Immature development of *Eretmocerus mundus* (Hymenoptera: Aphelinidae)

Dan Gerling, Michael B. Blackburn

Department of Zoology, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

Invasive Insect Biocontrol and Behavior Laboratory, USDA, ARS, Beltsville, MD 20705, USA

**Abstract**

The development from egg to pupation is followed for the wasp *Eretmocerus mundus*, parasitizing the whitefly *Bemisia tabaci*. We elucidate and describe structural details, histological developments and changes that the different parasitoid and host tissues have undergone during parasitism. These include the presence and apparent function of very large salivary glands, which probably produce substances that help to regulate the host’s decomposition and parasitoid nutrition. Moreover, the gut of all instars is devoid of both peritrophic membrane and microvilli and, in the early instars, it has squamous rather than columnar epithelial cells. Differing from many other parasitoids, the *E. mundus* larva usually does not come into contact with the host tissues and does not devour the entire host during its development.

The possible reasons for the developmental mechanisms, as well as the functions of the host capsule that envelopes the parasitoid, are discussed.

**1. Introduction**

Few studies of immature parasitoid histology exist. These include an early study of several braconids (Weissenberg, 1909; Gatenby, 1919), aphelinids (Gerling et al., 1990, 1991; Hu et al., 2003), the trichogrammatid *Trichogramma australicum* Girault (Jarjees et al., 1998) and the eulophid *Euplectrus separatae* (Nakamatsu and Tanaka, 2003). All of these deal with larval morphology while studying parasitoid development and host relations. From these, it is evident that the internal structures of at least some chalcidoid immatures differ greatly in comparison to the known data on the midgut structure in insects (e.g. Chapman, 1998). These differences probably arise from the parasitic way of life: for example, all whitefly parasitoids depicted in three of the aforementioned publications have a modified midgut that may reflect their exploitation of liquid food.

The genus *Eretmocerus* comprises parasitoids of whiteflies whose developmental biology has been outlined by Clausen and Berry (1932) and Gerling et al. (1990, 1991). The solitary females oviposit on the leaf under a whitefly nymph and develop within it from first instar till emergence. In contrast to all other known parasitoid genera, the whitefly host forms a viable cellular capsule of epidermal origin, whose formation is induced by the first instar before and while penetrating its host (Gerling et al., 1990). The larva then develops within this capsule which later disintegrates before the parasitoid reaches its pupal stage.

This study complements the studies of Gerling et al. (1990, 1991) on *Eretmocerus mundus* (Mercet) in which the penetrations into the hosts, the formation of the capsule and its origin have been described. Hereunder we describe structural details of the immature development of the parasitoid from the egg stage till pupal formation, concentrating on its unique histological features.

**2. Materials and methods**

**2.1. Insect culture**

The host whitefly (*Bemisia tabaci* Gennadius) and the parasitoid were reared on cotton plants in greenhouses and incubators at Tel Aviv University, Israel and at the USDA ARS laboratory in Beltsville MD, USA. Young parasitoid stages were studied by cutting out leaf pieces containing 4th-instar whitefly hosts, 2-5 days following parasitization. Older parasitoid immatures were studied by removing the parasitized whitefly with a fine pin 5-12 days after parasitization. The parasitized whiteflies were fixed and then sectioned as detailed in Gerling et al. (1990, 1991). Altogether, 20-30 parasitoid immatures of each stage were examined.
2.2. Histology

Slides were examined under a Nikon Eclipse 600 compound microscope, and photomicrographs were taken using a Nikon DMX 1200 CCD camera. Measurements were taken on images using the scale provided by the camera’s software Nikon ACT-1 (v.2.12).

3. Results and discussion

3.1. Feeding and the alimentary canal

All three larval instars feed on a liquid diet. The first and second instar’s mandibles are needle shaped, being adapted for piercing. Those of the third instar are sickle-shaped and might assist grasping tissue and/or cells. The mandibular shape corresponds with the larval location; up to the third instar they are totally immersed in a liquid matrix surrounded by capsule cells (see below). Only in the third instar, after the capsule’s decomposition, do they have access to solids.

Feeding, as evidenced by the presence of stained material in the midgut, occurs from hatching onwards. The cells forming the midgut epithelium are never columnar and do not possess a brush border or any visible microvilli. Rather, they always form a squamous or cuboidal epithelium in which as few as 2-3 cells may appear to span the whole midgut circumference.

Like other chalcidoids, the digestive system of the *E. mundus* larva shows no connection between the mid and hind guts until the late third instar.

