

# Influence of the parasitoid *Chelonus inanitus* and its polydnavirus on host nutritional physiology and implications for parasitoid development

Martha Kaeslin, Rita Pfister-Wilhelm, Beatrice Lanzrein\*

*Institute of Cell Biology, University of Berne, Baltzerstrasse 4, CH-3012 Bern, Switzerland*

Received 3 May 2005; received in revised form 10 August 2005; accepted 11 August 2005

## Abstract

*Chelonus inanitus* is a solitary egg-larval endoparasitoid, which feeds on host haemolymph during its internal phase. Parasitization induces in the host *Spodoptera littoralis* a precocious onset of metamorphosis and a developmental arrest in the prepupal stage. At this stage the parasitoid larva emerges from the host and consumes it. We show here that parasitization and the co-injected polydnaviruses affect the nutritional physiology of the host mainly in the last larval instar. Polydnaviruses cause a reduced uptake of food and an increase in the concentration of free sugars in the haemolymph and of glycogen in whole body. The parasitoid larva, along with polydnaviruses, causes a reduction of proteins in the host's plasma and an accumulation of lipids in whole body. Dilution of host haemolymph led to a reduced concentration of lipid in parasitoid larvae and a reduced survival rate. Thus, a sufficient concentration of nutrients in the host's haemolymph appears to be crucial for successful parasitoid development. Altogether, the data show that the parasitoid and the polydnavirus differentially influence host nutritional physiology and that the accumulated lipids and glycogen are taken up by the parasitoid in its haematophagous stage as well as through the subsequent external host feeding.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** Free sugars; Glycogen; Lipids; Nutrient uptake

## 1. Introduction

Parasitoids are free-living as adults and parasitic as larvae and are found mainly in the orders Hymenoptera and Diptera; the larvae develop inside (endoparasitoids) or outside (ectoparasitoids) their hosts, which are mostly insects of various developmental stages. Hosts do not survive and thus parasitoids play an important role in regulating the population of the hosts. Many endoparasitoids influence growth and development of their hosts but the intensity and time frame of host manipulation varies greatly between various parasitoid-host systems (Lawrence and Lanzrein, 1993; Vinson, 1990; Vinson and Iwantsch, 1980). Endoparasitic wasps use various means to manipulate the physiology of their hosts such as polydnaviruses, venom and teratocytes whereby the relative importance

and mode of action of these parasitoid-associated factors is to some extent dependent on the system (Quicke, 1997). Polydnaviruses replicate only in the calyx cells of endoparasitic wasps of the families Ichneumonidae and Braconidae and are injected into the host at oviposition along with the parasitoid egg. These symbiotic viruses play an essential role in abrogating the host's immune response and in affecting host development (Kroemer and Webb, 2004; Espagne et al., 2004; Turnbull and Webb, 2002). Venoms produced by the venom gland are also injected into the host at oviposition and in some systems seem to synergize the polydnaviruses (Stoltz, 1993; Soller and Lanzrein, 1996; Zhang et al., 2004). Teratocytes are cells derived from the parasitoid's serosal membrane and have only been observed in certain members of the families Braconidae, Scelionidae, and Platygasteridae (Dahlman and Vinson, 1993) and of the Chalcidoidea (Pedata et al., 2003). They are involved in causing host developmental arrest in several systems (Lawrence and Lanzrein, 1993; Pennacchio

\*Corresponding author. Tel. +41 31 631 46 77; fax: +41 31 631 46 16.  
E-mail address: [beatrice.lanzrein@izb.unibe.ch](mailto:beatrice.lanzrein@izb.unibe.ch) (B. Lanzrein).

et al., 1994) but were also seen to play a role in parasitoid nutrition by causing the breakdown of the fat body (Nakamatsu et al., 2002). In general, two different strategies to use the host as a nutritional source exist: idiobiont parasitoids paralyse their hosts and/or kill them quickly while koinobiont parasitoids develop in hosts that continue to feed and develop. In hosts of koinobiont parasitoids various effects of the parasitoid and parasitoid-associated factors on food consumption and metabolism have been observed, and these effects seem to vary according to the developmental stage of the parasitoid in many cases (Quicke, 1997; Vinson et al., 2001; Thompson, 1993; Harvey et al., 2004).

We are working with the solitary koinobiont egg-larval parasitoid *Chelonus inanitus* parasitizing *Spodoptera littoralis*, a major cotton pest. This parasitoid and its polydnavirus influence host growth and development markedly as hosts enter metamorphosis precociously and become developmentally arrested in the prepupal stage (Grossniklaus-Bürgin et al., 1994; Grossniklaus-Bürgin et al., 1998). Parasitoid removal and transplantation experiments showed that the parasitoid larva plays a key role in induction of the precocious metamorphosis and that it causes a premature drop in juvenile hormones (Pfister-Wilhelm and Lanzrein, 1996; Steiner et al., 1999). Furthermore, the parasitoid larva releases proteins into the host (Hochuli et al., 1999; Kaeslin et al., 2005a) and one of them appears to be involved in the precocious onset of metamorphosis (Kaeslin et al., 2005a). The developmental arrest in the prepupal stage is caused mainly by polydnaviruses (Soller and Lanzrein, 1996). However, coinjection of venom had a strong synergistic effect (Soller and Lanzrein, 1996) but venom proteins disappear within 1–2 days after parasitization (Kaeslin and Bühler, unpublished). The synergistic effect of venom appears thus to be restricted to the initial phase of parasitization when polydnaviruses enter host tissues. In the phase when the developmental arrest is induced, ecdysone production is reduced (Grossniklaus-Bürgin et al., 1998), several viral genes are upregulated (Bonvin et al., 2004) and for two an involvement in arresting development is suggested (Bonvin et al., 2005). Teratocytes are only seen during embryonic development (Kaeslin et al., 2005b) and do not appear to play a role in host regulation. Here, we investigated whether the developmental alterations caused by polydnavirus and the parasitoid larva are accompanied by alterations in the nutritional physiology of the host. We compared food intake and nutrient concentrations between nonparasitized, parasitized and polydnavirus containing larvae of the penultimate and last larval instar. The quantity of free sugars was measured in haemolymph and the plasma proteome was analysed. The concentration of lipids and glycogen was measured in whole body extracts of hosts as well as in parasitoid larvae and pupae. Furthermore, the effect of diluting host haemolymph on parasitoid nutrient concentrations and survival was investigated.

## 2. Materials and methods

### 2.1. Insects and X-ray irradiation

*S. littoralis* (Noctuidae) was reared at  $27 \pm 1^\circ\text{C}$  at a photoperiod of 14 h and fed an artificial diet. Adult *S. littoralis* larvae and diet were kindly provided by Syngenta AG, Stein, Switzerland. *S. littoralis* is a natural host of *C. inanitus* (Braconidae), which is a solitary egg-larval parasitoid. For parasitization, 27–32 h old eggs (kept at  $20^\circ\text{C}$  until parasitization) were used. Parasitization was always verified by dissection of a few host eggs, and an egg clutch was only used when 2–3 *C. inanitus* eggs were found per dissected egg. The parasitoid larva hatches approx. 16 h after parasitization (Kaeslin et al., 2005b) and remains in its first instar while the host develops from the first to early fifth instar. During its long first instar the parasitoid larva undergoes remarkable morphological changes (Grossniklaus-Bürgin et al., 1994). In the feeding phase of the host's fifth instar the parasitoid is in its second instar and feeds on host haemolymph. The host then precociously enters metamorphosis, digs into the soil, constructs a pupal cell and becomes a prepupa; the parasitoid emerges as a freshly moulted third instar larva from the precocious prepupal host. Nonparasitized larvae, on the other hand, pass through six larval instars. For details about parasitoid and host rearing and development see Grossniklaus-Bürgin et al. (1994). To study the role of polydnaviruses in the absence of a parasitoid larva, female wasps were irradiated with X-ray ( $146 \text{ Gy} \pm 10\%$ ) as described by Soller and Lanzrein (1996). These wasps parasitize normally and inject polydnaviruses and venom but the egg is nonviable. Larvae developing from eggs parasitized with X-ray irradiated wasps are designated as X-ray parasitized. These larvae, similarly to larvae developing from eggs injected with calyx fluid and venom, pass through six larval instars, dig into the soil, construct a pupal cell and then become developmentally arrested in the prepupal stage (Soller and Lanzrein, 1996). An overview on the effects of parasitization and X-ray parasitization on host development has been presented earlier (Lanzrein et al., 2001). In many Figures the data are presented by comparing the physiologically corresponding instars, i.e. the last instar of nonparasitized and X-ray parasitized larvae is the sixth instar and for parasitized larvae it is the fifth instar.

