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DEVELOPMENTAL ANALYSIS OF *GANASPIS XANTHOPODA*, A LARVAL PARASITOID OF *DROSOPHILA MELANOGASTER*

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Summary

***Ganaspis xanthopoda* is a solitary larval parasitoid wasp of the fruit fly *Drosophila melanogaster*. The life cycle of *Ganaspis xanthopoda* in the wild-type and developmental mutant *ecdysoneless* strains of *Drosophila melanogaster* is described. The female infects a second-instar host larva. The parasitoid embryo hatches into a mobile first-instar (L1) larva. The L1 parasitoid has fleshy appendages and, while mobile, it remains confined within the wandering larval host. The second-instar larva (L2) is an endoparasite within the host prepupa and lacks appendages. The L2-to-L3 molt is dependent on pupation and marks the transition of the endoparasite into an ectoparasite. The third-instar larva (L3) is a sessile ectoparasite, develops an extensive tracheal system and consumes the host as it progresses through its prepupal and pupal stages. A single adult male or female emerges from the host puparium. The developmental analysis of *Ganaspis xanthopoda* reveals a tight synchrony between host and parasitoid development which is, at least in part, dependent on the ecdysone levels of the host.**

Key words: development, parasitoid wasp, *Ganaspis xanthopoda*, mutant, ecdysone, fruit fly, *Drosophila melanogaster*.

Introduction

Parasitic wasps are possibly one of the most diverse groups of insects, constituting a largely unstudied number of extant species in tropical, subtropical and temperate climates (Quicke, 1997). This high degree of speciation and global presence in a variety of habitats indicates that parasitic wasps occupy many ecological niches. Parasitic wasps (Hymenoptera) are obligate parasitoids and kill their host as they complete their development. Their pre-imaginal stages are found on or within the host. Upon completion of their development, free-living adults disperse to mate and seek new hosts (Godfray, 1994).

Approximately 50 drosophilid parasitoids were reported in the 1970s and 1980s (Basden, 1972; Carton et al., 1986). Some parasitic wasps of *Drosophila* spp. belong to the superfamily Cynipoidea and family Eucoilidae. Eucoilidae is a monophyletic group and, within Cynipoidea, it has the largest number of parasitic species, mostly found in the tropics (Nordlander, 1980, 1982). More than 20 eucoilid genera have been identified (Nordlander, 1980, 1982; Carton et al., 1986). Of these, *Leptopilina* is the most thoroughly studied genus. *Leptopilina heterotoma* and *L. boulardi* are eucoiliform endoparasitoids of *Drosophila* spp. Many aspects of the biology of *Leptopilina* and *Leptopilina–Drosophila* system, including morphological and molecular phylogenetics, host detection behavior, superparasitism and interaction with the host immune response, have been documented (Nordlander, 1980; Rizki and Rizki, 1990; Vet and Bakker, 1985; Carton et al., 1986; van Alphen and Visser, 1990; Carton and Nappi, 1991; Schilthuizen et al., 1998; and references therein). The development of *Leptopilina* has also been described previously (*L. heterotoma*, Jenni, 1951; Nostvik, 1954; *L. boulardi*, Kopelman and Chabora, 1984). However, the biology of most other eucoilid genera is not well understood. In the present paper, we provide a description of the development of the eucoilid *Ganaspis xanthopoda*.

Several *Ganaspis* species are solitary larval parasitoids of *Drosophila* species (Nordlander, 1980, 1982; Vet and Bakker, 1985; Carton et al., 1986). Because of its wide geographical distribution, *G. xanthopoda* is classified as a tramp species (G. Nordlander, personal communication). *G. xanthopoda* parasitizes *D. melanogaster* and *D. sturtevanti* (Carton et al., 1986). Superficially, *Ganaspis* and *Leptopilina* share many morphological characteristics, although the phylogenetic relationship between these genera is regarded as being fairly distant (Schilthuizen et al., 1998). Where comparative information is available, marked differences between species of these genera have been found. For example, *Leptopilina* and *Ganaspis* spp. differ in the manner in which they detect their hosts (Vet and Bakker, 1985). The fact that these genera are quite different from each other, but can still successfully parasitize *D. melanogaster*, provides an interesting context for comparative analysis of these parasitoid wasps as well as their interactions with the same host.

Despite their potential as a powerful tool in *Drosophila* research and their significant ecological importance, our understanding of the basic developmental biology of fruit fly parasitoids is limited. In the present study, we have studied the life cycle stages of *G. xanthopoda* in *D. melanogaster* with special emphasis on embryonic and larval stages. Using wild-type and mutant *D. melanogaster* strains, we find that the overall developmental strategy of *G. xanthopoda* is quite similar to that described for *L. heterotoma* and *L. boulardi*, and that the developmental progression of *G. xanthopoda* is closely tied to that of its host.

Materials and methods

Insects, egg lays and wasp infections

Drosophila melanogaster were reared in vials on cornmeal/yeast 'fly food' at room temperature (22–25 °C). At 21 °C, the life cycle of wild-type *D. melanogaster* ranges from 14 to 20 days, whereas at 29 °C, it ranges from 9 to 12 days. *ecdysoneless*^{1} (*ecd*^{1} *st*/*ecd*^{1} *st*) was raised at 25 °C. *Ganaspis xanthopoda*, *Leptopilina heterotoma* and *L. boulardi* were reared at room temperature on second-instar larvae of *Canton S* or *rosy*^{506} strains of *D. melanogaster*. Variation in host and parasite development was limited by minimizing the host egg lay and the wasp infection periods. In general, 40–60 female and 30–50 male flies were placed at 25 °C for 3–8 h. At 48 h after the beginning of egg lay, wasps were added to the vials for a timed (3–6 h) infection period.

Analysis of life cycle stages

To study the life cycle stages of *G. xanthopoda*, eight 3 h host egg lays were prepared. Wasp infection lasted for 2–3 h and involved 10–12 females and 6–10 males. Wasp development was monitored at 21 °C. To observe early embryonic stages of development, infected host larvae were dissected at 1 h intervals during the first 8 h. Subsequent dissections were at numerous time points; at the following times, at least eight samples were collected and their dimensions measured using a micrometer reticule: 27, 44, 76, 96, 144, 170, 196, 213, 237, 245, 263, 270, 288, 300, 314, 336, 360, 450, 528, 576, 672, 744 and 800 h. Mean dimensions were calculated and plotted. To analyze size variation of a particular stage, a standard deviation analysis was performed. For the 18 embryonic and 63 larval measurements in Fig. 2, the standard deviation/mean value varied from 0.001 to 0.20 for all but four data points; this value for the latter four measurements ranged from 0.2 to 0.25. Dissections were made in glass depression dishes in phosphate-buffered saline (PBS, pH 7.4) under a Zeiss Stemi 2000-C stereomicroscope. Samples were fixed in 4% glutaraldehyde/PBS solution for 5 min and mounted in 20% glycerol/2% glutaraldehyde/PBS. Embryonic stages were stained with the nuclear dye Hoechst 33258 (10 μg ml^{-1}, Molecular Probes) for 5 min. Acridine Orange (5 μg ml^{-1}; Sigma) was applied to unfixed embryos. Samples were washed once with PBS and observed. A Zeiss Axioplan with phase contrast, Nomarski and fluorescence optics was used to examine samples under higher magnification. To describe the morphology of the mandibles, dissected larval preparations were mounted in Hoyer's medium (Wieschaus and Nüsslein-Volhard, 1986) and incubated at 65 °C for 1–2 days. A small weight was placed on the coverslip to flatten the structures. E. Duverge utilized living specimens to make the hand drawings. These sketches were scanned and crafted into Fig. 1.

ecd^{1} *temperature-shift experiment*

A temperature-sensitive allele of *ecdysoneless* (*ecd*^{1}) causes a dramatic reduction in ecdysone release in the host. Lowered ecdysone levels result in developmental arrest during larval or pupal stages (Garen et al., 1977). To evaluate whether host ecdysone levels play a role in parasitoid development, *ecd*^{1} larvae were infected. Because this mutation also causes partial female sterility, more females than those used for wild-type egg lays were used in 6–8 h egg lays at 25 °C. Wasp infection involved 6–12 female and 6–8 male *G. xanthopoda* for 3–5 h. To arrest host development at various times, vials were transferred to 29 °C according to the following schedule: (A) 51–53 h (four vials), (B) 72 h (12 vials) and (C) 96 h (three vials) after removal of wasps. The vials remained at 29 °C for the rest of the experiment and were periodically hydrated to prevent larval desiccation. Host larvae at 29 °C were closely monitored for arrest or death and were allowed to remain in their arrested state for as long as possible. When some larvae began to show external signs of death (days 8–12), the vial was removed from 29 °C. The stage and behavior of each host was recorded separately prior to dissection. All *ecd* experiments were repeated at least three times. Eight control *Canton S* 5–7 h egg lays were prepared and infected with 6–10 female and 6–8 male *G. xanthopoda* at 25 °C for 3–5 h. Host *ecd* and control larvae were dissected. Wasp larvae within were staged and pooled on the basis of host larval stage.

