

[REVIEW]

Strategies during Larval Development of Hymenopteran Parasitoids in Ensuring a Suitable Food Resource

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An essential requirement for the successful growth and development of living organisms is to acquire sufficient food. But, unlike most organisms, parasites are constrained in this regard because of the necessity to obtain their food from a single source (the host). The nutritional relationship that is established between hosts and their parasites is never simple and often reflects a long co-evolutionary 'arms-race' between each other (Godfray, 1994). In this arms-race, hosts evolve mechanisms that enable them to escape from their parasites or to eliminate them; on the other hand, the parasites need to overcome host defenses and utilize the host as a nutritional reservoir. Although parasites are often divided in ecto- and endo-parasites according to their parasitic lifestyle, this division is inapplicable from a nutritional perspective. Parasitoids (primarily in the insect orders Diptera and Hymenoptera) are different from true parasites that do not kill the hosts, because parasitoid larvae feed exclusively on the body of another insect as host, eventually killing it (Vinson, 1975; Godfray, 1994), and the adults are free-living (Godfray, 1994). Haeselbarth (1979) was the first to define differences in the way parasitoid larvae exploit host resources and divided parasitoid species into two macro-evolutionary groups called koinophytes and idiophytes. Later, Askew and Shaw (1986) refined these terms by classifying parasitoids as koinobionts or idiobionts.

Idiobiont parasitoids mainly attack egg, pupal, or adult host stages, and paralyze the host preceding oviposition. Idiobionts thus develop in non-growing hosts where the amount of food resource does not increase after parasitization. On the other hand, koinobionts develop in hosts that continue to grow and metamorphose during at least the initial stages of parasitism. These include egg-larval and larval-pupal parasitoids, or larval parasitoids that do not permanently paralyze their hosts at oviposition (Godfray, 1994). Thus, parasitized hosts continue to feed and grow, while the koinobiont parasitoid larvae can use the food resources increased after parasitization. The parasitoid larvae feed selectively on certain host tissues in order to keep a minimal damage to the host. Severe damage of the host during early development may lead to precocious death of the parasitoid. Hence, the parasitoid larvae commence destructive feeding, leading eventually to host death, only later during their development. Feeding exclusively on the nutrient-rich hemolymph is one strategy that parasitoid larvae employed to minimize host damage. Hemolymph retains a high nutritional value since it efficiently retrieves nutrients, which have already been or are to be stored in organs like the fat body *via* a specialized metabolic pathway (Thompson, 1993), through inhibition of absorption of nutrients by the fat-body, or only by slowly reducing the amount of fat body. The koinobiont larvae, however, possess well developed gut to digest some foods during their late stages. Also, during late larval stages, the parasitoid larvae exhibit rapid growth just prior to pupation. These facts are difficult to explain on the sole basis of nutrient uptake from host hemolymph. Furthermore, it is unlikely that the consumption of the fat body results in significant physical damage that affects the survival of lepidopteran host larvae. Because the fat body is an organ responsible for the storage and the synthesis of

carbohydrates, proteins, and lipids (Keeley, 1985; Haunerland and Shirk, 1995), it is also possible that parasitoid larvae can take their food effectively with minimum damage, by directly consuming the fat body of their host.

However, there is another factor that cannot be overlooked. It is commonly observed that the parasitized hosts continue to feed for extended periods during parasitism, but that the amount of food intake and subsequent weight gain is lower in parasitized than in healthy hosts (Vinson, 1990; Tanaka *et al.*, 1992; Harvey and Thompson, 1995; Harvey *et al.*, 1999; Harvey and Strand, 2002). This is another important consideration implicating that parasitoids must exploit resources more effectively avoiding physical damage to the parasitized hosts, which are smaller than unparasitized cohorts.

The utilization of the host as a nutritional food resource may be dichotomously categorized as follows: (1) the parasitoid larvae consume most or all of the host resources before pupation or (2) the parasitoid larvae consume at most a fraction of host resources and emerge from host larvae that are still physically intact and may continue to remain active and alive for variable periods, but do not feed and eventually perish. Elucidation of how ecto- and endoparasitic koinobionts utilize the hosts as a food resource will be useful in clarifying the evolution of common or different strategies in koinobionts.

