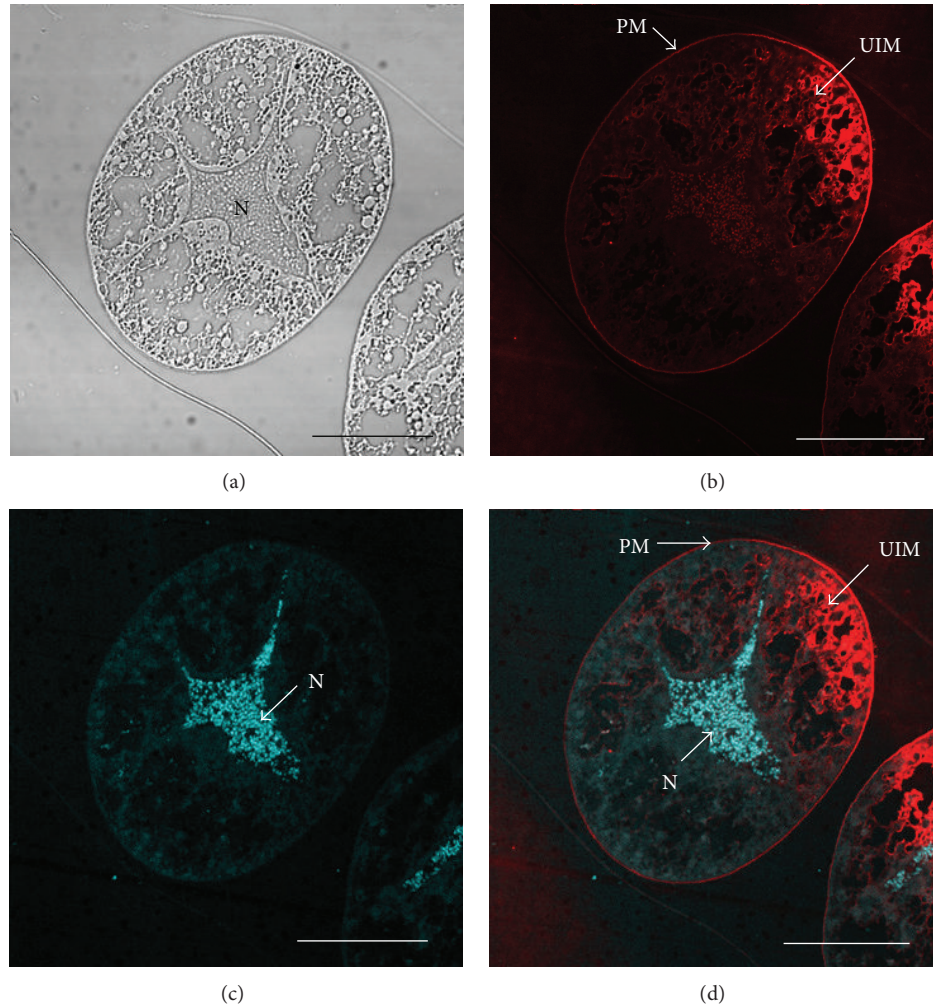
(a)

(b)

(c)

(d)

FIGURE 3: Semithin sections of teratocytes were dual-stained with Dil for plasma membrane and with TO-Pro3 for nucleus. (a) Transmitted light micrograph of teratocyte showing internal structure of the cell. (b) Same cell section stained with Dil showing strong staining of plasma membrane and an unidentified internal membranous network. (c) Same section stained with TO-Pro3 showing intense staining of chromosomal DNA within the large amorphous nucleus. (d) Overlaid images from (a) to (c) showing red plasma membrane and blue amorphous nucleus. N: nucleus, PM: plasma membrane, UIM: unidentified internal plasma membrane network. Scale bars = 20 μm.

was undertaken based on light and fluorescent microscopic examination of a large number of semithin sections.

Semithin sections were stained with toluidine blue/acid fuchsin to obtain resolution of intracellular structures (Figures 2(a)–2(d)). A large stellate nucleus occupied the center of each teratocyte, with ramifications extending throughout the cytoplasm to the plasma membrane. The remainder of the interior was occupied by densely staining granules. In all teratocytes examined, a curious unstained internal membranous network at one end of each teratocyte and occupying a substantial volume was present in close proximity to the plasma membrane (Figures 2(b)–2(d)).