Results

The life cycle of *G. xanthopoda* can be divided into five phases: embryonic, larval endoparasitic, larval ectoparasitic, prepupal/pupal and independent adult (Figs 1, 2). At 21 °C, the life cycle takes approximately 33–35 days to complete. The embryonic phase of *G. xanthopoda* (0–96 h) begins with the injection of an egg into a second-instar host larva. The embryo hatches into the first-instar (L1) endoparasitic eucoiliform larva (96–288 h). The L1 molts into a hymenopteriform second instar (L2) just before or shortly after pupariation of the host and this marks the beginning of tremendous parasitoid growth. Short-lived, the L2 molts into a third-instar larva (L3) in synchrony with host pupation and emerges from the host body cavity, marking the endo-to-ectoparasitic transition and the death of the host. During the ectoparasitic phase (288–360 h), the L3 feeds externally on the host, until it is consumed entirely. Host remains are excreted, and the L3 molts to give rise to a prepupa. The prepupal/pupal phase (360–800 h) is characterized by a halt in growth and rapid differentiation. The adult phase begins with the eclosion of free-living adult male

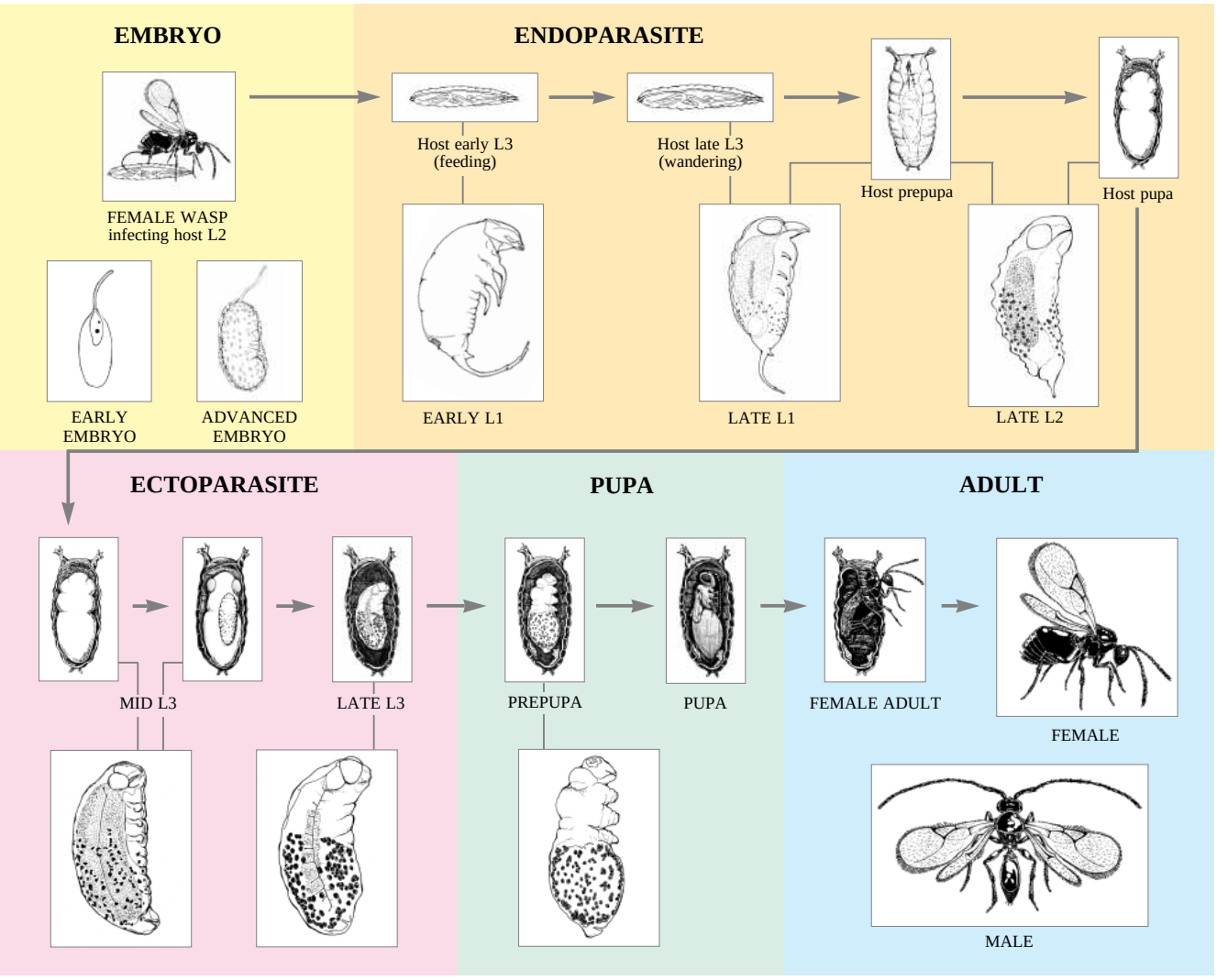

Fig. 1. Life cycle. Schematic representation of life cycle stages of *Ganaspis xanthopoda*. Development is divided into five phases, each represented in a different color. Representative specimens were collected from freshly dissected hosts and hand-sketched. Wasp instar L1 is eucoiliform, while instars L2 and L3 are hymenopteriform. The dimensions of the wasp relative to the host are drawn to scale. Wasp stages shown in the insets are presented to highlight gross morphology and are not to scale relative to each other. Parasitoid growth and the duration of each stage are depicted in Fig. 2.

(>800 h) and female (>824 h) wasps. Even with tightly timed egg lays and infection periods, the degree of variation in both host and parasitoid development is remarkable. Therefore, the time ranges (see Fig. 2 and the text) do not reflect the significant overlap between adjacent stages.

Embryonic stages

The embryonic stage of the life cycle of *G. xanthopoda* begins with the injection of one or more eggs into the hemocoel of a second-instar *D. melanogaster* larva. Approximately similar in size to the *L. boulardi* egg (approximately 0.3 mm long, including stalk; approximately 0.07 mm wide), the *G. xanthopoda* egg is also stalked on the anterior end and, like the *L. boulardi* egg, is surrounded by a transparent, flaccid chorion (Ch, Fig. 3C). The chorion and stalk of both species are sticky. The host gut seems to be the most common site of adherence (HG, *L. boulardi*, Fig. 3A,C; *G. xanthopoda*, Fig. 3B), although embryos may adhere to other host tissue throughout their early development (HT, Fig. 3D, *G. xanthopoda*). These host tissues are immunologically protected because host hemocytes cannot easily reach and form a complete capsule around the developing egg.

To characterize nuclear divisions during early embryogenesis, specimens were stained with Hoechst. A 1-h-old embryo (Fig. 4A) has a single large nucleus, located centrally. In addition, a brightly staining ‘accessory nucleus’ is found, often in close apposition to the plasma membrane (arrowhead, Fig. 4A). As in most other insects, early embryogenesis is a period of rapid synchronous nuclear divisions, apparently without cellular cleavage. At 2 h after oviposition, the embryo can be seen with four nuclei (Fig. 4B). The brightly staining ‘accessory nucleus’ is still present (arrowhead, Fig. 4B). By 8 h, 48–130 nuclei in mitotic synchrony are observed in the embryonic periphery (Fig. 4C).