### 2.2. Measurement of food intake

Diet was weighed before being offered to a certain number of larvae at the beginning of an instar. At the end of the instar diet was weighed again and the amount of ingested food per larva was calculated. To correct for the loss of humidity, diet was weighed and incubated under the same conditions but without larvae.

### 2.3. Collection of insect material and analysis of free sugars, glycogen, lipids and proteins

Larvae were weighed and anaesthetized on ice. For whole body extracts, individual larvae were put into test tubes (16 mm diameter). In the case of parasitized larvae, the parasitoid was first removed, washed in insect Ringer, weighed and then placed into a test tube. To reduce loss of host material, the slide on which the parasitoid had been removed was washed with ethanol, which was added to the test tube. All collected larvae were covered with 100% ethanol which was then evaporated at 90 °C. Thereafter, 200–1000 µl (depending on the size of the larva) of 2% Na<sub>2</sub>SO<sub>4</sub> were added. *S. littoralis* larvae were homogenized with a motor driven Polytron (PT 1300D, Kinematica AG, Switzerland) while *C. inanitus* were homogenized with a glass mortar. For collection of haemolymph, one of the last pseudopodia of a larva was cut away and 5 µl of the out flowing haemolymph was taken up with a Gilson pipette and put into a test tube. Twenty microlitres of ethanol was added and then evaporated at 90 °C.

For the analysis of free sugars in haemolymph and glycogen in whole body extracts, the anthrone reaction (Van Handel, 1985a) with glucose as standard (0.1% glucose in 25% ethanol) was used; lipids of whole body extracts were measured by the vanillin–phosphoric acid reaction as described (Van Handel, 1985b) with soybean oil as standard (0.1% soybean oil in chloroform; Sigma). For measurement of glycogen and lipids, 2.8 ml chloroform-methanol (1:1) was added to the test tubes containing the whole body extracts, followed by vortexing and centrifugation at 1300g for 10 min. The pellet was used for measurement of glycogen while the supernatant was mixed with 2 ml H<sub>2</sub>O, vortexed and centrifuged at 1300g for 10 min. The upper phase was then discarded and the lower phase was dried at 90 °C and used for measurement of lipids. Data were statistically tested with student *t*-Test. For analysis of plasma proteins by 2D-gel electrophoresis, haemolymph was collected as described above and centrifuged at 100g for 10 min at 4 °C to gently spin down the haemocytes (Cook et al., 1984). Plasma was stored at –20 °C. The plasma of at least three larvae of a particular stage was pooled and three different pools were analysed per stage. 2D-gel electrophoresis was carried out as described (Kaeslin et al., 2005a; Görg et al., 2000).

### 2.4. Haemolymph dilution experiments

A fine cut was made in one of the last pseudopodia of fourth instar hosts at the head capsule slippage (hcs) stage and 5–10 µl haemolymph were gently squeezed out. As hosts contain approx. 25–30 µl haemolymph at that stage (Steiner et al., 1999) the quantity removed represents between a fifth to a third of the total haemolymph. Five microlitres insect Ringer was then injected with a 10 µl Hamilton syringe, which was inserted through the same opening in the pseudopodium. As a negative control the

Hamilton syringe was inserted, haemolymph was drawn out and injected again. The Hamilton syringe was washed with distilled H<sub>2</sub>O after each control injection. To measure the survival rate of the parasitoids, host larvae were kept on diet and emergence of the wasps was registered. Statistical analysis was with  $\chi^2$ -test. To analyse the effect of host haemolymph dilution on parasitoid nutrients, parasitoids were removed from their hosts at the digging stage, weighed and then their lipid and glycogen content was measured as described above.

## 3. Results

### 3.1. Uptake of food

We already know that parasitized larvae reach a much lower weight than nonparasitized larvae as they enter metamorphosis precociously in the fifth instar; interestingly, also X-ray parasitized larvae, which contain polydnavirus only, reach a lower weight than nonparasitized larvae (Lanzrein et al., 2001; Grossniklaus-Bürgin et al., 1998). To find out whether this is due to altered feeding activity we compared the food intake in the penultimate and last larval instar between nonparasitized, parasitized and X-ray parasitized larvae. Fig. 1 shows that parasitized larvae eat significantly less in their penultimate and last instar compared to nonparasitized and X-ray parasitized larvae; but when values are compared for the fifth instar, parasitized larvae take up similar quantities of food as nonparasitized and X-ray parasitized larvae. In the sixth instar, parasitized larvae take up similar quantities of food as nonparasitized and X-ray parasitized larvae. In the sixth instar, X-ray parasitized larvae consumed significantly less diet than nonparasitized larvae while no difference was seen in the fifth instar indicating that polydnaviruses influence food uptake only in the last instar.

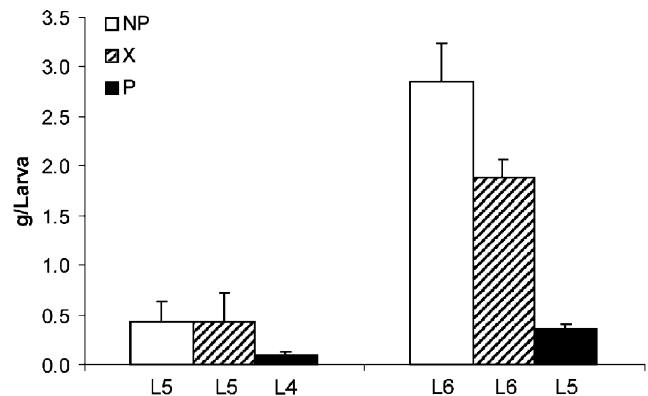


Fig. 1. Uptake of food in g per larva in the penultimate and last larval instar of nonparasitized (NP), X-ray parasitized (X) and parasitized (P) larvae. Data are means + SD from three independent experiments and are based on 10 measurements each; data were analysed with student *t*-Test. In the penultimate instar, parasitized larvae eat less than nonparasitized ( $P = 0.023$ ) and X-ray parasitized larvae ( $P = 0.06$ ). In the last instar, parasitized larvae eat significantly less than nonparasitized and X-ray parasitized larvae ( $P$  for both  $<0.001$ ) and X-ray parasitized larvae eat significantly less than nonparasitized larvae ( $P = 0.009$ ).