3.2. First instar

The eggs hatch on the leaf surface under the whitefly nymphs about 3 days after oviposition, at 25 °C. The chorion is equipped with one or more 5–6 μm long stout protruding spines (Fig. 1, s), with the hatched larva lodging its posterior in the chorion remnants. The pointed mandibles (Fig. 2) are ca. 33 μm long and 1.5 μm thick at their widest part and protrude from the 13 μm oral opening. They are directed at the venter of the whitefly, usually near the insertion points of the whitefly nymph’s mouthparts into the leaf. The pressure that the presence of the parasitoid egg and larva exerts upon the soft venter of the host nymph usually creates a concave region surrounding the larva (Fig. 1). The epidermal cells, normally squamous and 1-3 μm thick, become cuboidal or columnar (Fig. 3) often measuring 16 μm or more in height. The enlarged cells that begin to proliferate opposite the parasitoid’s mouthparts form a globe that partially surrounds the parasitoid larva, leaving an opening of ca. 20 μm. The parasitoid larva can be seen moving through this narrow opening in an amoeboid fashion (Fig. 3A). Once it has completed penetration, the larva becomes globular, surrounded by the enlarged epidermal cells that form a capsule-like structure (Fig. 3B, c). The larval midgut is lined with a few very large, flat cells with prominent nuclei (Fig. 3B, *'). All larvae have large salivary gland cells flanking the gut. These very prominent cells (Fig. 3B, sg) may have round or crescent-shaped nuclei that measure about 15 μm across in the first instar. They remain visible from the pre-penetration stage on throughout larval development. Shortly after the larva enters the host, it molts to the second instar.

3.3. Second instar

The second-instar stage (Fig. 4) lasts ca. 2 days, from the 4th to the 6th post oviposition day, residing completely within the cellular capsule. The capsule measures 112.9 ± 8.26 μm × 113.3 ± 9.05 μm (n = 17; avg. ± SE), the gut measures 14.74 ± 3.47 μm × 17.38 ± 4.1 μm (n = 18; avg. ± SE), and the esophagus is about 40 μm long lined by 2–4 μm cuboid cells. The globular larva, together with its capsule, occupies most of the host’s thickness (e.g. parasitoid = 43 μm vs. host = 49 μm in a cross-section of the whitefly). It has a pair of pointed mandibles that occupy a recessed section of the head that is opposed by extensive cellular proliferation of the capsule (Fig. 4A, md). This differs from Gerling (1966), who claimed that only the mandibles of the first instar were lancet-shaped. In all sections, as well as in the live whitefly when observed in transmitted light, the parasitoid larva appears to be surrounded by an empty space separating it from the capsule cells and the host (Fig. 4). The parasitoid larva changes its position during development: after penetration, its head and mouth parts are directed upward and later they redirect, first sidewards and then backwards towards the host’s abdomen. The prominent salivary cells may appear singly or in groups anterior or lateral to the midgut (Fig. 4A and B, sg). Two anterior cell masses are present lateral to the mouth and one posterior to the gut (Fig. 4B). These probably represent future nerve centers and the hind gut, respectively.

In a frontal section (plane of section parallel to the leaf) the round parasitoid larvae occupies only a fraction of the whitefly nymph which, in the fourth instar, is typically ca. 500 μm wide and over 800 μm long. The tissues and cellular structure of the fat body and blood cells of the host are strongly affected by parasitism, with the cells becoming vacuolated. This process is progressive, with the capsules of the early second-instar larvae still adjacent to some normal-looking tissues (Fig. 5A) whereas older (3rd instar) host-parasitoid associations are surrounded exclusively by disintegrating cells (Fig. 5B). The nervous tissue, the gut, the mycetomes (=bacteriome), and the gonads appear unaffected by the parasitoid’s presence.

3.4. Third instar

The third-instar larva develops from the sixth to the ninth post-oviposition days. During development, the larva changes its position so that its head and mouthparts occupy the emptying head region of the whitefly host. Pupation later occurs in this position, with the parasitoid mouthparts facing the dorsal anterior surface of the host.
The third-instar larva measures $161.37 \pm 54.84 \, \mu m \times 146.63 \pm 48.72 \, \mu m \ (n = 5; \text{avg. } \pm \text{SE}).$ Like the second instar, it appears within a narrow non-cellular matrix, which now measures $30.3 \pm 10.58 \, \mu m$ to $4.1 \pm 1.83 \, \mu m$ in thickness at the widest vs. narrowest points ($n = 5; \text{avg. } \pm \text{SE}).$ However, the capsule that typified the second instar now disintegrates, with only occasional cell groups of the original capsule remaining (Fig. 5B). This facilitates direct contact between the remaining host tissues and the developing *E. mundus* larva.

The gut lumen measures ca. $100 \times 70 \, \mu m$ and includes some major developmental and structural differences as compared to the second instar. The epithelial cells are more numerous, are often round, and many are heavily vacuolated (Fig. 5C). The foregut section appears as a straight tube about $55 \, \mu m$ long, lined with cuboidal cells. No solid material is visible in the gut lumen until pupation. Thereafter, particles start appearing, culminating in globules containing nitrogenous excretion that remain till adult emergence.