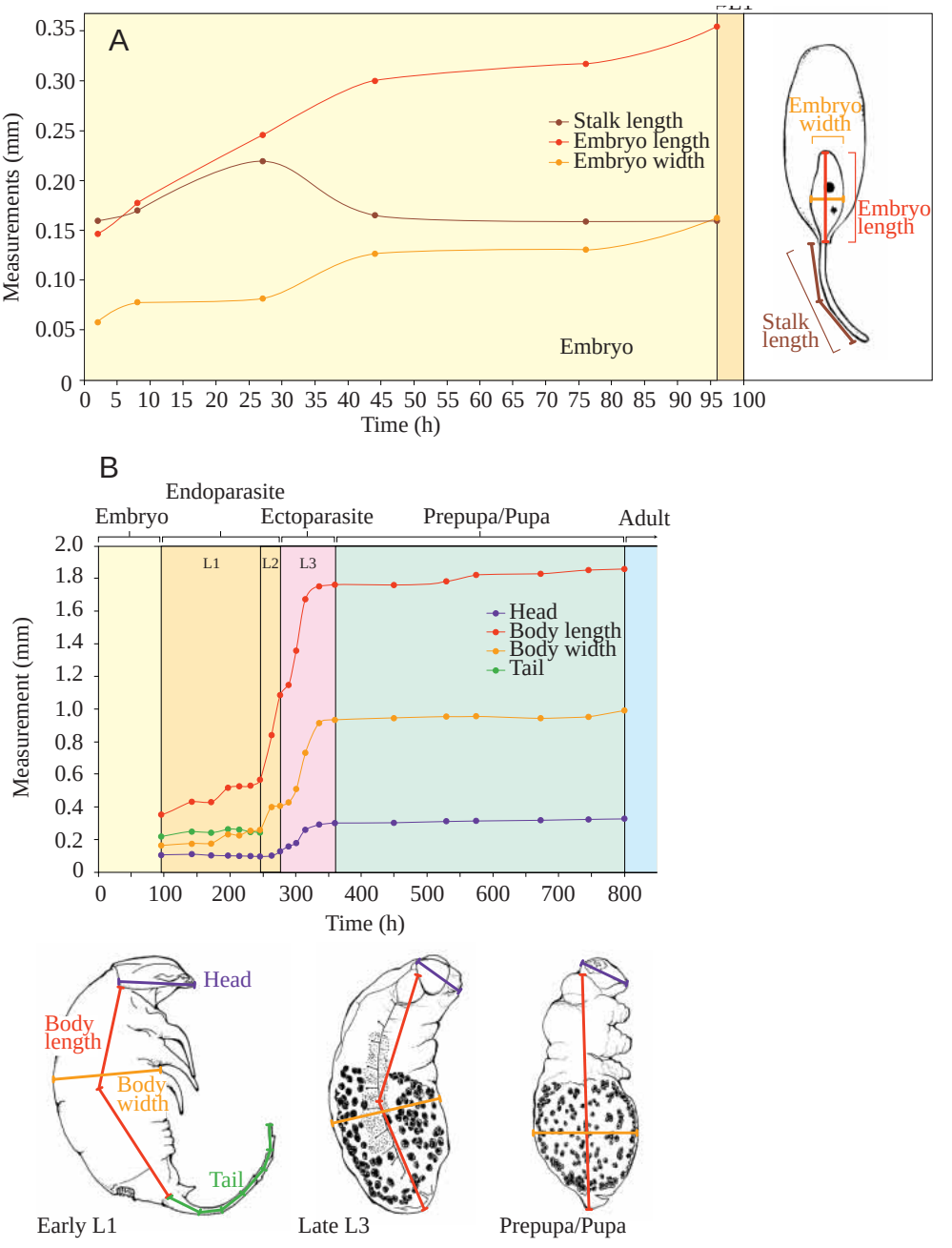

Fig. 2. (A) Graphic representation of growth (0–96 h) during the embryonic stages of development. Number of hours on the *x*-axis refer to parasitoid age ±1.5 h (as oviposition was allowed for 3 h). The first-instar parasitoid larva hatches approximately 96 h after oviposition, when the host is approximately 6 days old. Subsequent growth is shown in B. (B) Graphic representation of growth (96–800 h) of the larval and pupal stages. Parasitoid age is ±1.5 h. Wandering larval, prepupal and pupal host transitions occurred at approximately days 7, 10 and 11 after parasitoid oviposition. The approximate duration of each parasitoid life cycle stage is indicated by vertical lines. Specimens were measured as shown in the inset. Results, based on 6–14 representative specimens at each time point, are presented as mean values. Body length and width increase rapidly after the onset of L2. This growth spurt levels off prior to parasitoid pupariation.

The 'accessory nucleus' is not apparent at this stage, possibly being masked by the bright embryonic nuclei.

Around 70 h after oviposition, larval segmentation (arrows, Fig. 4D) becomes apparent and a cleft develops posteriorly (arrowhead, Fig. 4D). Peripheral embryonic cells appear to delaminate and are organized into an envelope that loosely surrounds the entire embryo. This structure is referred as the 'delaminating membrane' (DM, Fig. 4D). The constriction between the head and the rest of the body then becomes more pronounced (Fig. 4E).

Between 76–96 h, recognizable structures of the first larval instar develop. In an 76-h-old embryo (Fig. 4F; 'delaminating membrane' removed), the head (Hd), oral cavity (Or), anus (An) and tail (T) are developing. The 'delaminating membrane' covering the embryo that is shown in Fig. 4F was removed and stained with Hoechst (Fig. 4G). Cells of this membrane stain brightly.

Further development results in the formation of an anatomically complex and muscular 90-h-old embryo (Fig. 4H) that has acquired many of the characteristics of a first-instar larva. With the 'delaminating membrane' (DM, Fig. 4H) partially removed, the tail and head are obvious, although not yet fully formed. Chitin mandibles are also developed. With a thickening cuticle around the larva, little of the Hoechst stain is able to penetrate, except in the anal region. By 96 h, the embryo is ready to exit the surrounding 'delaminating membrane' which, along with the chorion and stalk, is cast off at the time of hatching.

Over the first 4 days, the embryo approximately doubles in length (from approximately 0.15 mm to approximately 0.30 mm) and triples in width (from approximately 0.06 mm to approximately 0.17 mm; Fig. 2A). Embryonic growth takes place primarily in the first 44 h and the last 20 h. The stalk shows evidence of elongation between 10 and 26 h, and then returns to approximately its previous length by 44 h.

The eucoiliform early L1 stage

The eucoiliform (thoracic segments bear a pair of long ventral processes; posterior abdominal segments taper into a fleshy caudal region) early first-instar larva (EL1; Fig. 5A) hatches from the membranes surrounding it at approximately 96 h *via* the strong muscular action of its tail, appendages and body. The EL1 stage was found between 96 and 170 h in hosts that were still feeding (late second to mid-third instar).

The EL1 head, not yet distinct from the thorax, is covered by a transparent 'helmet' (He, Fig. 5A). The muscular body is highly flexible, able to bend significantly backwards and also to curl into a spherical shape. A pair of functional appendages (Ap, Fig. 5A), each approximately one-third of the body length, is present on each of the first three thoracic segments. A spiked tail (T, Fig. 5A) that is up to half of the body length emerges from the caudal region. The number of spikes on the tail increases with distance from the body, and the spikes are not consistent in number or in placement among different individuals. In addition to the spikes, a ventral process (VP, Fig. 5A) is present close to the caudal region. The anus (An, Fig. 5A) is located dorsally in the caudal region, immediately posterior to the tenth segment.

The late L1 stage

The EL1 parasitoid undergoes an abrupt change in its morphology to give rise to a late first-instar larva (LL1; Fig. 5B; 210 h sample). This change occurs within very late feeding or wandering L3 hosts. Even though the morphological differences in appearance and behavior of the LL1 are sufficiently abrupt and distinct from those of the EL1, we found no evidence of a molt between these stages and have, therefore, placed them within the same instar. This LL1 phase of the first larval instar is long-lived (170–263 h), existing in this stage through early host pupariation (Tables 1, 2).