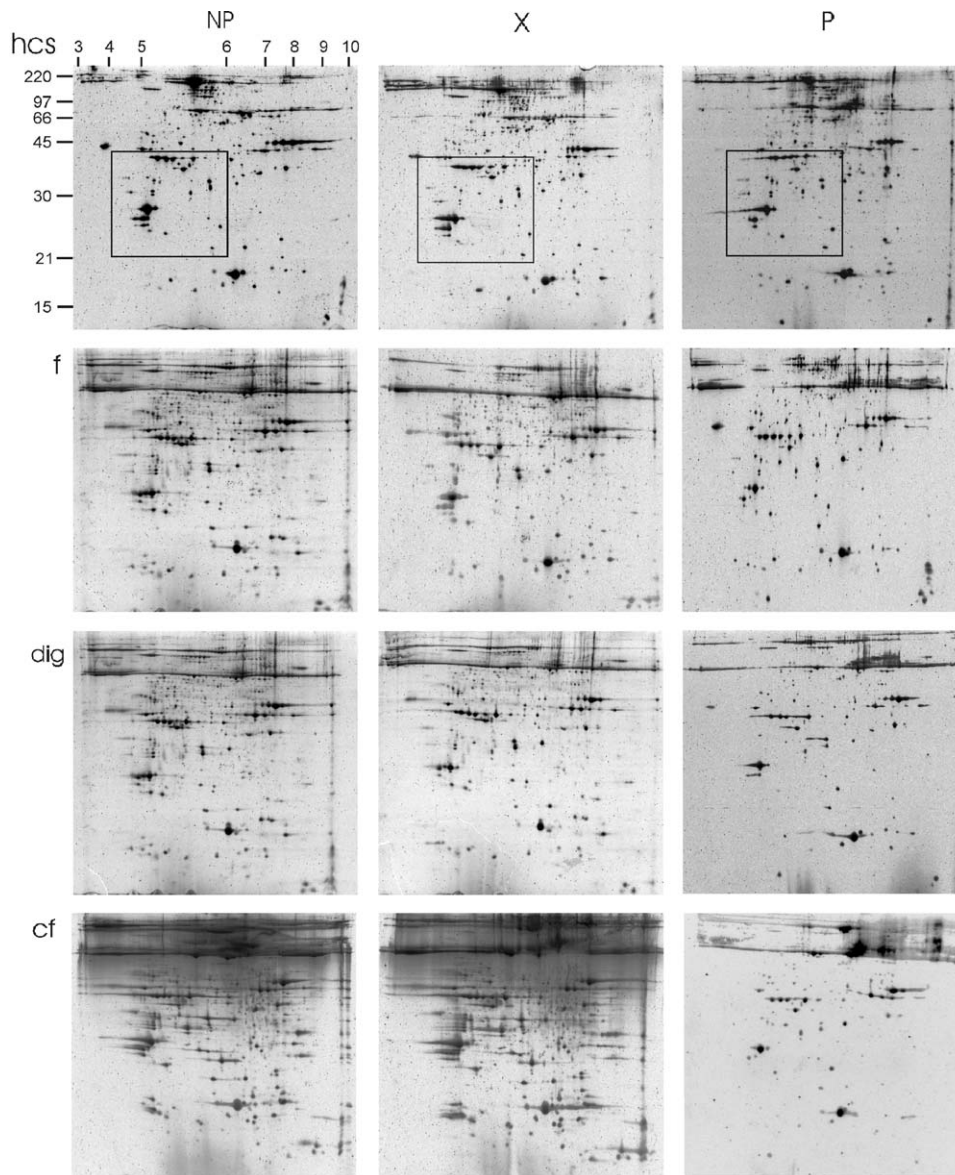


Fig. 2. Ruthenium-stained 2D-gels of plasma of nonparasitized (NP), X-ray parasitized (X) and parasitized (P) *S. littoralis* larvae in the penultimate and last larval instar. Parasitized larvae enter metamorphosis precociously in L5 and the gels show the head capsule slippage (hcs) stage of the penultimate larval instar (= L4 for P and L5 for NP and X) and the feeding (f), digging (dig) and cell formation (cf) stage of the last larval instar (= L5 for P and L6 for NP and X). The marked sections of the hcs stage are shown in more detail in Fig. 3.

### 3.2. Analysis of proteins and free sugar in the haemolymph

In an earlier investigation we had observed that the concentration of proteins is significantly lower in parasitized larvae than in X-ray parasitized and nonparasitized larvae from the digging stage onwards; in the pupal cell formation stage, values for the latter were between 60–70 mg/ml while those for parasitized larvae were around 20 mg/ml (Kaeslin et al., 2005a). Here we analysed whether the lower protein concentration in parasitized larvae is due to the presence of fewer proteins or to smaller quantities or both. Fig. 2 shows a comparison of the plasma proteome of nonparasitized, X-ray parasitized and parasitized larvae from the end of the penultimate larval instar up to the pupal cell formation

stage. It reveals a similar pattern at the head capsule slippage stage; from then on fewer proteins were seen in the plasma of parasitized larvae compared to nonparasitized and X-ray parasitized larvae. The difference was most obvious at the cell formation stage when both the number and the quantity of proteins were much smaller in parasitized larvae. Nevertheless, some proteins were abundant in all stages analysed even in parasitized larvae, and these thus seem to be essential for the host. To find out whether the plasma proteome at the hcs stage of parasitized fourth instar larvae and of X-ray parasitized and nonparasitized fifth instar larvae (Fig. 2 top) is typical for the penultimate instar but different from previous larval instars we made a comparison including the fourth instar hcs stage

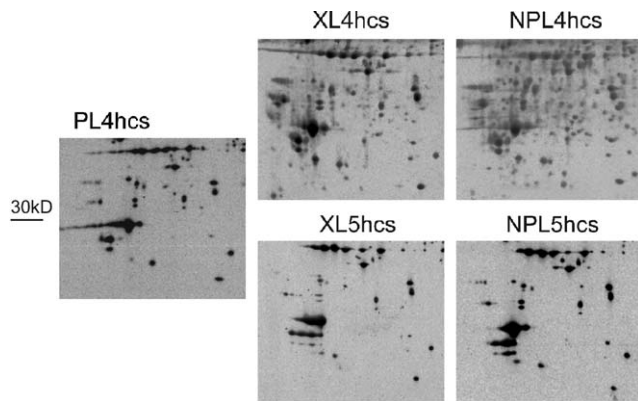


Fig. 3. Sections as depicted from the top of Fig. 2 of Ruthenium-stained 2D-gels of plasma of nonparasitized (NP), parasitized (P) and X-ray parasitized (X) *S. littoralis* larvae in the 4th and 5th larval instar in the head capsule slippage (hcs) stage.

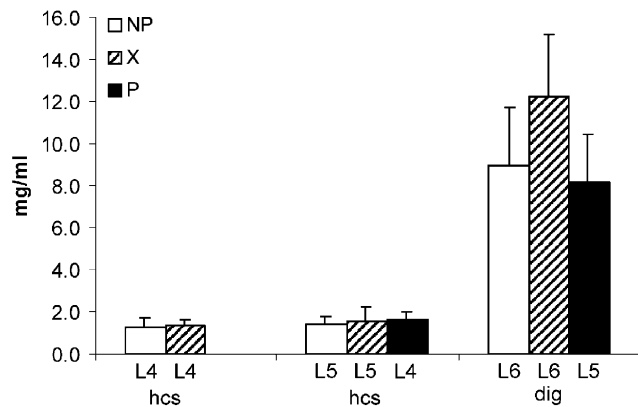


Fig. 4. Concentration of free sugars in the haemolymph of nonparasitized (NP), X-ray parasitized (X) and parasitized (P) larvae at the head capsule slippage (hcs) stage of L4 and L5 and at the digging (dig) stage. As parasitized larvae enter metamorphosis precociously in L5, the bars of parasitized larvae are shown along with the physiologically corresponding stages of nonparasitized and X-ray parasitized larvae. Data are means + SD and are based on 10–20 measurements each. X-ray parasitized larvae had a significantly higher haemolymph sugar concentration in the digging stage compared to nonparasitized and parasitized larvae (student *t*-Test,  $P < 0.001$ ).