Organogenesis towards the mature insect is evident in the third instar. The salivary cells undergo changes, including union with the malphigian tubules to form the ileolabial glands as described by Zinna (1961, 1962) for the aphelinid parasitoids *Coccophagus bivittatus* Compere and *Coccophagoides utilis* (Mercet). These glands, which apparently serve in secreting the transparent, brittle covering that surrounds the pupae of the parasitoids, can be seen along the gut margins (Fig. 5B and C, ilg).

Extensive growth of nerve centers is visible at the anterior part of the body and along the venter (Fig. 5B, br). The parasitoid body is replete with highly vacuolated fat body cells possibly acting as metabolic and storage centers for the developing pupa. Imaginal discs are apparent along the body margins, both lateral and posterior to the gut (Fig. 5B, id). The disintegration of the host continues and all cellular tissue becomes highly vacuolated, except the central nervous system, parts of the gut, the bacteriomes and part of the reproductive system (Fig. 5B, b, go, gu).

### 3.5. Superparasitism

Superparasitism by *E. mundus* in our greenhouse culture is relatively frequent and may reach over 20% of the parasitized whiteflies. Although two larvae are usually seen in a host, as many

---

**Fig. 2.** Mandibles of the first instar larva. Bar = 25 μm.

**Fig. 3.** First instar larvae entering the host. A. Larva showing constriction while penetrating the hole formed in the host. B. Larva within the forming capsule, C = capsule, g = gut of the parasitoid, sg = salivary glands of the parasitoid. All bars = 25 μm.
as four separate second instar larvae have been observed, each within its separate capsule. Since eggs are often laid adjacent to each other, causing penetration of several larvae to be adjacent, the ensuing capsules may be contiguous. Moreover, the cellular components of the capsule may be absent where the larvae abut each other and only a thin membrane then separates between the non-cellular layers of the capsule wall. The number of capsules that may be formed is directly dependent upon the number of parasitoid larvae that penetrate the host; in cases of superparasitism, each penetrating larva has its own capsule.

The presence and function of the cellular capsule in the genus *Eretmocerus* are unique phenomena. As discussed by Gerling et al. (1991), it is of epidermal origin and thus differs from the mesodermally-originating capsules known to develop in parasitized hosts. These latter structures that are formed through migration of blood cells to surround the intruding organism, function in the defense of the host through formation of a non-cellular isolating structure around the intruder [e.g. *Drosophila* – Vass and Nappi (1998), scales - Blumberg (1977), and Lepidoptera - Salt (1968)]. Therefore, these are two very different kinds of capsules. Nevertheless it is possible that the *E. mundus* capsule, which presently serves the parasitoid’s development, originated as a host defense mechanism.

### 3.7. Effect on host tissues

As described above, the parasitoid larva is surrounded by a layer of liquid medium around which the host-originating capsule separates it from the host tissues. Vinson (1975) and Thompson (1993) recognized two types of ingestion of the host by the parasitoid: feeding on the host tissue and feeding on the host hemolymph. In *E. mundus* neither definition fits well. The parasitoid, probably through secretions from its salivary glands, affects the host tissues indirectly causing their disintegration. The lack of direct contact between the parasitoid’s mouthparts and the capsule cells suggests that materials are secreted by the parasitoid larva into the surrounding liquid and reach the capsule cells. From there, either directly by passing through the cells to the host tissues or indirectly by activating the capsule cells, cause host tissue disintegration. The digested host disintegration products reach the liquid medium that surrounds the parasitoid larva through the capsule cells and presumably serve as the sole nutrient for the parasitoid larva. Similar need for predigested host tissues as in *E. mundus* larvae was also noted in *Eupelmus orientalis* (Doutry et al. 1997).

Host disintegration is continuous and becomes most visible in the later stages of parasitoid development. Healthy fourth-instar *B. tabaci* have visible eye spots, start developing wing buds and have extensive fat bodies. In parasitized individuals, the fat body...
appears in histological sections as heavily vacuolated cells throughout the parasitoid development. Eyespots are visible side-by-side with first- and second-instar *E. mundus* larvae but not later. Wing bud formation is not seen with the exception of occasional tissue fragments. Frontal sections of the whitefly’s ventral region, taken during parasitoid penetration and second instar development, show some normal epidermal cells (Figs. 4B and 5A). These are no longer visible after the parasitoid molted into the third instar (Fig. 5B.), and possibly even earlier in the late second instar. At these stages, the only apparently unaffected cells are those of the nervous tissues (CNS), the gut, the bacteriomes, and the gonads. As stated previously, second-instar parasitoid larvae occupy the entire thickness of the host from venter to dorsum. However, much of the whitefly width and length remain unoccupied by the parasitoid prior to pupation.