Here we describe strategies that enable parasitoids to ensure a sufficient food resource in both ecto- and endoparasitic koinobionts and explain how the parasitoid larvae utilize the host resource to enhance successful parasitism.

1. Strategy of food resource acquisition in the ectoparasitoid, *Euplectrus separatae* (Hymenoptera: Eulophidae)

Euplectrus separatae is a gregarious ectoparasitoid, which uses the common armyworm *Pseudaletia separata* (Lepidoptera: Noctuidae), as a host and oviposits on the dorsal side of the host thorax (Fig. 1A) (Nakamatsu and Tanaka, 2005). Preceding oviposition, the female wasps inject venom into the host to regulate the host's physiological condition by inhibiting larval molting at the stage parasitized (Nakamatsu and Tanaka, 2003a). Absence of this regulation allows the host to ecdyze to next instar, and the parasitoid eggs or larvae are disposed along with host

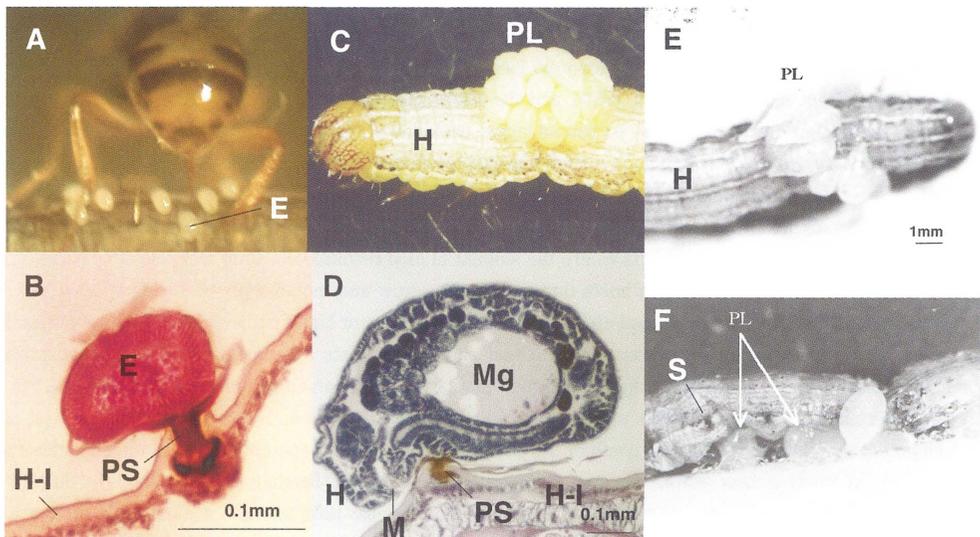


Fig. 1 Development of ectoparasitoid, *Euplectrus separatae* (Hymenoptera: Eulophidae). A. Oviposition on dorsal surface of the host, *Pseudaletia separata* (Lepidoptera: Noctuidae). E: egg. B. A section of a laid egg. Each egg (E) is anchored in the host integument (H-I) through a proteinaceous stalk (PS). C. Fully grown larvae attached on the dorsal site of the host (H) with the stalk. D. A section of second instar of *E. separatae* on the stalk (PS). H: head of parasitoid larva, H-I: host integument, M: mouth of parasitoid larva, Mg: midgut of parasitoid larva. E. Parasitoid larvae (PL) start to move down from dorsal to ventral side of the host body (H). F. Lateral view of parasitoid larvae (PL) under the host body, which spin each cocoon. S: Spiracle of the host.

exuviae. The eggs of *E. separatae* are anchored to the oviposition site *via* a proteinaceous stalk, and develop on the host cuticle until pupation (Fig. 1B, C) (Nakamatsu and Tanaka, 2003b). The parasitoid larvae on the dorsal side grow by perforating the host cuticle and imbibing host hemolymph as food (Fig. 1D). Just prior to pupation, the parasitoid larvae descend from the dorsal to the ventral side of the host body (Fig. 1E). The host then dies when the parasitoid larvae reach the underside of its body. The host body, subsequently, flattens against the surface where the parasitoids spin and construct a thin cocoon and pupate under the host body (Fig. 1F) (Nakamatsu and Tanaka, 2003b).