The width of the LL1 head is reduced. This makes the head more compact, appearing dwarfed in size compared with the burgeoning body (Fig. 5B). The 'helmet' also becomes more constricted, lending further to the compact appearance of the head. The pair of antennal orbitals is clearly visible just posterior and dorsal to the head (Fig. 5B). There is growth of fleshy tissue, and the larval body assumes a stretched and bloated appearance. This tissue eventually grows over the head completely. Segmentation becomes more pronounced and appears wave-like ventrally and dorsally. Fat globules appear in the posterior half of the larva and grow in number and size throughout this stage (FG, Fig. 5B). Although the appendages and tail are still present, they become nonfunctional and transparent. Evidence of increased LL1 feeding is seen in the prevalence of dark material in the gut (Gu, Fig. 5B), now discernible in the mid-portion of the body. A spherical area, enclosed by a wrench-shaped gonadal region (GR, Fig. 5B) just internal to the anus, is devoid of fat globules, signifying the development of the gonads.

The mandibles of L1 form a complex series of six asymmetrical chitinous structures (total of eight points; Fig. 5D,E). The upper points are larger and longer than the lower points, and the outermost mandibles (1 and 6, Fig. 5E) appear to be a single unit, with the points connected by an arch between them. Because of the lack of necessary optical resolution, it is not

Table 1. *Correlation between wild-type host and parasitoid developmental stages*

Host stage	Wasp EL1	Wasp LL1	Wasp L2	Wasp L3
L3 feeding (*N*=62)	55 (89 %)	7 (11 %)	0	0
L3 wandering (*N*=177)	14 (8 %)	163 (92 %)	0	0
White/tan prepupae (*N*=76)	0	59 (80 %)	17 (20 %)	0
Pupae (*N*=72)	0	5 (7 %)	51 (71 %)	16 (22 %)

Ganaspis xanthopoda were placed on second-instar hosts as described in Materials and methods.

Parasitoids were dissected from staged hosts.

N refers to the number of hosts dissected; absolute numbers and percentages (in parentheses) are shown.

Wasp early first (EL1), late first (LL1), second (L2) and early third (EL3) larval instars were identified.

Table 2. *Development of* Ganaspis xanthopoda *in developmentally arrested* ecd/ecd *mutant larvae*

Host stage	Wasp EL1	Wasp LL1	Wasp L2	Wasp L3
L2 (*N*=27)	24 (89 %)	3 (11 %)	0	0
L3 wandering (*N*=160)	8 (5 %)	125 (79 %)	25 (16 %)	0
White/tan prepupae (*N*=27)	0	10 (37 %)	17 (63 %)	0

Infections and temperature shifts of infected hosts were performed as described in Materials and methods.

Hosts and parasitoids were staged only when arrested at their terminal stage of development. If the mutants were not temperature-shifted, they would have progressed into adult stages by 9–12 days.

Arrest of host L2 causes arrest of parasitoid EL1. Arrest of wandering host L3 causes arrest of parasitoid primarily as LL1, although some L2 larvae develop.

Arrested host prepupae do not allow parasitoid L2 to molt to L3.

N refers to the number of hosts dissected; absolute numbers and percentages (in parentheses) are shown.

Wasp early first (EL1), late first (LL1), second (L2) and early third (EL3) larval instars were identified.

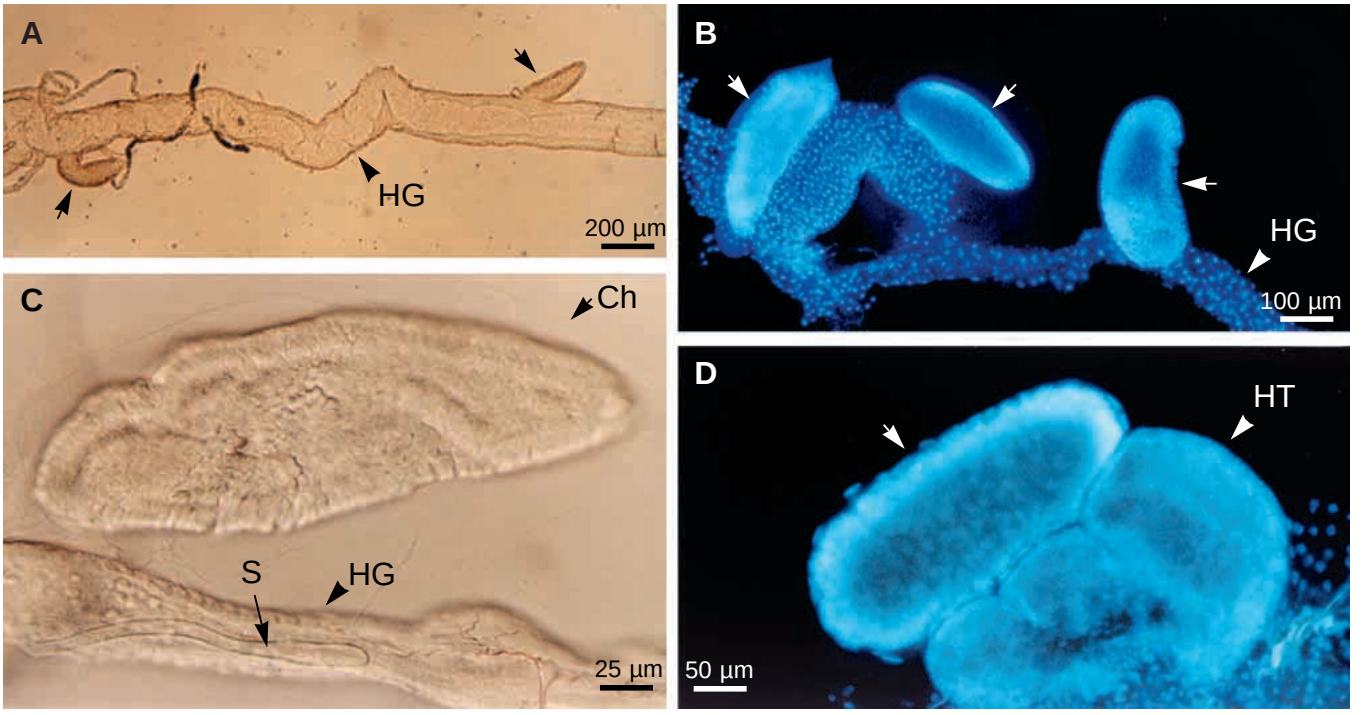

Fig. 3. Adherence of embryos. Embryos of *Leptopilina boulardi* (A,C) and *Ganaspis xanthopoda* (B,D; stained with Hoechst) adhere tightly to endogenous host tissue. (A) Two *L. boulardi* embryos (approximately 30 h) adhere to host gut. (B) Three *G. xanthopoda* embryos (44 h) in association with host gut and fat body. (C) The stalk of the *L. boulardi* embryo (approximately 30 h) is looped around the host gut. (D) A *G. xanthopoda* embryo (76 h) tightly stuck to host tissue. Host tissue is indicated with an arrowhead, parasitoid tissue with an arrow. HG, host gut; Ch, chorion; S, stalk; HT, host tissue.

clear whether the inner teeth (2/3 and 4/5, Fig. 5E) are also connected *via* the same arch-like structure. The anus of the EL1 and the LL1 is oval in shape, with hair-like protrusions that grow from the outside towards the center (A, Fig. 5G). The development of the LL1 culminates in a molt of the entire cuticle, including the appendages, ventral process, tail and mandibles.

The hymenopteriform L2 stage

The LL1 molt gives rise to the second-instar larva (L2) that does not have thoracic appendages and, therefore, is hymenopteriform (Fig. 5C, 270 h sample). This stage was found in early prepupal to early pupal hosts (Table 1). It appears to be short-lived (263–288 h).

The external anatomical definition of the L2 (Fig. 5C) is less pronounced than that of the preceding eucoiliform stages (Fig. 5A,B). The compact late L1 head is completely covered by fleshy tissue, although with careful observation the borders of this head may still be seen through the semi-transparent tissue. The antennal orbitals (AO, Fig. 5C) are conspicuous. The L2 body has wave-like segmentation on both ventral and dorsal sides. Larval movement is slow, but the body is still flexible. The increasing size and dark material of the gut (Gu, Fig. 5C) are prominent, occupying a significant portion of the body. The fat globules continue to grow in number and size. The anus (An, Fig. 5C) is submerged by the fleshy tissue growth and is evident only by an indentation in the contour on the posterior dorsal side. The larva has a pair of unidentate mandibles (Fig. 5F). The development of the endoparasitic L2 culminates in a molt of the entire cuticle, including the tail and mandibles.