Further examination of teratocyte intracellular structure was undertaken by fluorescence microscopy of semithin sections using dyes chosen for their ability to stain specific organelles (Figures 3(a)–3(d)). Sections were dual-stained with the plasma membrane specific stain, Dil, and with the

nuclear stain TO-Pro3. A wide plasma membrane surrounding each cell was observed, and a polar structure comprised of an internal membranous network heavily stained by Dil was present at the periphery of each teratocyte examined (Figure 3(b)). The same sections stained with TO-Pro3 exhibited intense staining of chromosomal DNA within the large stellate nucleus (Figure 3(c)). The nucleus did not appear to overlap with the unidentified internal membranous network (Figure 3(d)).

Semithin sections were dual-stained with the Golgi specific stain $NBDC_6$ and with the lysosomal specific stain Lysotracker (Figures 4(a)–4(d)). Within each teratocyte multiple strongly staining Golgi structures were observed distributed along the periphery of the nucleus (Figure 4(b)). Numerous lysosomal-staining structures were distributed throughout the volume of the teratocyte (Figure 4(c)). When the images were digitally overlaid, the perinuclear position of
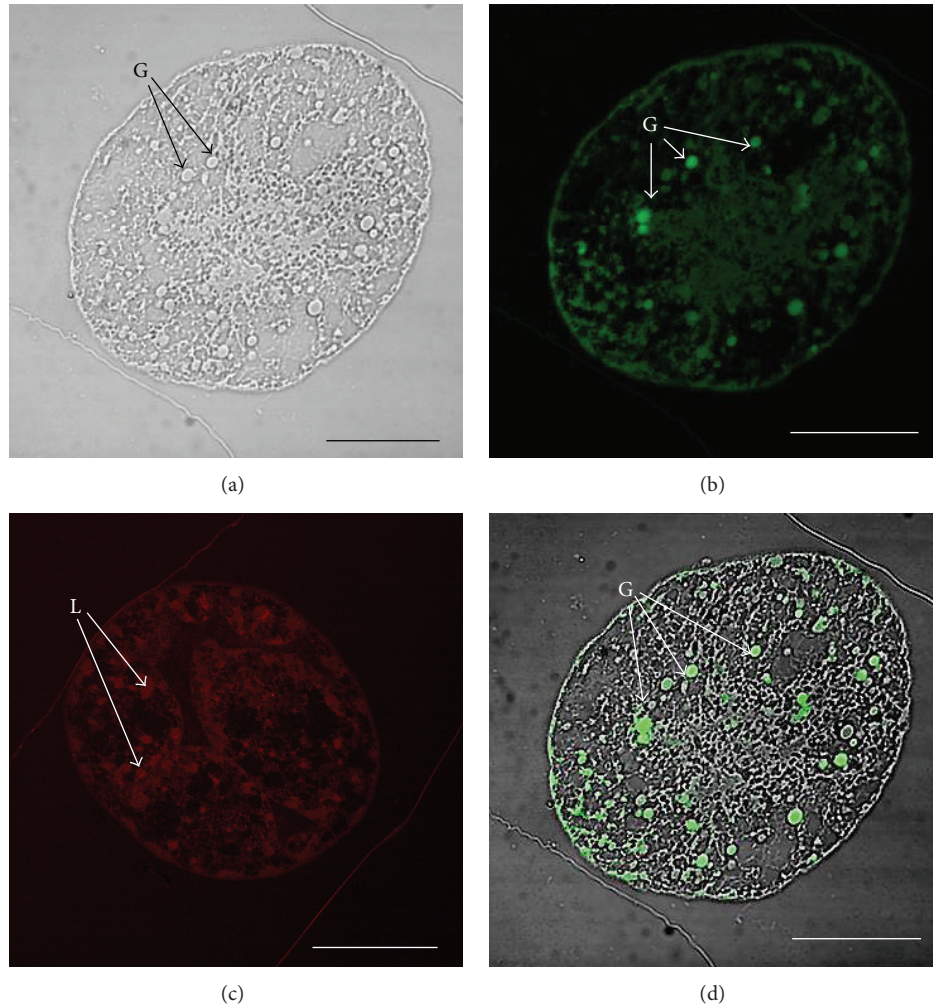
(a)

(b)

(c)

(d)