How does this ectoparasitoid species obtain food from its host? The hosts are initially injected with venom that causes hyperlipidemia in the hemolymph (Nakamatsu and Tanaka, 2004b), which is characterized by gradual increase of lipid and protein contents. The parasitoid larvae then obtain nutrient-rich hemolymph from the host through a slow disruption of fat body cells, which is facilitated by the function of the venom. Disruption of the intercellular matrix of fat body is aided by venom proteolytic enzymes. This occurs when venom matrix metalloproteinase (MMP), which is activated by several components of the host hemolymph, makes an initial attack on a specific site in the fat body matrix. Subsequently, hyaluronidase and phospholipase B, which have high activity in the venom, cause lysis of fat body cells that allows lipid droplets to be released into the host hemolymph (Nakamatsu and Tanaka, 2004b). Venom affects only microtubules in the fat body cells (Fig. 2) and has no effects on other organs or tissues such as muscles, guts, malpighian tubules. However, it has not yet been clarified how venom affects the cell matrix of the host fat body alone.

Microtubules are involved in several basic cellular processes such as intracellular transport, maintenance of cell shape or positioning of cell organelles with the co-action of other components of the cytoskeleton such as actin microfilaments (Unger *et al.*, 1990). Immuno-staining with anti-tubulin or anti-actin antibody reveals that venom disrupts the cytoskeleton of fat body (Fig. 2). This disruption is set on the fusion of lipid droplets with each other and finally enables the fat body cell to release the large lipid droplets. Lipid droplets are not carried by lipophorin, but are phagocytosed by granulocytes or plasmatocytes in the host hemolymph. Because the amount of lipid carried with lipophorin in parasitized hosts is the same as that of nonparasitized hosts, the parasitoid larvae consume hemolymph including hemocytes with lipid droplets.

The parasitized hosts became 'flat' after parasitoid larvae descended from the dorsal to ventral sides (Fig. 1F). All of the contents in the host body cavity have been pulled by the parasitoid larvae under the host body. Almost all parasitoid larvae pupate under and are shielded by the flattened host body. The parasitoid larvae inject saliva into the host to digest all of the contents nonspecifically, just before moving to the ventral site. The digestive enzyme shows trypsin-like activity and all internal organs of the host are digested (Nakamatsu and Tanaka, 2004a). This tactic assures that the parasitoids acquire enough nutrient source to achieve the developmental maturity of parasitoid larvae before pupation. Further, it is likely that this 'pulling' behavior of the host contents that are to be digested under the host body

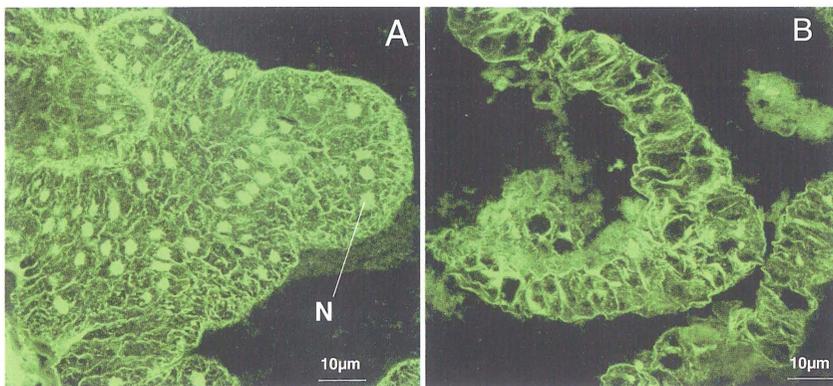


Fig. 2 Sections of fat bodies of caterpillar, *Pseudaletia separata*, nonparasitized (A) and parasitized (B) by *Euplectrus separatae*. Each section is stained with anti-actin antibody, which exposes the actin microtubules in the fat body cells. Fat body cell of nonparasitized host has a nucleus (N) with well developed actin filaments in the center of cells (A). In fat body cells of the host 6 days after parasitization, when the parasitoid larva is second instar, actin filaments are broken with venom, and the cellular contents are leaked out (B).

reduces the risk of being attacked by other predaceous insects during the pupal stage.