of nonparasitized and X-ray parasitized larvae. Fig. 3 shows a representative section of the gel which clearly illustrates that the plasma proteome of fourth instar parasitized larvae is very similar to that of fifth instar X-ray parasitized and nonparasitized larvae but very different from that of fourth instar X-ray parasitized and nonparasitized larvae. In the latter two, many proteins are seen which are no longer visible in the penultimate instar. These findings show that, at the plasma proteome level, the precocious onset of metamorphosis in parasitized larvae is already manifested at the head capsule slippage stage of the fourth instar. Data on the concentration of free sugars in haemolymph are presented in Fig. 4. They show that concentrations are similar in nonparasitized, X-ray parasitized and parasitized larvae at the head capsule slippage stage of the penultimate

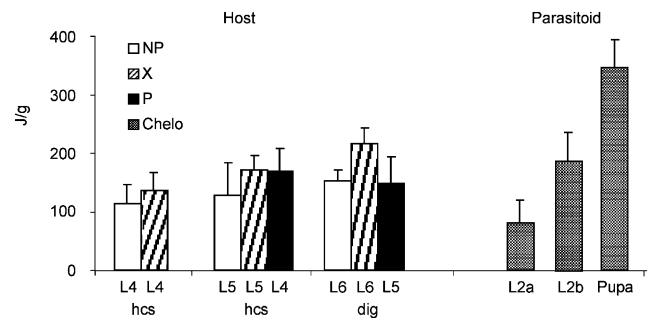


Fig. 5. Glycogen concentration in J/g fresh weight in whole body homogenates of nonparasitized (NP), X-ray parasitized (X) and parasitized (P) larvae at the head capsule slippage (hcs) stage of L4 and L5 and at the digging (dig) stage. Bars of parasitized larvae are shown along with the physiologically corresponding stages of nonparasitized and X-ray parasitized larvae. At the right hand side values for the parasitoid larva (Chelo) dissected out of digging stage hosts are shown whereby L2a are small second instar larvae with a weight between 0.6–5.9 mg and L2b are large second instar larvae with a weight between 6–16 mg. Pupae are young, namely of stages 1–2 according to Albrecht et al. (1994). Data are means + SD and are based on 10–15 measurements each. Data were analysed with student *t*-Test. In the hcs stage of the penultimate larval instar, X-ray parasitized and parasitized larvae had a significantly higher glycogen concentration than nonparasitized larvae ( $P = 0.014$ ). In the digging stage, X-ray parasitized larvae had a significantly higher glycogen concentration than nonparasitized and parasitized larvae ( $P < 0.001$ ).

instar. In the digging stage, the concentrations were much higher in all, but values were significantly higher in X-ray parasitized larvae than in parasitized and nonparasitized larvae. This indicates that polydnviruses positively affect free sugars in haemolymph in the last instar; the lower value in parasitized larvae suggests that these sugars are taken up by the parasitoid larva.

### 3.3. Analysis of glycogen and lipid in whole body homogenates of host and parasitoid

Glycogen concentrations in penultimate and last instar larvae and in parasitoid larvae and pupae are presented in Fig. 5. Values were higher in parasitized and X-ray parasitized larvae than in nonparasitized larvae at the head capsule slippage stage of the penultimate instar; at the digging stage, values were significantly higher in X-ray parasitized larvae than in nonparasitized and parasitized larvae. These data indicate that polydnviruses cause an increase of the glycogen concentration in the host and that the additional presence of a second instar parasitoid larva counteracts this effect. In the parasitoids the glycogen concentration raised rapidly in the final endoparasitic haematophagous phase and also in the phase of external host feeding (Fig. 5). The results of the parallel lipid analysis are presented in Fig. 6 and give a different picture. At the head capsule slippage stage of the penultimate instar, the lipid concentration was significantly higher in parasitized and X-ray parasitized larvae than in nonparasitized larvae indicating that polydnviruses stimulate the accumulation of lipids. In the digging stage, the lipid concentration was much

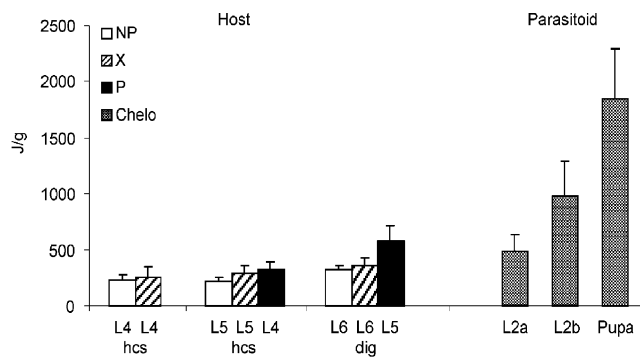


Fig. 6. Lipid concentration in J/g fresh weight in whole body homogenates of nonparasitized (NP), X-ray parasitized (X) and parasitized (P) larvae at the head capsule slippage (hcs) stage of L4 and L5 and at the digging (dig) stage. Bars of parasitized larvae are shown along with the physiologically corresponding stages of nonparasitized and X-ray parasitized larvae. At the right-hand side values for parasitoid larvae and pupae (Chelo) are shown (for details see caption to Fig. 5). Data are means + SD and are based on 10–15 measurements each. Data were analysed with student *t*-Test. In the hcs stage of the penultimate larval instar X-ray parasitized and parasitized larvae had a significantly higher lipid concentration than nonparasitized larvae ( $P < 0.001$ ). In the digging stage, parasitized larvae had a significantly higher lipid concentration than nonparasitized and X-ray parasitized larvae ( $P < 0.001$ ).

higher in parasitized larvae than in X-ray parasitized and nonparasitized larvae, and no difference was seen between the latter two. These results show that the parasitoid larva along with polydnnaviruses, push the host into an obese direction at the end of the endoparasitic life. The values measured in the parasitoid indicate that it accumulates very high quantities of lipids towards pupation (Fig. 6).

### 3.4. Haemolymph dilution experiments

Apparently, the parasitoid larva and polydnnavirus influence host nutritional physiology in the late endoparasitic phase when the parasitoid larva consumes host haemolymph. To analyse whether these alterations in the composition of host haemolymph are vital for parasitoid development we artificially diluted the haemolymph of parasitized larvae at the head capsule slippage stage of the penultimate larval instar with insect Ringer. As a control, larvae were similarly treated without dilution of haemolymph (see Material and Methods for details of the procedure). Fig. 7 shows that from control-treated larvae approx. 75% adult wasps emerged. However, from larvae with diluted haemolymph, only approx. 50% adult wasps emerged. Inspection of the remnants of the 50%, which did not survive revealed that 35% died as larvae inside the host, 49% as external third instar larvae or prepupae and 16% as pupae. Among the 25% dying after the control treatment, 32% died in the host, 50% as third instar larvae or prepupae and 18% as pupae. To test to which extent dilution of host haemolymph affects the nutrient concentration in the parasitoid larva, host haemolymph was diluted in penultimate instar larvae at the head capsule slippage stage as described above. In the digging stage of the host, parasitoids

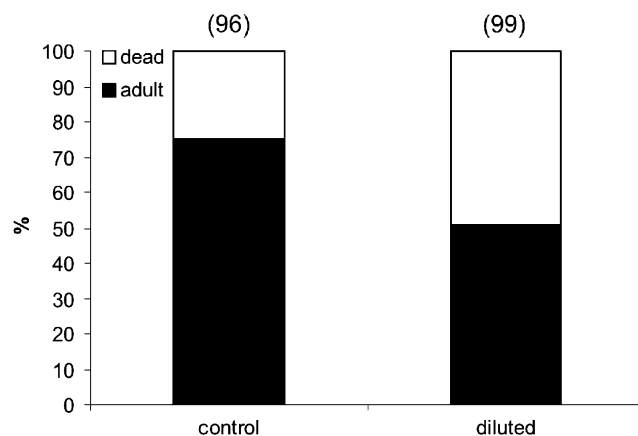


Fig. 7. Effect of dilution of the host haemolymph in the L4 head capsule slippage stage on parasitoid survival. The number of adult wasps emerging (black bars) and of dead larvae or pupae (white bars) was registered. Numbers in parentheses represent numbers of treated larvae from five independent experiments. The proportion of wasps emerging after host haemolymph dilution was significantly lower than after the control treatment according to  $\chi^2$ -test:  $df = 1$ ;  $\chi^2 = 12.5$ ;  $P = 0.0004$ .