The ectoparasitic L3 stage

Soon after the host undergoes pupariation, the L2-to-L3 molt is observed. The wasp third instar (L3; 288–360 h) can be divided into three substages: early, mid and late. The early third instar (EL3; not shown) remains endoparasitic for a short period. The transition from the endoparasitic to ectoparasitic phase of the wasp life cycle occurs when the EL3 protrudes its head from the host pupal body. In our classification, this is the beginning of the mid third-instar larva, ML3 (Fig. 6A), and signifies the beginning of the ectoparasitic phase. Emergence usually occurs in the middle region of the host, the wasp being aligned antero-posteriorly with the host. The host has acquired recognizable pupal structures, and it is possible to distinguish the outline of the developing abdomen, head, eyes and legs (Fig. 6B). However, the endo- to ectoparasitic transition results in the death of the host.

The EL3 is slightly smaller than, but is morphologically similar to, the ML3. Being an ectoparasite, the ML3 can be distinguished from the EL3 by the presence of a single spiracle on the first prothoracic segment (Sp, Fig. 6A). The ML3 head becomes redefined; the antennal orbitals protrude anteriorly from the head into bulb-like structures. The origins of three leg buds (LB, Fig. 6A) are visible on the anterior ventral side. Feeding intensity increases dramatically, and the

gut (Gu, Fig. 6A) is now enormous relative to the body, occupying over half of the parasitoid body. The fat globules (FG, Fig. 6A) are segregated to the posterior half of the larva. The anus (An, Fig. 6A) is reorganized and now exits from the posterior apex of the larva.

The ML3 (arrow, Fig. 6B) is sessile, facing inwards and feeding aggressively from outside the host, although still inside the puparium (arrowhead, Fig. 6B). As the ML3 matures, an extensive tracheal system is visible. In addition to the single spiracle that surfaces on the first prothoracic segment, submerged spiracles can also be seen through the cuticle (1, 2, Fig. 6C). The posterior-most pair of spiracles emerges next, followed by the intervening pairs, resulting in a total of 10 pairs (one pair for each segment, except for the head and the caudal segments). Careful observation of the mandibles reveals a pair of bidentate chitinous teeth, with the larger, outer tooth closely associated with a smaller tooth nearer the oral cavity (Fig. 6D,E). The mandibles move in relentless motion, consuming the host entirely.

After the host has been completely consumed, the wasp larva is termed a late third instar (LL3; Fig. 6F–H). The larva at this stage is immobile, able to bend only slightly and to move its mandibles, which it continues to do even after the host has been completely consumed. The head is dominated by the protruding antennal orbitals (AO, Fig. 6F) and the muscular mandible system (M, Fig. 6F,G). The imaginal eyes are visible just posterior to each antennal orbital (Fig. 6F). Larval segmentation is pronounced (Fig. 6G). This is particularly true in the thoracic region, where the six leg buds (LB) stick out further than the abdominal segments. The remains of the host (meconium) are clearly seen through the cuticle of the wasp larva as a dark, cylindrical mass (Fig. 6F–H). The spiracles and associated

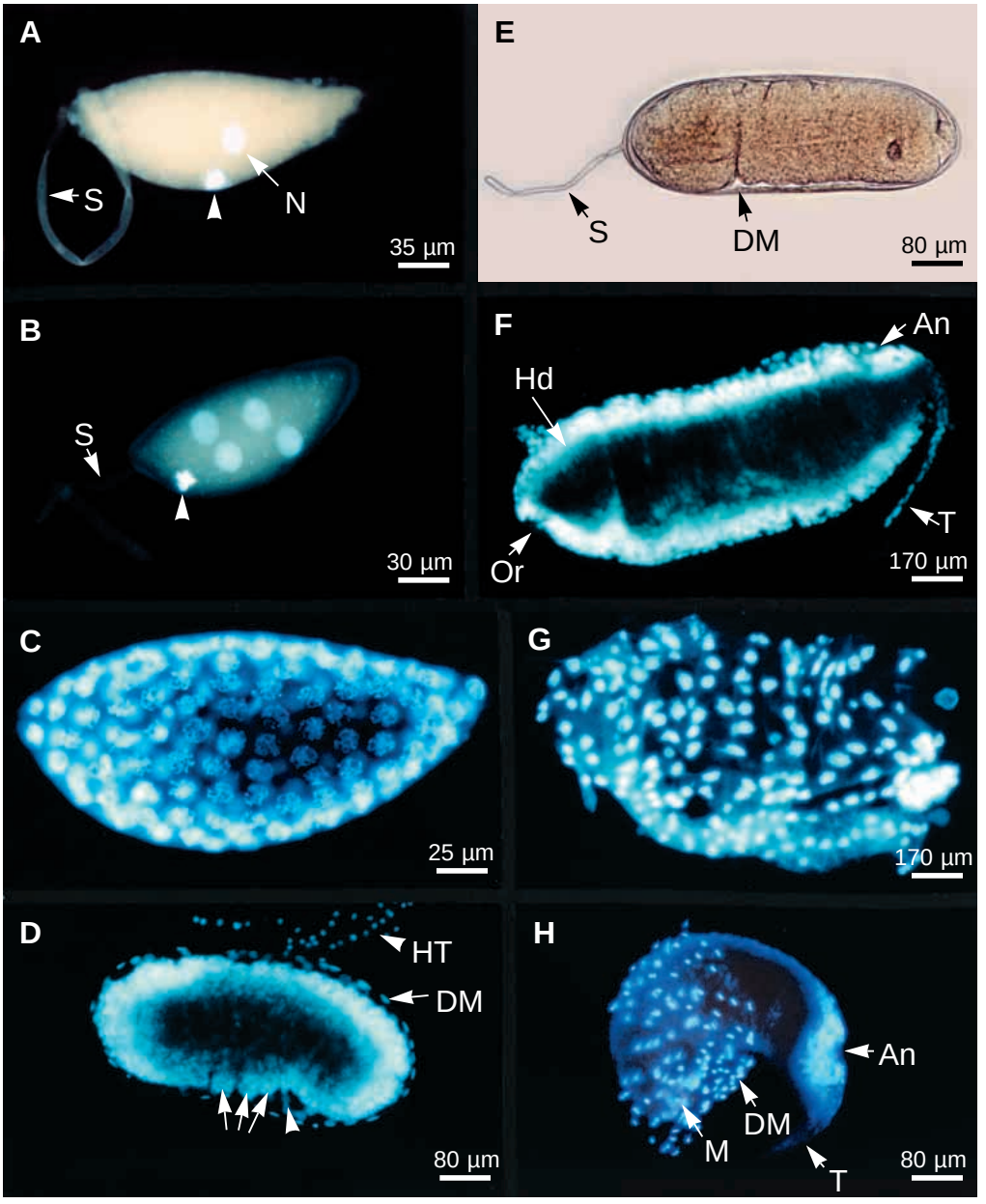

Fig. 4. Embryonic stages. (A–H) Embryonic development of *Ganaspis xanthopoda* from 0 to 96 h. Anterior is to the left. A–D and F–H are stained with Hoechst. (A) A 1-h-old stalked embryo. One nucleus (arrow) is present. A brightly staining 'accessory nucleus' is in close apposition to the membrane (arrowhead). S, stalk. (B) A 2-h-old embryo. Four nuclei are present; a brightly staining 'accessory nucleus' persists (arrowhead). (C) An 8-h-old embryo showing synchronous mitosis. Chromosomal condensation is apparent. (D) A 66-h-old embryo showing the 'delaminating membrane' (DM). A posterior cleft (arrowhead) and segmentation (arrows) are apparent. Host tissue (HT) is associated with the embryo. (E) A 66-h-old embryo, unstained. (F,G) A 76-h-old embryo with the 'delaminating membrane' removed and mounted separately in G. (F) The oral cavity (Or), head (Hd), body segments, anus (An) and tail (T) are evident. (G) Membrane cells and nuclei are variable in size and shape. (H) A 90-h-old embryo, with the 'delaminating membrane' (DM) partly removed. Mandibles (M) are visible under the membrane.