### 3.8. Interpreting the midgut structure

The insect midgut is typically reported to contain cuboidal and columnar cells that are usually equipped with a brush border of microvilli and often lined internally by a peritrophic membrane (Snodgrass, 1935; Terra, 1990; Chapman, 1998). Gut cells have both a secretory and an absorptive function. They secrete enzymes and electrolytes into the gut lumen through both merocrine and holocrine secretion. In the process they age and, upon losing their functional ability, are replaced by new cells that develop from regenerative cell centers located at the cell base (Snodgrass, 1935; Wigglesworth, 1972; Chapman, 1998). The microvilli greatly increase the cell wall surface and are instrumental in the secretion of digestive enzymes and absorption of the nutrients through the gut epithelium (Smith, 1968; Wigglesworth, 1972). *Eretmocerus mundus* larvae, like some other fluid-feeding insects, most notably Hemiptera and adult Thysanoptera (Gullan and Cranston, 2010) and the proctotrupoid *Phaenoserphus viator* Hal (Eastham, 1929), lack microvilli. This is in contrast to larval Thysanoptera (Müller, 1927), honeybees (Snodgrass, 1956), *Vespa vulgaris* (Green, 1931), several braconids (Gatenby, 1919; de Eguileora et al., 2001), and chalcidooids (our observations on *Encarsia formosa* larvae). Moreover in contrast to many of the above-mentioned groups, the gut epithelium of *E. mundus* is squamous in the young larvae and partially cuboidal in older ones, never reaching the typical columnar shape.

The peritrophic membrane is considered an element that protects the gut epithelium from physical damage by the ingested food and by microorganisms (Chapman, 1998) and is functional in compartmentalization of the digestive region (Terra, 1990). Recently, it has been shown that it is also functional in protecting from damage by plant allelochemicals and antioxidants (Barbehenn, 2001; Barbehenn and Stannard, 2004). This membrane is sometimes very delicate and difficult to observe, as in the Coleopteran family Carabidae, in which it was considered absent until its discovery as a partially fluid structure (Terra, 1990). Insect groups that have no peritrophic membrane include the Hemiptera and Thysanoptera (Gullan and Cranston, 2010), a feature commensurate with their feeding on fluids. However, many Hymenoptera, including the honeybees, have a well-developed membrane (Snodgrass, 1956; Wigglesworth, 1972). Likewise, it was found in some parasitoids such as the proctotrupoid *Phaenoserphus viator* Hal. (Eastham, 1929) and the larva of the braconid (Aphidiid) *Aphidius ervi* Haliday (de Eguileora et al., 2001). It is lacking in *E. mundus*, and possibly in some other Chalcidoidea, as can be seen in *E. mundus*, and possibly in some other Chalcidoidea, as can be seen in

---

**Fig. 5.** Second and third instar larvae. A. second instar larva showing position of the parasitoid, its relative dimensions in the host and the yet intact host tissues. B. Third instar parasitoid larva showing the disintegrating capsule cells and host tissues except for nervous tissues, gut, gonads and bacteriomes. In the larva, forming imaginal discs brain and ileo-labial glands. C. An enlarged section of B, showing ileolabial gland elements: salivary glands anteriorly and forming malphigian tubes posteriorly and vacuolated gut epithelial cells. b = bacteriomes, br = brain, dc = disintegrating capsule, g = parasitoid gut, go = host gonads, gu = host gut, id = imaginal disc, ilg = ileolabial gland, L₂, L₃ = second and third instar parasitoid larvae, m = membrane surrounding the parasitoid larva. All bars = 50 μm.
in the pictures published by Hu et al. (2003), Jarjeees et al. (1998) and Nakamatsu and Tanaka (2003). The distribution of the discussed midgut-associated structures throughout the insect orders demonstrates the flexibility of their development in relation to their apparent use. Thus, P. viator has no microvilli but a developed membrane, whereas E. mundus has neither. The capsule in which the larva develops for much of its life and which filters possible deleterious substances and facilitates the decomposition of host organs and molecular structures, may preclude the necessity for developing the microvilli and peritrophic membrane.

3.9. Host utilization

As shown by Gerling et al. (2007), there is no correlation between the size of the host nymph and that of the emerging parasitoid. Our present finding, showing that the host whitely is not completely consumed when the parasitoid pupates helps to explain this observation.

Acknowledgements

We thank Ms. T. Orion for her extensive histological work and Ms. N. Paz for linguistic assistance.

References


Eastham, L.E.S., 1929. The post-embryonic development of Phaedrosolus viator Hal. (Proctotrupidae), a parasite of the larva of Pterostichus niger (Carabidae) with notes on the anatomy of the larva. Parasitology 21, 1–21.