FIGURE 4: Semithin sections of teratocytes were dual-stained with NBDC$_6$ for Golgi apparatus and Lysotracker for lysosomes. (a) Transmitted light micrograph of teratocyte showing internal structure of the cell. (b) Same cell section stained with NBDC$_6$ showing strong staining of the Golgi distributed mostly around the periphery of the nucleus. (c) Same section stained with Lysotracker showing distribution of lysosomes (red) within the teratocyte. (d) Overlaid images of (a)–(c) showing Golgi around nucleus. G: Golgi apparatus, L: lysosomes. Scale bars = 20 μm.

the multiple Golgi was observed (Figure 4(d)). Sections dual-stained with the mitochondrial specific dye Mitotracker and the ER specific dye DiOC$_6$ revealed the close proximity of protein synthesis with energy production (Figure 5(a)). Dark unstained regions were seen within lobes of the teratocyte bordered by intense ER staining (Figure 5(b)). Mitochondria are present throughout the same volume of teratocyte occupied by intensely staining ER (Figure 5(c)).

Ultrastructural studies were undertaken to obtain a more detailed view of the highly complex teratocyte cytoplasm. The plasma membrane appeared to be composed of a dense lawn of microvilli. Substantial amounts of rough ER cisternae and mitochondria were closely associated with plasma membrane in close proximity to the microvilli (Figures 6(a) and 6(d)). The membranous network located at one end of the teratocytes was composed of highly complex and folded membranes with a tubular appearance that could perhaps be invaginations of the plasma membrane involved in uptake of

materials from the host hemocoel (Figure 6(b)). The function of this structure has yet to be determined. The stellate nucleus was surrounded by rough ER, Golgi (Figure 6(c)), and lipid droplets (Figure 6(d)). Higher magnification revealed mitochondria, lipid droplets, and darkly staining granules throughout the cytoplasm between the nucleus and the plasma membrane (Figure 6(e)). The internal structure of the same area of the teratocyte showed large numbers of starch and pigment granules and of lipid droplets (Figure 6(f)).

4. Discussion

Teratocytes collected from the hemolymph of field-collected adult alfalfa weevils at approximately seven months after parasitization were examined by bright-field microscopy. When parasitized weevils were dissected under saline or tissue culture medium numerous teratocytes were easily visualized. No attempt was made to determine the mean number of
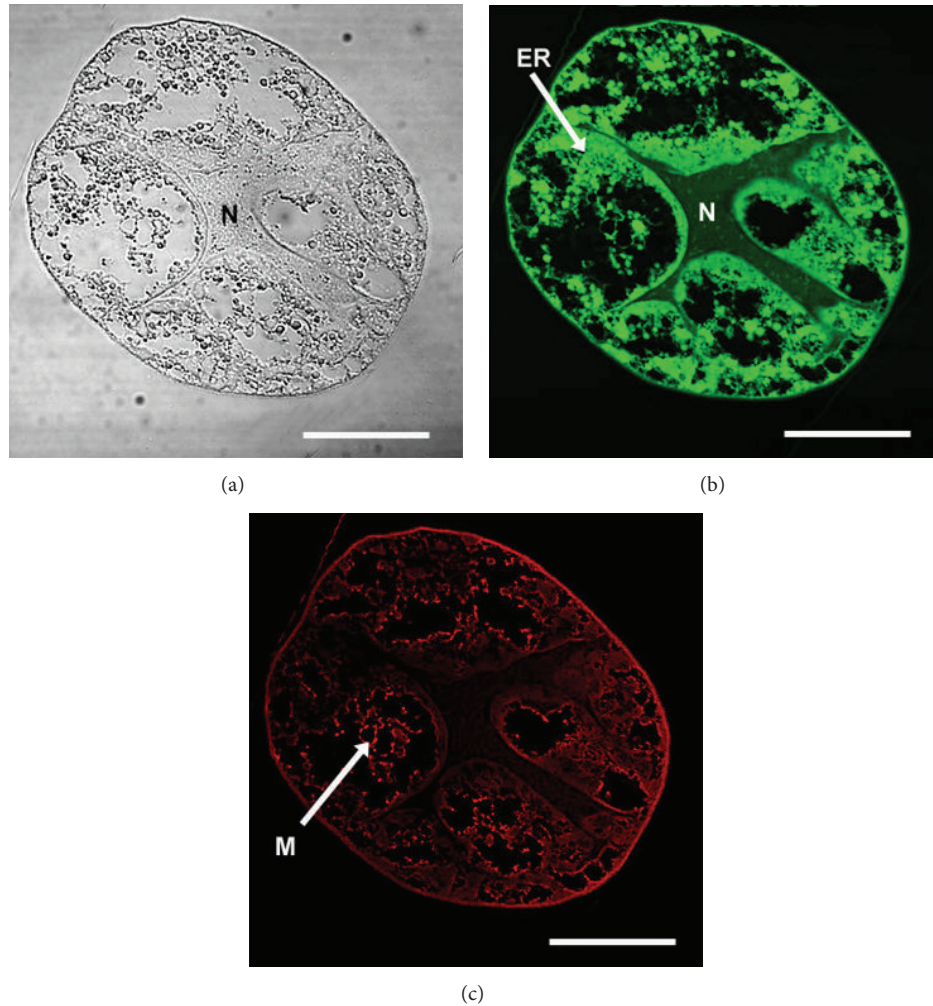
(a)