2. Strategy of food resource acquisition in endoparasitoid, *Cotesia kariyai* (Hymenoptera: Braconidae)

Some endoparasitoid taxa, including *Cotesia* spp., oviposit their eggs into the host hemocoel along with venoms and polydnviruses (PDVs). PDV penetrates the host cell membranes and expresses its genes to regulate the host's physiological condition [see reviews by Turnbull and Webb (2002) and Webb and Strand (2004)]. PDV is one of the most important factors for successful parasitism in a number of endoparasitoid clades.

The braconid parasitoid, *Cotesia kariyai* can successfully parasitize second instar to day 3 terminal (= sixth) instar of *Pseudaletia separata*. Development of the parasitoid takes approximately 10 days from oviposition to emergence at 25°C when parasitization occurs in any instar (Tanaka *et al.*, 1987). Under these conditions, egg hatching usually occurs about 3.5 days after oviposition. Ecdysis from first to second parasitoid instars occurs about 6.5 days after oviposition. The gut of the first parasitoid instar is immature and is, therefore, void of content (Fig. 3C-1), in contrast to gut of second instar filled with food contents (Fig. 3C-2). Figure 3 shows that amaranth dye ($C_{20}H_{11}N_2Na_3O_{10}S_3$, MW 604.48) is absorbed into the body cavity through the caudal vesicle in first instar (Fig. 3A) and in second instar of parasitoid (Fig. 3B). The first instar appears to absorb nutrients from the host hemolymph through the caudal vesicle or integument (Fig. 3A). Second parasitoid instar also incorporates amaranth dye through the caudal vesicle because it is stained slightly pink comparing to control (Fig. 3B). A paraffin section of second parasitoid instar after incubation with amaranth dye revealed the connection between hemocoel and caudal vesicle (Fig. 3D).

Aphidius ervi takes nutrients as amino acids through both the integument and the midgut (Caccia *et al.*, 2005). It is