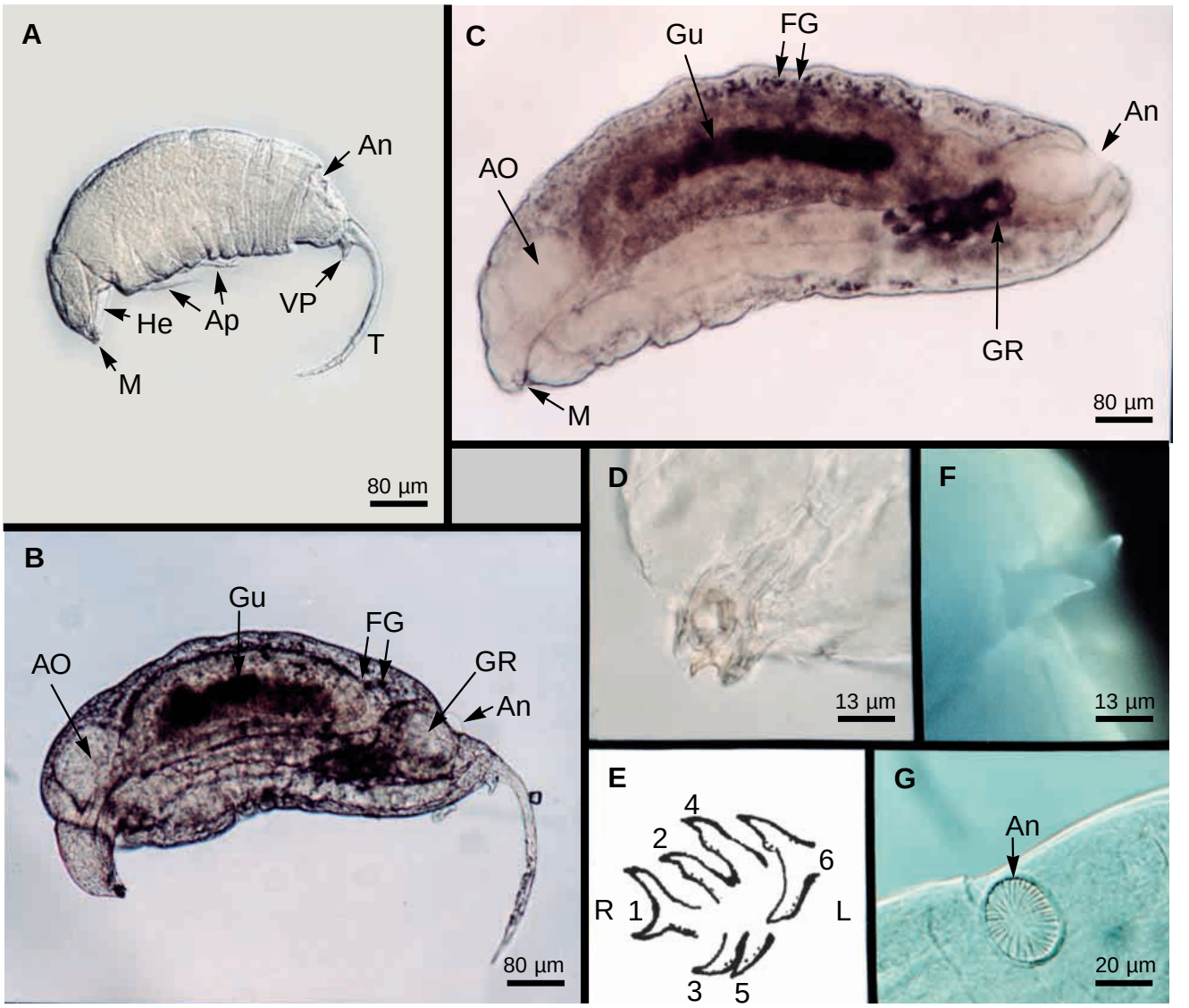

Fig. 5. Endoparasitic first and second instars. Anterior is to the left. (A) Eucoiliform early first-instar larva, EL1. M, mandibles; Ap, appendages; An, anus; VP, ventral process; T, tail. The head is surrounded by a transparent integument 'helmet' (He). (B) Late first-instar larva, LL1. Abrupt developmental changes include constriction of the 'helmet' around the head, more obvious antennal orbitals (AO), the growth of fleshy tissue, an enlarged gut (Gu), fat globules (FG) and visibility of gonadal region (GR). (C) Second larval instar, L2. Hymenopteriform. Fleshy tissue surrounds the head of LL1, which is now barely visible. Antennal orbitals (AO), gut (Gu), gonadal region (GR), fat globules (FG) and the dorsal anus (An) are visible. (D) Mandibles of the L1 stage. Six sharp chitinous structures, arranged in a whorl. (E) Drawing of L1 mandibles. L, left; R, right. (F) L2 mandibles. Unidentate, photographed under ultraviolet light. (G) The anus of L1 shows inward-growing projections.

tracheae are clearly visible (3–10, Fig. 6H). The anus forms a characteristic 'cup' shape that faces the ventral side (An, Fig. 6G). The bidentate mandibles have become larger and more distinct (M, Fig. 6I). *L. heterotoma* mandibles are recognizably different, being unidentate and serrated (M, Fig. 6J). The eventual ecdysis of the meconium appears as a dark crust in the posterior apex of the puparium. The LL3 stage culminates in a molt of the entire cuticle, including the mandibles.

Prepupal and pupal stages

The L3 molt gives rise to the prepupa, shown here with the molted integuments and mandibles of the LL3, while still surrounding it (Fig. 7A). The prepupa undergoes rapid anatomical differentiation. The head begins to take the adult wasp form, and antennal development is visible through the transparent cuticle of the antennal orbitals, appearing as a coiled tubular structure (not visible in Fig. 7A). The developing eyes appear as clear oval structures towards the posterior portion of the head (Fig. 7A). The region between the thorax and abdomen constricts, and the emerging legs (L, Fig. 7A) appear as three protruding mounds on the ventral thoracic side. The scutellum is visible (Sc, Fig. 7A).

A 613 h male pupa shows external adult wasp characteristics, including legs, wings, scutellum and full-length

antennae (Ant, Fig. 7B). This pupa has incomplete body coloration, although deep red eye pigmentation is evident (Fig. 7C). A 648 h female, still partially inside the puparium, shows increasing body pigmentation. Expelled meconium is located within the pupal case, towards her posterior end (Fig. 7D). The mandibles are pigmented and have at least three serrations (M, Fig. 7D,E). A 785 h male shortly before eclosion is shown in Fig. 7F. The wasp is darkly pigmented. The eyes change from red to almost black. Males eclose approximately 24–48 h before females. After eclosion, adult wasps are usually at least half the size of their adult *Drosophila melanogaster* hosts (Fig. 7G).

Parasitoid growth and molts are linked to host ecdysone peaks

To examine whether the growth of the parasitoid is controlled by that of the host, we measured the growth of the parasitoid as a function of time. Coincident with the host