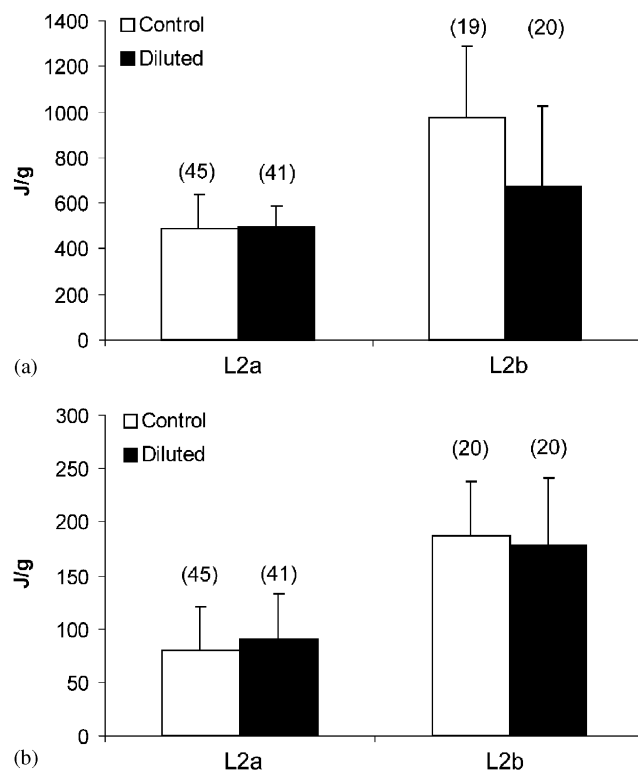


Fig. 8. Lipid (a) and glycogen (b) concentration in whole body homogenates of *C. inanis* larvae in J/g fresh weight after dilution of the host's haemolymph in the L4 head capsule slippage stage. Small (L2a) and large (L2b) second instar larvae were dissected out of digging stage hosts (for details see caption to Fig. 5). Data are means + SD and the number of measurements is given in parentheses. The lipid concentration of L2b parasitoids was significantly lower after haemolymph dilution (student *t*-Test,  $P < 0.003$ ).

were dissected out and their lipid and glycogen concentrations were analysed (Fig. 8). Parasitoid larvae of the weight class 6–16 mg (= L2b), which are close to egression from

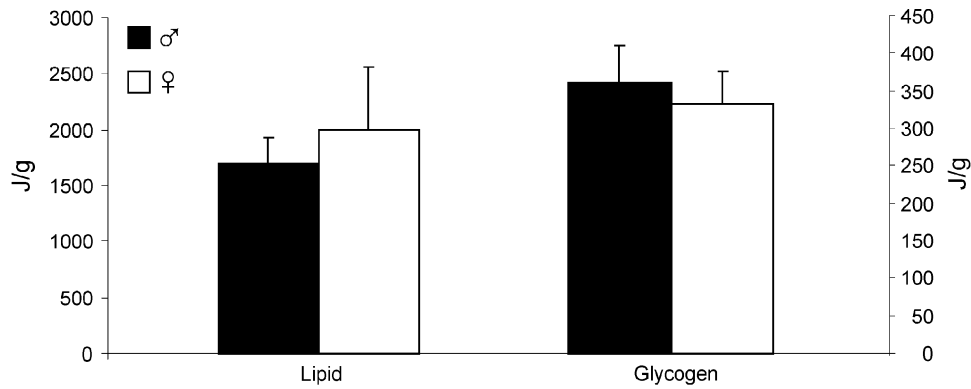


Fig. 9. Lipid and glycogen concentration in J/g fresh weight in whole body homogenates of female and male *C. inanitus* pupae of stages 1–2 according to Albrecht et al. (1994). Data are means+SD and are based on 20 measurements each. Lipid concentration is higher in female pupae (student *t*-Test,  $P < 0.02$ ) while glycogen concentration is higher in male pupae ( $P < 0.02$ ).

the host, had a significantly lower lipid concentration when the haemolymph of their hosts had been diluted; in the L2a stage (weight class 0.5–5 mg) this difference was not yet seen (Fig. 8a). No difference was seen in glycogen concentrations (Fig. 8b). Thus, dilution of host haemolymph leads to a lower lipid concentration in the parasitoid and this may be related to the reduced survival rate observed in Fig. 7. We also compared glycogen and lipid concentrations between female and male pupae (Fig. 9). The data show that females had a significantly higher concentration of lipids than males while males had a significantly higher concentration of glycogen than females. This indicates a sex-specific accumulation of lipid and glycogen.

## 4. Discussion

### 4.1. General observations

We show here that parasitization by *C. inanitus* influences the nutritional physiology of its host *S. littoralis* in several respects. Alterations were seen in all major physiochemicals and were either induced by the polydnavirus alone or the parasitoid larva together with the polydnavirus. Major effects of parasitization and X-ray parasitization were seen in the last instar whereby the polydnavirus appeared to act mainly on the carbohydrate metabolism while the parasitoid larva, along with the polydnavirus, stimulated lipid accumulation and caused a reduction in plasma proteins. It was observed also in other parasitoid-host systems that parasitization has strongest effects on host nutritional physiology in the last instar of the host (Vinson and Iwantsch, 1980; Vinson et al., 2001). The nutrient demands of the parasitoid increases towards the end of the endoparasitic phase and selective depletion of particular host resources has been described (Quicke, 1997). In various species, including *C. inanitus*, the parasitoid moults into its second instar only after the host has moulted into its last instar (Grossniklaus-Bürgin et al., 1994; Vinson et al., 2001). In first instar parasitoids, nutrients are possibly taken up from the host haemolymph

through the epidermis and/or the anal vesicle (de Eguileor et al., 2001; Edson and Vinson, 1977; Giordana et al., 2003; Vinson and Iwantsch, 1980) while in second instar larvae direct feeding on haemolymph is often observed (Quicke, 1997; Hawlitzky and Boulay, 1974a). This appears also to be the case for *C. inanitus* which has sucking mouth parts in its second instar (Grossniklaus-Bürgin et al., 1994).

### 4.2. Parasitism-induced alterations of host proteins

Parasitization by *C. inanitus* has a marked effect on the plasma proteome in the last instar as the number and quantity of proteins decreased from the feeding to the cell formation stage in parasitized larvae while it increased in nonparasitized and X-ray parasitized larvae (Fig. 2). Similarly, measurement of protein concentrations in haemolymph showed that values increased from approx. 18 mg/ml at feeding to 60 mg/ml at cell formation in both nonparasitized and X-ray parasitized larvae while they stayed around 15 mg/ml in parasitized larvae in the same interval (Kaeslin et al., 2005a). These observations suggest that the parasitoid larva feeds intensively on haemolymph proteins in the final phase of its endoparasitic development. In the closely related *Chelonus* sp., arylphorins were seen to be taken up by the parasitoid second instar larva (Kunkel et al., 1990). A reduction in haemolymph proteins in the host in the late endoparasitic phase was also observed in other parasitoid-host systems (Bae and Kim, 2004; Nakamatsu et al., 2001; Vinson, 1990). The most obvious developmental effect of *C. inanitus* on its host is induction of a precocious onset of metamorphosis in the fifth instar; interestingly, the comparative analysis of the plasma proteome revealed that this developmental manipulation is already manifested at the head capsule slippage stage of the penultimate instar (Fig. 3). At this stage, the juvenile hormone titre of parasitized larvae drops to undetectable levels and remains low while it immediately increases again in nonparasitized and X-ray parasitized larvae (Steiner et al., 1999). These observations suggest that the precocious

onset of metamorphosis is already initiated in the course of the fourth larval instar.