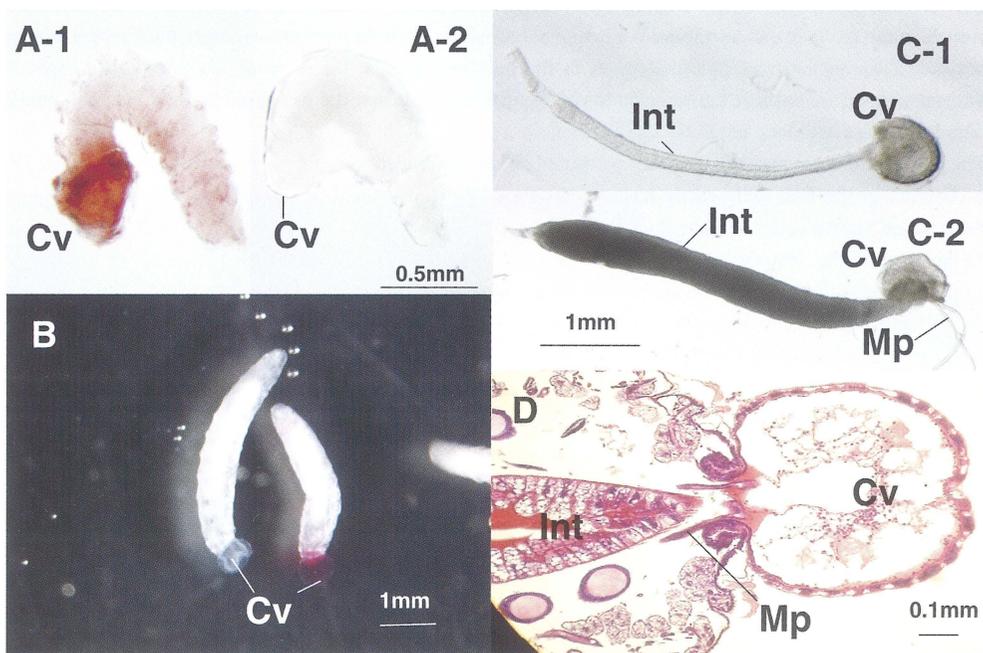


Fig. 3 Dye-uptake of first and second instars of *Cotesia kariyai* through caudal vesicle. A-1. Amaranth dye is incorporated through caudal vesicle of first instar. Parasitoid larvae are incubated in physiological saline contained 0.5% amaranth (red) dye. The caudal vesicle (Cv) and inside of first instar turned to red. A-2. A first instar of parasitoid was incubated in physiological saline without dye. B. Second instars of parasitoid treated with same condition as 3A. Caudal vesicle (Cv) is strongly stained with amaranth dye that has been incorporated through caudal vesicle (right). Nonparasitized control larva was incubated with no dye (left). C-1. Intestine (Int) and Cv drawn out from first instar body. C-2. Int and Cv of second instar. Mp: malpighian tubule. D. A paraffin section of second parasitoid instar after incubation with amaranth dye to elucidate the connection between hemocoel and caudal vesicle. Finally paraffin section was stained with Mayer's hematoxylin and 1% eosin. Cv: caudal vesicle, Int: intestine, Mp: malpighian tubule.

unclear whether amino acids in the parasitoid's midgut are produced by a digestion process in the midgut of the parasitoid or not. It remains to be determined how much of these amino acids were absorbed by *C. kariyai* larvae through their integument.

Artificial injection of PDV plus venom, as with parasitism, results in reduced host growth, although its effect depends on the amount injected into the host larva. The expression of PDV genes affects weight gain of the host and leads to a reduction in the amount of feeding damage. However, the approximate digestivity [AD: food consumption/food consumption minus frass, cf. Waldbauer (1968)] in parasitized hosts is elevated in hosts parasitized by *C. kariyai*, compared with the healthy larvae. Further, artificial injection of PDV plus venom increased trehalose titer in host hemolymph during late parasitism (*i. e.*, 7 or 8 days after parasitization) (Nakamatsu *et al.*, 2001). This indicates that PDV stimulates the fat body to elevate trehalose titers in hemolymph. On the other hand, trehalose titers in the parasitized hosts are reduced to zero during late parasitism, suggesting that the parasitoid larvae consume different amount of trehalose contained in the hemolymph between the hosts parasitized and injected with PDV plus venom (Nakamatsu *et al.*, 2001). A similar effect of PDV was reported in the egg-larval parasitoid and lepidopteran host association of *Chelonus inanitus*-*Spodoptera littoralis* (Kaeslin *et al.*, 2005). PDV causes an increased accumulation of glycogen in the whole host body from the production of dietary glucose. In the host final instar, it increases gluconeogenesis, which in turn leads to the increased level of free sugars circulating in the hemolymph. However, in the *C. kariyai*-*P. separata* association, the fat body of the parasitized host was completely consumed at 8 days post-parasitism while leaving the outer membrane of fat body tissue (Fig. 4) (Nakamatsu *et al.*, 2002). With the observation that the gut of second instar of parasitoid is well developed and filled with contents (Fig. 3C-2), it appears that the second instar of the endoparasitoid consumes the fat body as food and digests it to obtain nutrients. The parasitoid larvae also incorporate elevated trehalose and proteins in hemolymph in this association. Second instars of *C. kariyai* ingest and digest almost all of the visceral fat body and its contents with the aid of teratocytes. Different strategies probably exist in parasitoids to optimally utilize the food resource. Hence, it is important to determine the amount consumed by parasitoid larvae and the amount that is left unconsumed after larval parasitoid emergence.

Another pertinent question to be addressed is how endoparasitoid larvae specifically consume fat body of the host.

