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

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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 *Euplecturs seperatae* (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.

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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. 3, 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.

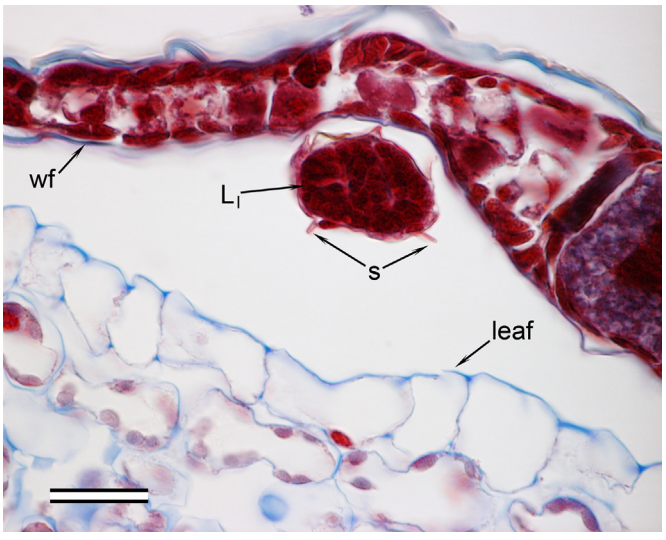

Fig. 1. An *in situ* egg of *E. mundus* on the leaf under the whitefly nymph. L_1 = first instar larva, s = spines on the chorion and wf = whitefly nymph. Bar = 50 μm.

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 sideways 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 (=bacteriomes), 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.