#### 4.3. Effect of parasitism and polydnavirus on food uptake

Parasitism has been observed to reduce food uptake and growth in several systems (Alleyne and Beckage, 1997; Bae and Kim, 2004; Beckage and Riddiford, 1978; Nakamatsu et al., 2001; Vinson and Iwantsch, 1980; Vinson et al., 2001). *S. littoralis* parasitized by *C. inanitus* consumed massively less food than nonparasitized and X-ray parasitized larvae, mainly because they enter metamorphosis precociously in the fifth instar (Fig. 1). Polydnavirus was seen to reduce food uptake, compared to nonparasitized larvae, only in the last instar (Fig. 1); in this instar, X-ray parasitized larvae weigh significantly less than nonparasitized larvae although differences in head capsule widths become manifested earlier (Grossniklaus-Bürgin et al., 1998; Lanzrein et al., 2001). The reduced food intake of parasitized and X-ray parasitized larvae is also reflected in the production of faeces: in the last larval instar nonparasitized larvae released approx. 0.3 g faeces (dry weight), X-ray parasitized larvae approx. 0.15 g and parasitized larvae approx. 0.04 g (Kwiatkowski and Lanzrein, unpublished). A growth retarding effect of calyx fluid has been described also in other systems (Vinson and Iwantsch, 1980; Vinson, 1990; Vinson et al., 2001) and in one system a reduced rate of faeces production after polydnavirus injection has been observed (Doucet and Cusson, 1996). It is not clear whether the reduced food consumption is due to a direct effect of polydnaviruses on feeding activity; it is also conceivable that it is mediated indirectly by the increase in concentration of free sugars in haemolymph of X-ray parasitized last instar larvae (Fig. 4). Interestingly, a polydnavirus-induced increase in haemolymph trehalose levels was also associated with a reduced growth in two other systems (Dahlman and Vinson, 1977; Nakamatsu et al., 2001).

#### 4.4. Effect of parasitism and polydnavirus on host sugars and lipids and uptake by the parasitoid

In the penultimate larval instar no difference in the concentration of free sugars in haemolymph was seen between nonparasitized, parasitized and X-ray parasitized larvae; in the last larval instar, however, the concentration increased massively and reached highest values in X-ray parasitized larvae (Fig. 4). This suggests that in the final endoparasitic phase polydnaviruses positively influence the quantity of haemolymph sugars and that the second instar parasitoid larva ingests them. In another system, injection of calyx fluid was seen to increase haemolymph trehalose levels (Dahlman and Vinson, 1977). The comparison between other nonparasitized and parasitized larvae reveals a mixed picture: in some, sugar levels in haemolymph were elevated but not in others, and the effect was also dependent on the time point of the measurement after parasitization (Hoch et al., 2002; Thompson, 1982;

Thompson and Binder, 1984; Thompson et al., 1990; Vinson, 1990; Vinson and Iwantsch, 1980). The glycogen concentration in whole body homogenates was significantly higher in parasitized and X-ray parasitized larvae than in nonparasitized larvae at the head capsule slippage stage of the penultimate larval instar; in the digging stage, however, it was higher only in X-ray parasitized larvae (Fig. 5). These data together with the data on haemolymph sugars (Fig. 4) could be interpreted as follows. Polydnavirus leads to an increased accumulation of glycogen from the dietary glucose. In the final instar it also increases gluconeogenesis, which in turn leads to the increased levels of free sugars in haemolymph. Some of them are then taken up by the second instar parasitoid larva drinking haemolymph; after emergence from the host, also the stored glycogen is ingested as the external third instar parasitoid larva eats its host up. This uptake is reflected by the high glycogen values measured in parasitoid young pupae (Fig. 5). In the related species *Phanerotoma flavitestacea* parasitization was also seen to lead to an increased accumulation of glycogen in the host which was then taken up by the parasitoid larva (Hawlitzky and Boulay, 1974b; Hawlitzky and Boulay, 1986). Elevated glycogen concentrations have been reported also for other parasitized larvae (Thompson et al., 1990; Thompson, 1982) and parasitism has been proposed to redirect exogenous glucose into trehalose on one hand and glycogen on the other hand (Thompson and Dahlman, 1998). Apparently this energy reserve is very important for pupal-adult development and adult life (Giron and Casas, 2003). The higher glycogen content seen in males compared to females (Fig. 9) suggests that glycogen stores may play an important role in providing males with energy to search for females.

Lipids are also of great importance for the life of adult parasitoids. Because de novo synthesis of lipids from the sugar they feed on as adults is not possible in meaningful quantities (Giron and Casas, 2003; Olson et al., 2000) the parasitoid has to accumulate the lipids in the larval stage. Effects of parasitization on host lipids have been reported for several systems (Thompson, 1986; Vinson and Iwantsch, 1980). In our system, the lipid concentration was much higher in parasitized than in nonparasitized and X-ray parasitized larvae in the digging stage (Fig. 6), indicating that the parasitoid larva, in presence of polydnavirus, induces an accumulation of lipids in the host. In the parasitoid, the lipid concentration was seen to increase massively in the second and third instar (Fig. 6); this indicates that some lipid is taken up in the late endoparasitic phase but that a large portion is ingested by feeding externally on the host. Uptake of lipids by the parasitoid through haemolymph drinking in the second instar and external feeding in the third instar have also been demonstrated in the closely related parasitoid *P. flavitestacea* (Hawlitzky and Mainguet, 1976; Hawlitzky and Boulay, 1974a; Hawlitzky and Boulay, 1979). Dilution of host haemolymph at the head capsule slippage stage of

the penultimate larval instar was seen to reduce the lipid concentration in late second instar parasitoids (Fig. 8). After such treatment less adult wasps emerged (Fig. 7) which points to the importance of lipids for successful parasitoid development. Some parasitoids died as prepupae or pupae but many did not manage to egress from the host. In the latter case, it is conceivable that death was caused by a reduced block of host pupation as dilution of haemolymph in X-ray parasitized larvae has been shown to partially prevent the developmental arrest in the soft prepupal stage (Kaeslin et al., 2005a). In the closely related species *P. flavitestacea*, it was shown that the accumulation of lipids during the internal and external larval stages influences adult size, fecundity and longevity (Hawlitzky, 1980). Young female pupae of *C. inanitus* contained more lipids than males (Fig. 9), which they might use in egg production. Other parasitoids feed on the host's fat body already in the endoparasitic stage (Blackburn et al., 2002; Nakamatsu et al., 2002); for *Cotesia kariyai* it was shown that teratocytes play a major role as they attach to the fat body and locally destroy it which allows the parasitoid to ingest the fat body components (Nakamatsu et al., 2002).

In conclusion, these data show that *C. inanitus* together with its polydnavirus influence host nutritional physiology along with effects on host development. All these manipulations contribute to successful parasitoid development and are well coordinated. The host consumes and converts nutrients for its own growth and development but also serves the needs of the developing parasitoid. Polydnavirus increases haemolymph and whole body sugars in the host in addition to protecting the parasitoid from encapsulation and preventing host pupation. The parasitoid larva, along with polydnavirus, induces an accumulation of lipids in addition to causing a precocious onset of metamorphosis.