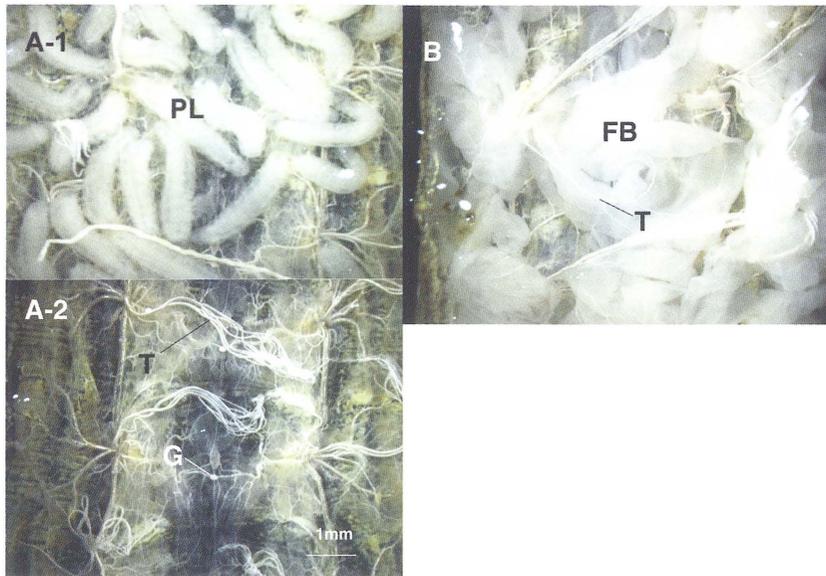


Fig. 4 Fat bodies from the hosts, *Pseudaletia separata*, parasitized (A) and nonparasitized (B) by *Cotesia kariyai*. A-1. Internal view of the host 1 day before parasitoid emergence. Host hemocoel is filled with second parasitoid larvae (PL). A-2. Internal view of the same host as shown in A-1, from which all parasitoid larvae were removed. Fat body completely consumed by parasitoid larvae. G: ganglion, T: trachea. B. Nonparasitized host at the same stage as shown in A. FB: fat body, T: trachea.

To begin with, histological studies have revealed that there are many teratocytes attached to the surface of the host fat body. Teratocytes are derived from serosal cells of the parasitoid embryo, and are released in the host hemolymph at hatching of the parasitoid and grow significantly in size in the host hemocoel during larval parasitoid development. It is known that teratocytes release many kinds of proteins to assist the growth of the parasitoid larvae (Dahlman, 1990; Dahlman and Vinson, 1993, Buron and Beckage, 1997; Hotta *et al.*, 2001; Hoy and Dahlman, 2002). In parasitized *P. separata* larvae, elevated lipid concentrations in the hemolymph are observed because regional digestion of the fat body matrix by teratocytes accelerated the release of the lipid granules from fat body. However, for teratocytes to function effectively, host tissues must be preconditioned with PDV plus venom (Wani *et al.*, 1990). PDVs are formed in calyx cells of female ovary (Tanaka *et al.*, 1987). In normal parasitization, PDV plus venom is injected with the eggs in the hosts at the time of oviposition. PDVs penetrate the host cells, such as fat body or hemocytes, with the help of venom and convert the physiological state of the cells with the expression of PDV genes (Hayakawa *et al.*, 1994; Webb and Strand, 2005).

Simultaneous transplantation of first instar with teratocytes proved that teratocytes are required for parasitoid larvae to achieve full growth in preconditioned hosts with PDV plus venom (Tanaka and Wago, 1990; Nakamatsu *et al.*, 2002). When parasitoid larvae alone are transplanted without teratocytes into preconditioned hosts, a large part of host fat body are remained, and the parasitoid larvae have poor growth. These studies show that parasitoid larvae feed on the available fat body of which cell matrix is broken down by teratocytes.

A further question arises as to how the teratocytes recognize the cell matrix of the fat body. Analysis of membrane fraction from the hosts injected with PDV plus venom by SDS-PAGE shows a different profile from that of nonparasitized control hosts (Fig. 5). Especially two bands, such as 53.1k and 30.7kDa proteins come out in membrane proteins from the hosts injected with PDV plus venom. These results may suggest a possibility that PDV function modifies the cell matrix of the fat body, which enables the teratocytes to recognize it.