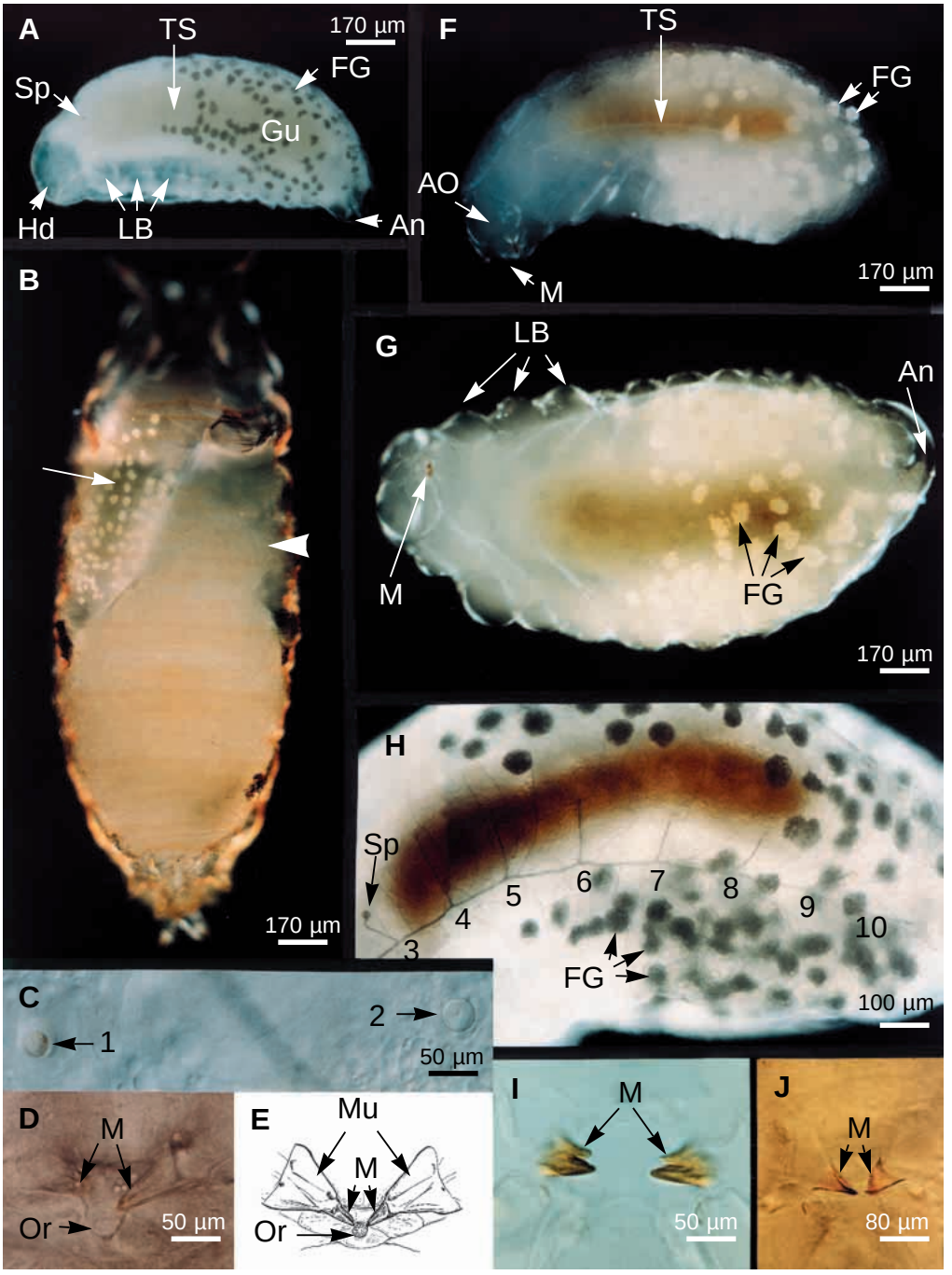

Fig. 6. Ectoparasitic third instar. (A) Mid-third-instar ectoparasite, ML3. The defined head (Hd) re-emerges; antennal orbitals protrude anteriorly. There are three leg buds (LB) in thoracic region, one prothoracic spiracle (Sp) has emerged, with an inconspicuous tracheal system (TS). A large gut (Gu) is visible with fat globules (FG); an abundant anus (An) at the posterior apex is apparent. (B) Ectoparasitic ML3, within the host puparium (arrowhead). The parasitoid (arrow) is lodged against the puparium casing, aligned antero-posteriorly with the host. The ventral side of the parasitoid faces inwards, immersed in host tissues. The host appears to have progressed into the pupal stage. (C) The first prothoracic spiracle, on the left, has surfaced (arrow 1), while the second prothoracic spiracle is still beneath the cuticle (arrow 2). (D,E) ML3 mandibles. (D) The mandibles (M) of the ML3 ectoparasite are bidentate and chitinous. The inner point is smaller than the outer point. (E) Drawing showing the anatomy of the oral region (Or) and the muscles (Mu) associated with mandibles (M). (F–H) Late third instar, LL3, after complete consumption of the host. F and G present lateral and ventral views, respectively. (F,G) Antennal orbitals (AO) and mandibles (M) are visible anteriorly. Spiracles have emerged from the tracheal system (TS). The consumed host is visible as a dark streak in the body. (G) Three pairs of leg buds (LB) bulge laterally in the thoracic region. The anus (An) is posterior and ventral. (H) Details of the branched tracheal system and associated spiracles (Sp). Eight of the ten spiracles (3–10) are clearly visible. (I,J) Flattened mandible preparations of *Ganaspis xanthopoda* (I) and *Leptopilina heterotoma* (J) at equivalent stages. (I) The mandibles of LL3 of *G. xanthopoda*. Bidentate morphology is more pronounced than that of the EL3 (C,D). (J). The mandibles of *L. heterotoma* are unidentate and serrated.

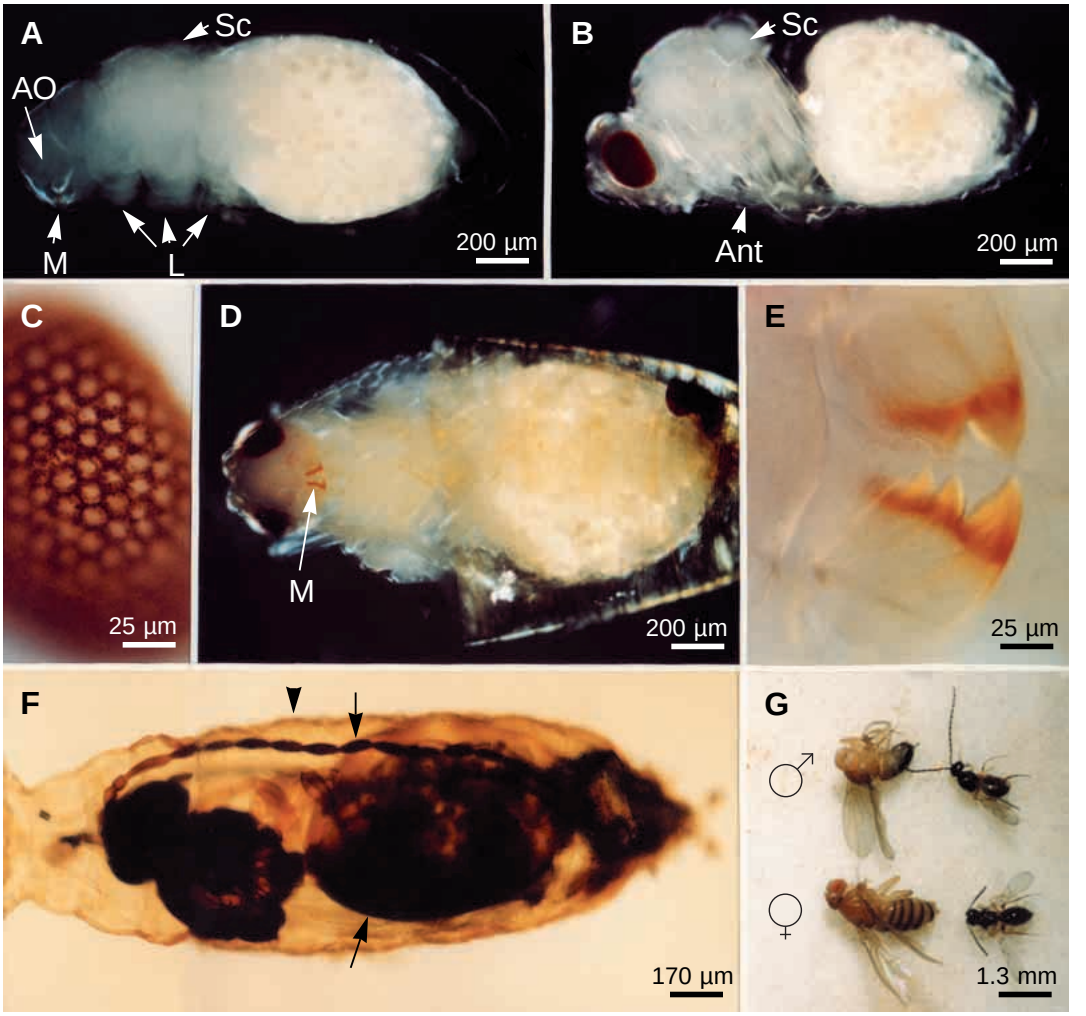

Fig. 7. Prepupal, pupal and adult stages. (A) Lateral view of a 360-h-old prepupa. Late L3 integument and mandibles (M) are about to be molted. The antennal orbitals (AO), the imaginal eye and the imaginal legs (L) are visible. A constriction between the abdominal and thoracic segments becomes apparent. A scutellum (Sc) is present. (B) Lateral view of a 528-h-old male pupa. The head, thorax, scutellum, abdomen and antennae (Ant) are distinct. Body coloration is pale. (C) The eye of the specimen from B, showing deep red pigmentation. (D) Ventral view of a 648-h-old female with the host puparium partly removed. The body and mandibles are darkening. Meconium is present at the bottom of the puparium. (E) The mandibles of C are pigmented and have at least three serrations. (F) A 785-h-old male (arrow) contained within an intact puparium (arrowhead). The body has darkened. (G) Adult *Canton S* flies (left) and *Ganaspis xanthopoda* wasps (right), showing relative sizes. Females are to the left, males to the right. The female wasp has shorter antennae than the male.

wandering, there is a small but significant amount of growth at the EL1-to-LL1 transition. At the onset of L2, there is a dramatic growth spurt that coincides with the metamorphosis of the host (Fig. 2B), and body length and width both increase. Dramatic growth continues into the L3 stages, with body length and width and head length all increasing to approximately three times their value compared with the LL2 stage (Fig. 2B).