## Acknowledgements

We express our thanks to Syngenta AG, Stein, Switzerland for providing us with adult *Spodoptera littoralis* and the diet for rearing the larvae. We also thank Prof. Hans Briegel, University of Zürich, for technical advices and support. Financial support from the Swiss National Science Foundation (grant 3100-063444.00 to B.L.) is also gratefully acknowledged.

## Reference

- Albrecht, U., Wyler, T., Pfister-Wilhelm, R., Gruber, A., Stettler, P., Heiniger, P., Kurt, E., Schümperli, D., Lanzrein, B., 1994. Polydnavirus of the parasitic wasp *Chelonus inanitus* (Braconidae): characterization, genome organization and time point of replication. *Journal of General Virology* 75, 3353–3363.
- Alleyne, M., Beckage, N.E., 1997. Parasitism-induced effects on host growth and metabolic efficiency in tobacco hornworm larvae parasitized by *Cotesia congregata*. *Journal of Insect Physiology* 43, 407–424.
- Bae, S., Kim, Y., 2004. Host physiological changes due to parasitism of a braconid wasp, *Cotesia plutellae*, on diamondback moth, *Plutella xylostella*. *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology* 138, 39–44.
- Beckage, N.E., Riddiford, L.M., 1978. Developmental interactions between the tobacco hornworm *Manduca sexta* and its braconid parasite *Apanteles congregatus*. *Entomologia Experimentalis et Applicata* 23, 139–151.
- Blackburn, M.B., Gelman, D.B., Hu, J.S., 2002. Co-development of *Encarsia formosa* (Hymenoptera:Aphelinidae) and the Greenhouse Whitefly, *Trialeurodes vaporariorum* (Hymenoptera: Aleyrodidae): a histological examination. *Archives of Insect Biochemistry and Physiology* 51, 13–26.
- Bonvin, M., Kojic, D., Blank, F., Annaheim, M., Wehrle, I., Wyder, S., Kaeslin, M., Lanzrein, B., 2004. Stage-dependent expression of *Chelonus inanitus* polydnavirus genes in the host and the parasitoid. *Journal of Insect Physiology* 50, 1015–1026.
- Bonvin, M., Marti, D., Wyder, S., Kojic, D., Annaheim, M., Lanzrein, B., 2005. Cloning, characterisation and analysis by RNAi of various genes of the *Chelonus inanitus* polydnavirus. *Journal of General Virology* 86, 973–983.
- Cook, D.I., Stoltz, D.B., Vinson, S.B., 1984. Induction of a new hemolymph glycoprotein in larvae of permissive hosts parasitized by *Campoletis sonorensis*. *Insect Biochemistry* 14, 45–50.
- Dahlman, D.L., Vinson, S.B., 1977. Effect of calyx fluid from an insect parasitoid on host hemolymph dry weight and trehalose content. *Journal of Invertebrate Pathology* 29, 227–229.
- Dahlman, D.L., Vinson, S.B., 1993. Teratocytes: developmental and biochemical characteristics. In: Beckage, N.E., Thompson, S.N., Federici, B.A. (Eds.), *Parasites and Pathogens of Insects*, vol. 1. Academic Press, San Diego, pp. 145–165.
- de Eguileor, M., Grimaldi, A., Tettamanti, G., Valvassori, R., Leonardi, M.G., Giordana, B., Tremblay, E., Digilio, M.C., Pennacchio, F., 2001. Larval anatomy and structure of absorbing epithelia in the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera, Braconidae). *Arthropod Structure and Development* 30, 27–37.
- Doucet, D., Cusson, M., 1996. Alteration of development rate and growth of *Choristoneura fumiferana* parasitized by *Tranosema rostrale*: role of the calyx fluid. *Entomologia Experimentalis et Applicata* 81, 21–30.
- Edson, K.M., Vinson, S.B., 1977. Nutrient absorption by the anal vesicle of the braconid wasp, *Microplitis croceipes*. *Journal of Insect Physiology* 23, 5–8.
- Espagne, E., Dupuy, C., Hugué, E., Cattolico, L., Provost, B., Martins, N., Poirie, M., Periquet, G., Drezen, J.M., 2004. Genome sequence of a polydnavirus: insights into symbiotic virus evolution. *Science* 306, 286–289.
- Giordana, B., Milani, A., Grimaldi, A., Farneti, R., Casartelli, M., Ambrosecchio, M.R., Digilio, M.C., Leonardi, M.G., de Eguileor, M., Pennacchio, F., 2003. Absorption of sugars and amino acids by the epidermis of *Aphidius ervi* larvae. *Journal of Insect Physiology* 49, 1115–1124.
- Giron, D., Casas, J., 2003. Lipogenesis in an adult parasitic wasp. *Journal of Insect Physiology* 49, 141–147.
- Görg, A., Obermaier, C., Boghuth, G., Harder, A., Scheibe, B., Wildgruber, R., Weiss, W., 2000. The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 21, 1037–1053.
- Grossniklaus-Bürgin, C., Wyler, T., Pfister-Wilhelm, R., Lanzrein, B., 1994. Biology and morphology of the parasitoid *Chelonus inanitus* (Braconidae, Hymenoptera) and effects on the development of its host *Spodoptera littoralis* (Noctuidae, Lepidoptera). *Invertebrate Reproduction and Development* 25, 143–158.
- Grossniklaus-Bürgin, C., Pfister-Wilhelm, R., Meyer, V., Treiblmayr, K., Lanzrein, B., 1998. Physiological and endocrine changes associated with polydnavirus/venom in the parasitoid-host system *Chelonus inanitus*-*Spodoptera littoralis*. *Journal of Insect Physiology* 44, 305–321.