Additionally, many lepidopteran hosts develop their testes during later larval stages. Inhibition of testis growth in the host is important because it enables the parasitoids to acquire more nutrients. In *P. separata*, the testis grows over ten-fold in volume during the last larval instar (Tanaka *et al.* 1994). This means that if the host testis develop normally, the food resource for parasitoid eggs or larvae decreases. In the *C. kariyai-P. separata* association, testis growth is inhibited from the start of parasitization. PDVs influence degeneration of the testis in parasitized hosts and have a direct effect on testis growth. In a study to prove this, the testis was transplanted into an isolated abdomen after 6 h-incubation with PDV plus venom *in vitro* and was stimulated with 20-hydroxyecdysone (20HE). The testis incubated with PDV plus venom lost the response to 20HE stimulus and gradually degenerated. In the testis growth of *P. separata*, spermatogonium repeats mitosis and becomes spermatocytes in the whole region until penultimate instar, or in the apical region of the last instar. The spermatocytes go through meiosis and become spermatids. PDV affects mitosis of the spermatogonium or meiosis of spermatocytes beyond G₂-M block in the cell cycle and leads to degeneration of the cells (Tagashira and Tanaka, 1998).

Parasitism by the solitary braconid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae), which attacks aphid hosts, also affects the host reproductive system. Artificial injection of venom has been shown to induce apoptosis in ovaries (Digilio *et al.*, 2000). *Manduca sexta* (Lepidoptera: Sphingidae) hosts parasitized by *Cotesia congregata* (Hymenoptera: Braconidae) also exhibit an inhibition in testicular growth and development (Reed and Beckage, 1997). In parasitized *Manduca* larvae, the degree of parasitic castration is correlated with the stage in which parasitization occurs (Reed and Beckage, 1997), which is found in as much as the same way in the *C. kariyai-P. separata* association. Inhibition of the reproductive organs at the time of parasitization is an effective mechanism that enables the parasitoid larvae to use the host in spite of a reduced food resource that is resulted from regulation with PDV and venom.

3. Relationship between the remaining amount of fat body and host behavior after parasitoid emergence

During development, endoparasitoids are bathed in an aquatic environment, but successful pupation can only be achieved by either consuming the host piecemeal or by emerging from the host into a dry environment. Parasitoid pupae are thus exposed and susceptible to attack from predatory insects and secondary hyperparasitoids. Some parasitoids, particularly species in the braconid subfamily Microgasterinae, appear to possess the 'use' of the living host larva after emergence, which has been suggested to act as a surrogate 'bodyguard,' and protect the parasitoid cocoon(s) (Brodeur and Vet, 1994). There are some associations involving microgasterines and their hosts where the

parasitized host die immediately after larval parasitoid emergence (for example, in the *Cotesia kariyai*-*Pseudaletia separata* association), and there are associations where the host may live for up to a week or more and behaves aggressively when disturbed (for example, in the *Cotesia glomerata*-*Pieris rapae* and *Manduca sexta*-*Cotesia congregata* associations). In the *M. sexta*-*C. congregata* association, the parasitoid cocoons are basally attached to the cuticle of the host dorsum (Adamo, 1998). When the parasitoids are about to emerge from the host, the parasitized *Manduca* larvae stop feeding and remain motionless. The cessation of feeding results in increased parasitoid survival during the pupal stages (Adamo *et al.*, 1997; Adamo, 1998). In the association between *P. separata* and its solitary endoparasitoid *Microplitis mediator*, the parasitized larva mounts the parasitoid cocoon with its caudal appendages and remains in this position until adult parasitoid emergence. The parasitized larva moves its head from side to side when disturbed (T. Tanaka, unpublished). In situations where the host larva remains alive and active after the emergence of parasitoid larva, over 50% of fat body is retained in the caterpillar. In contrast, larvae of *C. kariyai* consume virtually all host fat body content, so that the parasitized *P. separata* larva dies within several hours of parasitoid emergence (Table 1). Thus, in some associations the parasitoid larvae allow the host to remain alive and active temporarily by redirecting the host metabolism as an adaptive strategy against other natural enemies.

4. Emergence from the host hemocoel

Few studies have examined the specific mechanisms that allow parasitoids to emerge from their hosts. The full-grown parasitoid larvae must emerge to pupate under dry condition from the host hemocoel (see above). A better understanding of emergence behavior will provide a better comprehension of how the availability and accessibility of host resources are controlled by the parasitoid.