To correlate parasitoid growth both with its development and with the development of the host, we documented the developmental status of both the host and the parasitoid (Table 1). Under our conditions of infection in which the second larval instar (48 h) of the host was infected, a majority (approximately 90 %) of the parasitoid larvae were in the early first instar as long as the host was actively feeding. As the host larvae ceased feeding and progressed into the wandering stage, more than 90 % of the parasites also progressed into the late first larval instar. This observation suggests that the early-to-late transition of the L1 is dependent on the ecdysone peak that controls the transition of the feeding stage to the wandering stage of the host larva. The majority (75–80 %) of the LL1 parasitoids underwent the first molt into the L2 only after the host puparium had been formed, just prior to pupariation. An L2 larva was never found in the wandering third-instar wild-type host. This result suggests that the L1-to-L2 transition is tied to the prepupal peak of ecdysone levels that precedes the metamorphosis of the host.

After the host had shown head eversion (pupation), more than 70 % of all parasitoids were in the L2 stage. As pupation progresses, the wasp L2 larva molts to the L3 stage. The L2-to-L3 transition is not observed prior to head eversion (Table 1). This correlation suggests that there is a developmental synchrony between the host prepupal/pupal and the parasitoid L2/L3 transitions.

Results from temperature-shift experiments on ecd^1/ecd^1 hosts support the idea that the early-to-late L1 transition is dependent on the hormonal status of the host (Table 2). When host development is arrested early (host L2), a majority (approximately 90 %) of the parasitoids remain in the EL1 stage. When host development is allowed to proceed into the third larval instar (wandering host L3), parasitoid development progresses into LL1, as in their wild-type counterparts (Table 1).

Upon pupariation, more than 60% of the parasitoids molt into the L2 stage. Few (16%) parasitoids can undergo the LL1-to-L2 molt even in the developmentally arrested wandering L3 hosts. Of these, none progresses into the L3 stage. Thus, just as in the wild-type infections (Table 1), the *ecd* temperature-shift experiment shows that the L2-to-L3 molt is dependent on host pupation and suggests that high ecdysone levels are needed for this L2-to-L3 wasp transition (Tables 1, 2).

Discussion

In describing the life cycle of *Ganaspis xanthopoda*, our goal was to provide a general analysis of its development and to compare its overall developmental strategy with published descriptions of *Leptopilina* spp. Although the embryonic stages of the *Leptopilina* species have not been described in as much detail as its post-embryonic stages (Jenni, 1951; Kopelman and Chabora; 1984), the overall mode of development adopted by species of *Ganaspis* and *Leptopilina* appears to be quite similar. When post-embryonic stages of *Leptopilina* spp. (Jenni, 1951; Kopelman and Chabora; 1984) are compared with those of *Ganaspis* spp., the similarity between the life histories is quite evident: both genera parasitize the second-instar larval host, undergo embryogenesis to hatch into endoparasitic eucoilid larvae, then progress into the hymenopteriform larva, which becomes ectoparasitic at the time of host pupariation. In these *Leptopilina* and *Ganaspis* species, the critical developmental transitions of the parasitoid appear to be controlled similarly by the host ecdysone levels (e.g. Kopelman and Chabora, 1984; this study).

The analysis of embryonic stages of the *G. xanthopoda* presented in this study merely provides an initial description of this process and leaves out many important questions regarding mechanisms and strategies of early development. Grbic and Strand (1998) have used molecular and cellular markers to examine whether ectoparasitic or endoparasitic lifestyles influence the course of early embryogenesis. In a comparative study of the development of the ectoparasite *Bracon hebetor* with an endoparasite *Aphidius ervi*, they found that the development of the ectoparasite, which lays its eggs on the integuments of its host, was much like that of the free-living, long-germband *Drosophila* or honeybee. In contrast, the development of the endoparasite, which develops within the host hemocoel, follows a different mode that is closer to that of short-germband species. Like the egg of *A. ervi*, the eggs of *Ganaspis* and *Leptopilina* spp. are small and covered by a thin chorion with a stalk at the anterior end. Our preliminary observations suggest that early embryogenesis of *G. xanthopoda* involves a series of synchronous nuclear divisions that appear not to be accompanied by cytokinesis. However, more rigorous evidence is required to establish when cleavage occurs, whether it is complete and whether the embryonic pattern is established before or after cellularization. The use of molecular markers to study the expression of segmentation and the homeotic genes that control segmentation and segmental identity in *Drosophila* will allow a comparison of early development between *G. xanthopoda* and *A. ervi* as well as between *G. xanthopoda* and its *Drosophila* host.

While the degree of hormonal interaction between the parasitoid embryo and its host is not known, the larval development of *G. xanthopoda* is synchronous with that of its host. Indeed, like many other insect parasitoids (for a review, see Beckage, 1985), *G. xanthopoda* is sensitive to its host hormonal *milieu*, and responds to fluctuations in ecdysone levels within the host hemolymph. In *Drosophila*, pulses of ecdysone control major postembryonic developmental transitions. In the context of parasitoid development, three peaks of hormone titers are important: the first pulse of ecdysone signals a prewandering third-instar larval host to leave its food and begin wandering. This is followed by a high-titer second peak that signals puparium formation. Approximately 10 h later, a third peak triggers the prepupal-to-pupal transition (Thummel, 1996). By documenting how parasitoid development proceeds as a function of host development in wild-type as well as ecdysone-deficient hosts, we found (1) that the first prewandering peak in the third-instar larval host controls the early-to-late transition of L1, (2) that the second high-titer peak that precedes host pupariation is required for the dramatic growth spurt at the onset of L2 and for the L1-to-L2 transition, and (3) that the third peak prior to host pupation is important for the parasitoid L2-to-L3 transition.

While these observations allow us to correlate parasitoid transitions to specific ecdysone peaks, it is clear that, under some conditions, wasp ecdysis may occur in the absence of molting of the host larva. For example, even though the wasp L2 is not found in the wild-type wandering third-instar host, the L1-to-L2 molt occurs in approximately 15% of the parasitoids in the ecdysone-deficient developmentally arrested wandering third-instar host (Tables 1, 2). This result can be explained by assuming that, at the non-permissive temperature, ecdysone level in some ecd^1 hosts is lowered, but that ecdysone is not eliminated (Garen et al., 1977). This would imply that the parasitoid can respond to ecdysone levels that are lower than those to which the host is sensitive. Therefore, wasp development proceeds even when that of the host is arrested. It is also possible that other factors, such as a critical body size of the parasitoid, play a role in ecdysis.

The effect of the host ecdysone hormone on parasitoid development raises interesting questions about the molecular mechanism of hormone action. In the host, a protein heterodimer of the ecdysone receptor (EcR) and ultraspiracle (Usp) complexes with the hormone and activates transcription at many specific loci. This initiates a sequential cascade of molecular events that regulates the development and differentiation of host tissue (Thummel, 1996). It would be informative to explore whether similar receptor molecules in the parasitoid mediate the ecdysone signal to the nucleus and how this nuclear signal coordinates parasitoid development.

The host organism *D. melanogaster* is understood better than any other insect, and it should therefore be possible to exploit host strains with specific physiological, cellular or molecular properties to enhance our understanding of the molecular genetic mechanisms behind parasitoid behavior and physiology, and the interactions between the parasitoid and its host.

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