(b)

(c)

FIGURE 5: Semithin sections of teratocytes were dual-stained with Mitotracker for mitochondria and with $DiOC_6$ for endoplasmic reticulum. (a) Transmitted light micrograph of teratocyte showing internal structure of the cell. (b) Same cell section stained with $DiOC_6$ showing green staining of ER surrounding the dark unstained bodies. (c) Same section stained with Mitotracker (red) showing distribution of mitochondria within the teratocyte. ER: endoplasmic reticulum, M: mitochondria, N: nucleus. Scale bars = 20 μm.

teratocytes per host. In comparison to the developing *M. aethiopoides* larva from the same host, the teratocytes were extremely large hypertrophied, opaque buoyant spherical cells, visible to the naked eye. Since only a single age was available for study, the initial stages of teratocyte release and growth were not observed. At the time of collection, all teratocytes appeared to have attained maximal diameter. No earlier teratocyte developmental stages were examined. An initial cytological investigation of the internal structures was undertaken. Semithin sections of the same material were stained with toluidine blue/acid fuchsin to obtain resolution of intracellular structures. In a manner similar to the teratocytes of *Toxoneuron* (=*Cardiochiles*) *nigriceps* (Viereck) [20], a large stellate nucleus occupied the center of each teratocyte, with ramifications extending throughout the cytoplasm to the plasma membrane. The remainder of the interior was occupied by densely staining granules. An unstained internal membranous network occupying a substantial volume was present in close proximity to the plasma membrane.