- Harvey, J.A., Bezemer, T.M., Elzinga, J.A., Strand, M.R., 2004. Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. *Ecological Entomology* 29, 35–43.
- Hawltzky, N., 1980. Rôle respectif de deux régimes alimentaires successifs de la larve sur divers caractères imaginaires, chez un insecte parasite ovarulaire, *Phanerotoma flavitestacea* Fisch. (Hym. Braconidae). *Zeitschrift für angewandte Entomologie* 90, 482–493.
- Hawltzky, N., Boulay, C., 1974a. Identification des régimes alimentaires de la larve d'un insecte entomophage *Phanerotoma flavitestacea* (Hym. Braconidae). *Entomophaga* 19, 395–408.
- Hawltzky, N., Boulay, C., 1974b. Localisation et évolution du glycogène chez la larve d'un parasite entomophage, *Phanerotoma flavitestacea* Fisch (Hym. Braconidae). *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 279, 1587–1590.
- Hawltzky, N., Boulay, C., 1979. Quelques données sur l'origine, la morphologie et l'évolution du tissu adipeux chez un parasite ovarulaire, *Phanerotoma flavitestacea* Fisch. *Revue de Zoologie Agricole et de Pathologie Végétale* 78, 1–5.
- Hawltzky, N., Boulay, C., 1986. Effects of the egg-larval parasite, *Phanerotoma flavitestacea* Fisch. (Hymenoptera, Braconidae) on the dry weight and chemical composition of its host *Anagasta kuehniella* Zell. (Lepidoptera, Pyralidae). *Journal of Insect Physiology* 32, 269–274.
- Hawltzky, N., Mainguet, A.M., 1976. Analyse quantitative des lipides, des substances azotées et du glycogène chez la larve d'un insecte parasite ovarulaire *Phanerotoma flavitestacea* (Hymenoptera, Braconidae). *Entomologia Experimentalis et Applicata* 20, 43–55.
- Hoch, G., Schafellner, C., Henn, M.W., Schopf, A., 2002. Alterations in carbohydrate and fatty acid levels of *Lymantria dispar* larvae caused by a microsporidian infection and potential adverse effects on a co-occurring endoparasitoid, *Glyptapanteles liparidis*. *Archives of Insect Biochemistry and Physiology* 50, 109–120.
- Hochuli, A., Pfister-Wilhelm, R., Lanzrein, B., 1999. Analysis of endoparasitoid-released proteins and their effects on host development in the system *Chelonus inanitus* (Braconidae)-*Spodoptera littoralis* (Noctuidae). *Journal of Insect Physiology* 45, 823–833.
- Kaeslin, M., Pfister-Wilhelm, R., Molina, D., Lanzrein, B., 2005a. Changes in the haemolymph proteome of *Spodoptera littoralis* induced by the parasitoid *Chelonus inanitus* or its polydnavirus and physiological implications. *Journal of Insect Physiology* 51, 975–988.
- Kaeslin, M., Wehrle, I., Grossniklaus-Bürgin, C., Wyler, T., Guggisberg, U., Schittny, J.C., Lanzrein, B., 2005b. Stage-dependent strategies of host invasion in the egg-larval parasitoid *Chelonus inanitus*. *Journal of Insect Physiology* 51, 287–296.
- Kroemer, J.A., Webb, B.A., 2004. Polydnavirus genes and genomes: emerging gene families and new insights into polydnavirus replication. *Annual Review of Entomology* 49, 431–456.
- Kunkel, J.G., Grossniklaus-Bürgin, C., Karpells, S.T., Lanzrein, B., 1990. Arylphorin of *Trichoplusia ni*: characterization and parasite-induced precocious increase in titer. *Archives of Insect Biochemistry and Physiology* 13, 117–125.
- Lanzrein, B., Pfister-Wilhelm, R., von Niederhäusern, F., 2001. Effects of an egg-larval parasitoid and its polydnavirus on development and the endocrine system of the host. In: Edwards, J.P., Weaver, R. (Eds.), *Endocrine Interactions of Insect Parasites and Pathogens*. BIOS Scientific Publishers Ltd., Oxford, pp. 95–109.
- Lawrence, P.O., Lanzrein, B., 1993. Hormonal interactions between insect endoparasites and their host insects. In: Beckage, N.E., Thompson, S.N., Federici, B.A. (Eds.), *Parasites and Pathogens of Insects*, vol. 1. Academic Press, San Diego, pp. 59–86.
- Nakamatsu, Y., Gytoku, Y., Tanaka, T., 2001. The endoparasitoid *Cotesia kariyai* (Ck) regulates the growth and metabolic efficiency of *Pseudaletia separata* by venom and Ck polydnavirus. *Journal of Insect Physiology* 47, 573–584.
- Nakamatsu, Y., Fujii, S., Tanaka, T., 2002. Larvae of an endoparasitoid, *Cotesia kariyai* (Hymenoptera: Braconidae), feed on the host fat body directly in the second stadium with the help of teratocytes. *Journal of Insect Physiology* 48, 1041–1052.
- Olson, D.M., Fadamiro, H., Lundgren, J.G., Heimpel, G.E., 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology* 25, 17–26.
- Pedata, P.A., Garonna, A.P., Zabatta, A., Zeppa, P., Romani, R., Isidoro, N., 2003. Development and morphology of teratocytes in *Encarsia berleseii* and *Encarsia citrina*: first record for Chalcidoidea. *Journal of Insect Physiology* 49, 1063–1071.
- Pennacchio, F., Vinson, S.B., Tremblay, E., Ostuni, A., 1994. Alteration of ecdysone metabolism in *Heliothis virescens* (F.) (Lepidoptera, Noctuidae) larvae induced by *Cardiochiles nigriceps* Viereck (Hymenoptera, Braconidae) teratocytes. *Insect Biochemistry and Molecular Biology* 24, 383–394.
- Pfister-Wilhelm, R., Lanzrein, B., 1996. Precocious induction of metamorphosis in *Spodoptera littoralis* (Noctuidae) by the parasitic wasp *Chelonus inanitus* (Braconidae): identification of the parasitoid larva as the key element and the host corpora allata as a main target. *Archives of Insect Biochemistry and Physiology* 32, 511–525.
- Quicke, D.L.J., 1997. *Parasitic Wasps*. Chapman & Hall, London.
- Soller, M., Lanzrein, B., 1996. Polydnavirus and venom of the egg-larval parasitoid *Chelonus inanitus* (Braconidae) induce developmental arrest in the prepupa of its host *Spodoptera littoralis* (Noctuidae). *Journal of Insect Physiology* 42, 471–481.
- Steiner, B., Pfister-Wilhelm, R., Grossniklaus-Bürgin, C., Rembold, H., Treiblmayr, K., Lanzrein, B., 1999. Titres of juvenile hormone I, II and III in *Spodoptera littoralis* (Noctuidae) from the egg to the pupal moult and their modification by the egg-larval parasitoid *Chelonus inanitus* (Braconidae). *Journal of Insect Physiology* 45, 401–413.
- Stoltz, D.B., 1993. The polydnavirus life cycle. In: Beckage, N.E., Thompson, S.N., Federici, B.A. (Eds.), *Parasites and Pathogens of Insects*, vol. 1. Academic Press, San Diego, pp. 167–187.
- Thompson, S.N., 1982. Effects of parasitization by the insect parasite *Hyposoter exiguae* on the growth, development and physiology of its host *Trichoplusia ni*. *Parasitology* 84, 491–510.
- Thompson, S.N., 1986. Nutrition and in vitro culture of insect parasitoids (review). *Annual Review of Entomology* 31, 197–219.
- Thompson, S.N., 1993. Redirection of host metabolism and effects on parasite nutrition. In: Beckage, N.E., Thompson, S.N., Federici, B.A. (Eds.), *Parasites and Pathogens of Insects*, vol. 1. Academic Press, San Diego, pp. 125–144.
- Thompson, S.N., Binder, B.F., 1984. Altered carbohydrate levels and gluconeogenic enzyme activity in *Trichoplusia ni* parasitized by the insect parasite, *Hyposoter exiguae*. *The Journal of Parasitology* 70, 644–651.
- Thompson, S.N., Dahlman, D.L., 1998. Aberrant nutritional regulation of carbohydrate synthesis by parasitized *Manduca sexta* L. *Journal of Insect Physiology* 44, 745–753.
- Thompson, S.N., Lee, R.W.K., Beckage, N.E., 1990. Metabolism of parasitized *Manduca sexta* examined by nuclear magnetic resonance. *Archives of Insect Biochemistry and Physiology* 13, 127–143.
- Turnbull, M., Webb, B.A., 2002. Perspectives on polydnavirus origins and evolution. *Advances in Virus Research* 58, 203–254.
- Van Handel, E., 1985a. Rapid determination of glycogen and sugars in mosquitoes. *Journal of the American Mosquito Control Association* 1, 299–301.
- Van Handel, E., 1985b. Rapid determination of total lipids in mosquitoes. *Journal of the American Mosquito Control Association* 1, 302–304.
- Vinson, S.B., 1990. Physiological interactions between the host genus *Heliothis* and its guild of parasitoids. *Archives of Insect Biochemistry and Physiology* 13, 63–81.
- Vinson, S.B., Iwantsch, G.F., 1980. Host regulation by insect parasitoids. *Quarterly Review of Biology* 55, 143–165.
- Vinson, S.B., Pennacchio, F., Consoli, F.L., 2001. The parasitoid-host endocrine interaction from a nutritional perspective. In: Edwards, J.P., Weaver, R. (Eds.), *Endocrine Interactions of Insect Parasites and Pathogens*. BIOS Scientific Publishers Ltd., Oxford, pp. 187–206.
- Zhang, G., Schmidt, O., Asgari, S., 2004. A novel venom peptide from an endoparasitoid wasp is required for expression of polydnavirus genes in host hemocytes. *The Journal of Biological Chemistry* 279, 41580–41585.