In the case of gregarious endoparasitoids, emergence from the host hemocoel is synchronized amongst the parasitoid brood. There must be some specific stimulus to emergence (such as increase of the dopamine titer) that is

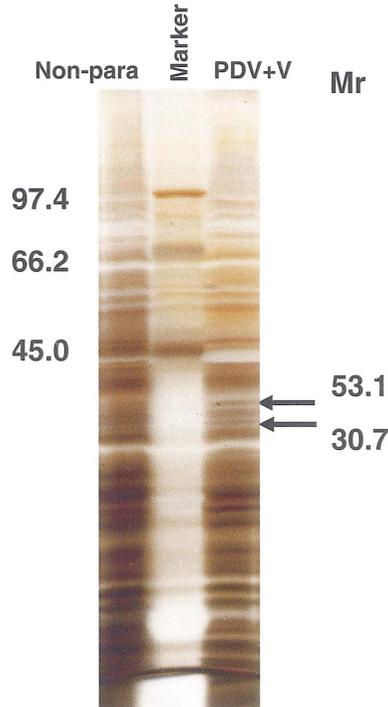


Fig. 5 Ten percent SDS-PAGE profile of proteins from fat body cell membrane fraction of nonparasitized control and 18 h after PDV plus venom injection. Host fat body after injection with PDV plus venom was homogenized, spinned at 10,000 g to remove cell debris, and supernatant was centrifuged at 100,000 g for 0.5 h to obtain membrane fraction.

Table 1 The ratio of the area of the fat body in the parasitized host to that in non-parasitized host abdomen measured on cross section.

Type*	Wasp	Host	Host fat body area ratio to whole (%)		Remaining fat body area (%)**
			Control (C)	Parasitized (P)	
I	<i>Cotesia congregata</i>	<i>Manduca sexta</i>	10.63	7.02	66.0
	<i>Microplitis mediator</i>	<i>Pseudaletia separata</i>	21.17	16.01	75.6
II	<i>Cotesia kariyai</i>	<i>Pseudaletia separata</i>	42.29	0.20	0.5
	<i>Cotesia ruficrus</i>	<i>Pseudaletia separata</i>	32.01	2.69	8.5

* Type I: The hosts survive for several days after parasitoid emergence. Type II: The hosts die immediately after parasitoid emergence.

** Remaining fat body area (%) = P/C × 100.

communicated amongst siblings and initiate them to begin emergence from the parasitized larva (Adamo, 1998). To successfully emerge from the host, the parasitoid larvae have to first perforate through the host integument with their mandibles. The parasitoid larva creates an emergence hole in the host integument by lifting up from inside the host and cutting a linear perforation with its mandibles. The parasitoid larvae ecdyse from the second to the third instar while emerging. A caudal 'foothold' is constructed by using exuviae of the second instar that adheres to the body of the parasitoid larva *via* a glycoprotein that it actively secretes. Furthermore, each foothold is combined with those of other siblings by decreasing the hemolymph volume of the host immediately before emergence (Nakamatsu *et al.*, submitted). This may have two implications. One is that the binding of the foothold by the entire parasitoid brood makes it easier to receive the initiation signal for emergence. Second, the parasitoid larvae absorb much of the remaining nutrients in the hemolymph just before emergence.

The emergence hole is finally sealed with exuviae immediately after emergence which prevents leakage of hemolymph from the wounds. If the hemolymph leaks from the emergence holes, this makes it difficult for the parasitoid larvae to construct cocoons and also increases the risk of bacterial infection. Moreover, in parasitized *M. sexta* larvae, *C. congregata* cocoons that are attached to dead host caterpillars experience much higher pupal mortality (90%) than cocoons attached to living caterpillars (18%) (Adamo, 1998). Successful cocoon formation in *C. kariyai* was also reduced when the hemolymph of the host larva leaked due to artificial removal of parasitoid larva with fine forceps during emergence (Nakamatsu and Tanaka, unpublished).

To better understand parasitoid host usage and food utilization strategies, particularly in koinobionts, attention should now be directed towards parasitoids in the large family Ichneumonidae, where the parasitoid larvae consume virtually all host contents before initiating pupation.

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