Ultrastructural studies were undertaken to obtain a more detailed view of the highly complex teratocyte cytoplasm. The plasma membrane appeared to be composed of a dense lawn of microvilli, similar in appearance to teratocytes of another braconid wasp, *Microplitis croceipes* (Cresson) [19]. Substantial amounts of rough ER cisternae and mitochondria were closely associated with plasma membrane. Close association of the microvilli, rough ER, and mitochondria at the plasma membrane indicates that both uptake of nutrients from and the synthesis/secretion of proteins into the host hemocoel are the primary activities occurring at this stage of teratocyte development [20, 25]. The membranous network located at one end of the teratocytes was composed of highly complex and folded membranes with a tubular appearance that could perhaps be invaginations of the plasma membrane involved in uptake of materials from the host hemocoel. The function of this structure has yet to be determined. The stellate nucleus was surrounded by rough ER, Golgi, and lipid droplets. Higher magnification revealed mitochondria,
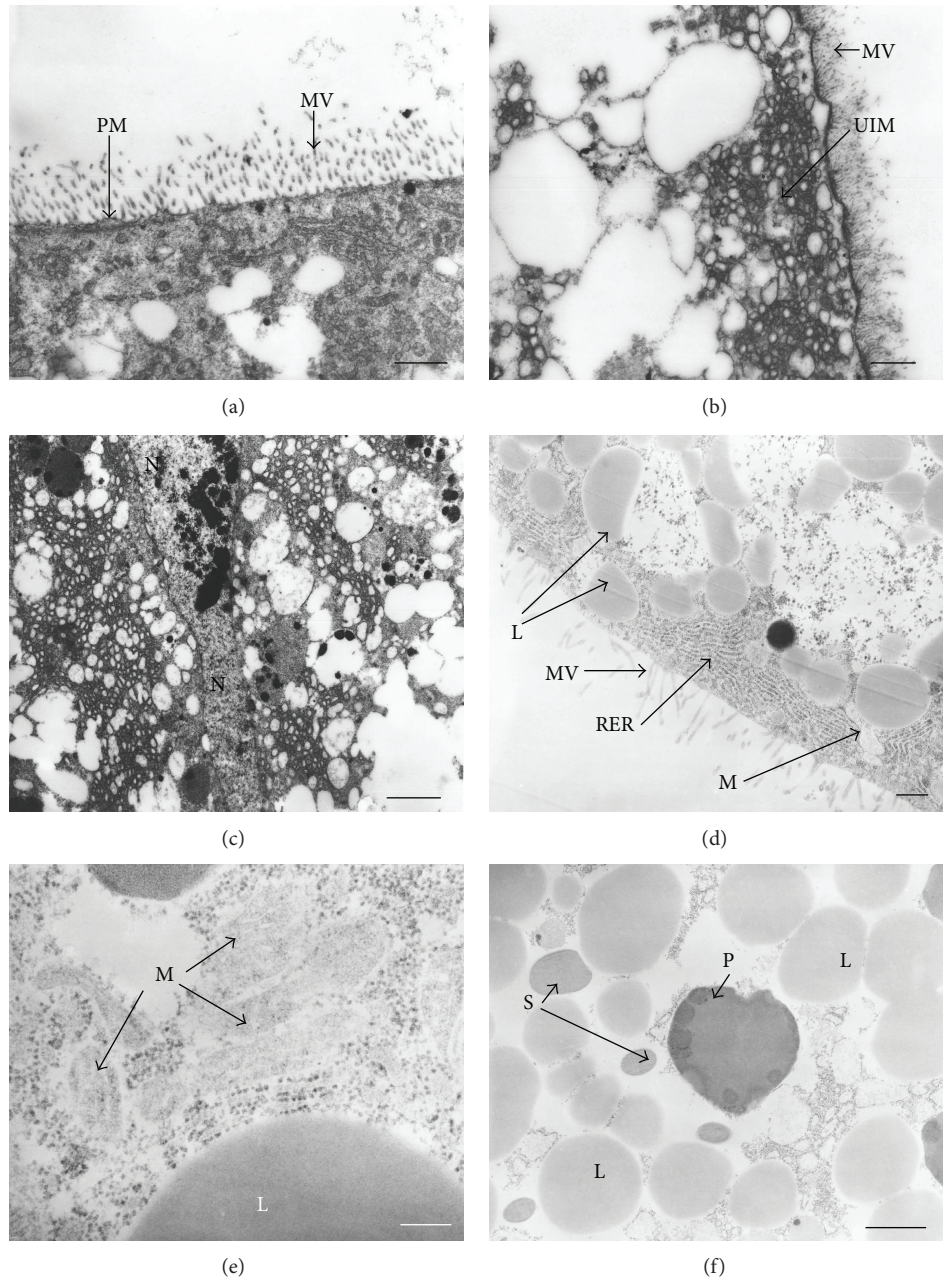
(a)

(b)

(c)

(d)

(e)

(f)

FIGURE 6: Ultrathin sections of teratocytes were stained with uranyl acetate and Lead citrate. (a) Dense microvilli, rough endoplasmic reticulum, and mitochondria associated with plasma membrane. Scale bar = 500 nm. (b) TEM image of unidentified membranous network located at opposite sides of the teratocyte. Scale bar = 1 μm. (c) TEM image of one arm of amorphous nucleus showing intense staining of heterochromatin, numerous lipid droplets, and pigment. Scale bar = 1 μ. (d) Same cell showing microvilli, rough ER, and associated mitochondria. Scale bar = 500 nm. (e) Same cell with higher magnification showing mitochondria and lipid droplets. Scale bar = 100 nm. (f) Electron micrograph of the internal structure of the same area of the teratocyte showing starch and pigment granules and lipid droplets. Scale bar = 500 nm. PM: plasma membrane; MV: microvilli; UIM: unidentified membranous network; N: nucleus; L: lipid droplets; RER: rough endoplasmic reticulum; M: mitochondria; S: starch; P: pigment granules. Scale bar = 20 μm.

lipid droplets, and darkly staining granules throughout the cytoplasm between the nucleus and the plasma membrane. Within this area of the teratocyte adjacent to mitochondria were putative energy storage depots such as lipid droplets and starch granules.

In conclusion, the extremely large *M. aethiopoides* teratocytes possess an internal structure well suited to their putative trophic and secretory function. The plasma membrane is studded with a dense array of microvilli which would facilitate efficient uptake of nutrients from the host hemocoel. Lysosomes, numerous lipid droplets, and granules of protein and starch crowd the cytoplasm. An amorphous membranous network staining heavily with an ER specific dye is present at one end of the cell. Arms of the large stellate

nucleus ramify throughout the volume of the cell abutting against rough ER and mitochondria. Finally, these teratocytes contain large amounts of Golgi, rough ER, and mitochondria that would be required for the synthesis and export of proteins into the host hemocoel that may be responsible for suppression of the host immune system.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.
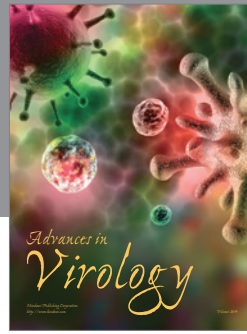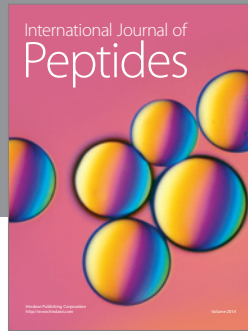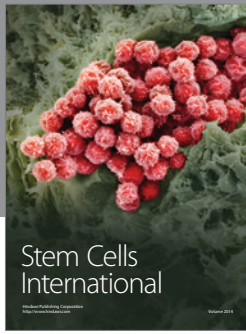
Acknowledgments

References

[1] L. W. Coles and B. Puttler, "Status of the alfalfa weevil biological control program in the Eastern United States," *Journal of Economic Entomology*, vol. 56, pp. 609–611, 1963.

[2] W. H. Day, L. W. Coles, J. A. Stewart, and R. W. Fuester, "Distribution of *Microtonus aethiops* and *M. colesi*, parasites of the alfalfa weevil, in the Eastern United States," *Journal of Economic Entomology*, vol. 64, pp. 190–193, 1971.

[3] O. J. Smith, "Biology and behavior of Microctonus vittatae Muesebeck (Braconidae)," in *University of California Publications in Entomology*, E. G. Linsley, S. B. Freeborn, E. A. Steinhaus, and R. L. Usinger, Eds., pp. 315–343, University of California Press, Los Angeles, Calif, USA, 1952.

[4] B. I. P. Barratt and P. D. Johnstone, "Factors affecting parasitism by *Microctonus aethiopoides* (Hymenoptera: Braconidae) and parasitoid development in natural and novel host species," *Bulletin of Entomological Research*, vol. 91, no. 4, pp. 245–253, 2001.

[5] B. I. P. Barratt and M. Sutherland, "Development of teratocytes associated with *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) in natural and novel host species," *Journal of Insect Physiology*, vol. 47, no. 3, pp. 257–262, 2001.

[6] B. I. P. Barratt, A. A. Evans, D. B. Stoltz, S. B. Vinson, and R. Easingwood, "Virus-like particles in the ovaries of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a parasitoid of adult weevils (Coleoptera: Curculionidae)," *Journal of Invertebrate Pathology*, vol. 73, no. 2, pp. 182–188, 1999.

[7] N. E. Beckage and D. B. Gelman, "Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control," *Annual Review of Entomology*, vol. 49, pp. 299–330, 2004.

[8] F. Rouleux-Bonnin, S. Renault, A. Rabouille, G. Periquet, and Y. Bigot, "Free serosal cells originating from the embryo of the wasp *Diadromus pulchellus* in the pupal body of parasitized leek-moth, *Acrolepiosis assectella*. Are these cells teratocyte-like?" *Journal of Insect Physiology*, vol. 45, no. 5, pp. 479–484, 1999.

[9] P. A. Pedata, A. P. Garonna, A. Zabatta, P. Zeppa, R. Romani, and N. Isidore, "Development and morphology of teratocytes in *Encarsia berlesei* and *Encarsia citrina*: first record for Chalcidoidea," *Journal of Insect Physiology*, vol. 49, no. 11, pp. 1063–1071, 2003.

[10] R. L. Rana, D. L. Dahlman, and B. A. Webb, "Expression and characterization of a novel teratocyte protein of the braconid, *Microplitis croceipes* (cresson)," *Insect Biochemistry and Molecular Biology*, vol. 32, no. 11, pp. 1507–1516, 2002.

[11] K. Kadono-Okuda, F. Weyda, and T. Okuda, "*Dinocampus (= Perilitus) coccinellae* teratocyte-specific polypeptide: its accumulative property, localization and characterization," *Journal of Insect Physiology*, vol. 44, no. 11, pp. 1073–1080, 1998.

[12] F. Pennacchio, S. B. Vinson, E. Tremblay, and A. Ostuni, "Alteration of ecdysone metabolism in *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) larvae induced by *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) teratocytes," *Insect Biochemistry and Molecular Biology*, vol. 24, no. 4, pp. 383–394, 1994.

[13] M. Hotta, T. Okuda, and T. Tanaka, "*Cotesia kariyai* teratocytes: growth and development," *Journal of Insect Physiology*, vol. 47, no. 1, pp. 31–41, 2001.

[14] Q. Qin, H. Gong, and T. Ding, "Two collagenases are secreted by teratocytes from *Microplitis mediator* (Hymenoptera: Braconidae) cultured *in vitro*," *Journal of Invertebrate Pathology*, vol. 76, no. 1, pp. 79–80, 2000.

[15] Y. Nakamatsu, S. Fujii, and T. Tanaka, "Larvae of an endoparasitoid, *Cotesia kariyai* (Hymenoptera: Braconidae), feed on the host fat body directly in the second stadium with the help of teratocytes," *Journal of Insect Physiology*, vol. 48, no. 11, pp. 1041–1052, 2002.

[16] D. L. Dahlman, "Teratocytes and host/parasitoid interactions," *Biological Control*, vol. 1, no. 2, pp. 118–126, 1991.

[17] D. L. Dahlman and S. B. Vinson, "Teratocytes. Developmental and biochemical characteristics," in *Parasites and Pathogens of Insects*, vol. I, pp. 145–165, Academic Press, New York, NY, USA, 1993.

[18] T. Tanaka, "Effect of the venom of the endoparasitoid, *Apanteles kariyai* Watanabe, on the cellular defence reaction of the host, *Pseudaletia separata* Walker," *Journal of Insect Physiology*, vol. 33, no. 6, pp. 413–420, 1987.

[19] D. Zhang, D. L. Dahlman, U. E. Järlfors, H. H. Southgate, and S. P. Wiley, "Ultrastructure of *Microplitis croceipes* (Cresson) (Braconidae: Hymenoptera) teratocytes," *International Journal of Insect Morphology and Embryology*, vol. 23, no. 3, pp. 173–187, 1994.

[20] S. B. Vinson and J. R. Scott, "Ultrastructure of teratocytes of Cardiochiles Nigriceps Viereck (Hymenoptera: Braconidae)," *International Journal of Insect Morphology and Embryology*, vol. 3, no. 2, pp. 293–304, 1974.

[21] F. Pennacchio, S. B. Vinson, and E. Tremblay, "Morphology and ultrastructure of the serosal cells (teratocytes) in *Cardiochiles*

nigriceps Viereck (Hymenoptera: Braconidae) embryos," *International Journal of Insect Morphology and Embryology*, vol. 23, no. 2, pp. 93–104, 1994.

[22] T. I. Baskin and J. E. Wilson, "Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules," *Plant Physiology*, vol. 113, no. 2, pp. 493–502, 1997.

[23] T. I. Baskin, J. E. Wilson, A. Cork, and R. E. Williamson, "Morphology and microtubule organization in arabidopsis roots exposed to oryzalin or taxol," *Plant and Cell Physiology*, vol. 35, no. 6, pp. 935–942, 1994.

[24] T. I. Baskin, C. H. Busby, L. C. Fowke, M. Sammut, and F. Gubler, "Improvements in immunostaining samples embedded in methacrylate: localization of microtubules and other antigens throughout developing organs in plants of diverse taxa," *Planta*, vol. 187, no. 3, pp. 405–413, 1992.

[25] S. B. Vinson, "Development and possible functions of teratocytes in the host-parasite association," *Journal of Invertebrate Pathology*, vol. 16, no. 1, pp. 93–101, 1970.

Submit your manuscripts at
http://www.hindawi.com