

**The influence of the endoparasitic wasp *Glyptapanteles liparidis*  
(Hymenoptera: Braconidae) on the growth, food consumption, and food utilization  
of its host larva, *Lymantria dispar* (Lepidoptera: Lymantriidae)**

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***Glyptapanteles liparidis*, Braconidae, *Lymantria dispar*, Lymantriidae, nutritional indices, growth,  
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**Abstract.** The influence of parasitism by *Glyptapanteles liparidis* (Bouché) on the development, consumption, growth and food utilization from the third to the fifth instar of its host *Lymantria dispar* L. was studied on three different host types, each characterized by the stage in which they were parasitized. The first host type was parasitized during premoult into the 2nd instar, the second during premoult into the 3rd instar and the third during the middle of the 3rd instar.

Depending on the relationship between the host's size and the volume of the parasitoid complex inside the host, different developmental patterns of the various host types were found. From the majority of early-parasitized hosts, the parasitoid larvae emerged during the hosts' fourth instar (90%), while parasitoids emerged mainly during the fifth instar (77% and 81%) of both later-parasitized host types. Hosts which were heavily parasitized during their premoult stage, avoided obvious nutritional constraints by inserting a supernumerary host instar. Only from one lightly parasitized host type did parasitoid larvae emerge, after developing through only two host instars. Third-midinstar-parasitized hosts had a supernumerary instar only when they contained a relatively low number of parasitoids compared with those from which the parasitoids emerged during the fifth instar. Unparasitized gypsy moth larvae also differed in their developmental pattern, as (78% of males and 72% of females) pupated after the 5th and 6th instars, respectively. Differences in growth and development time permitted the differentiation between sexes, before larvae moulted to the fifth instar.

Parasitism most influenced host nutrition in early-parasitized hosts, as they showed increased rates of consumption and growth during the 3rd, penultimate host instar. Higher biomass gains during their final fourth instar of these early-parasitized hosts were due to prolongation of both development and the feeding period, which resulted in a relative consumption and growth rate significantly lower than in unparasitized female larvae and later-parasitized larvae. Growth and consumption rates during the 3rd and 4th instar of later-parasitized host types did not differ from female control larvae. In their final 5th instar these host types did not prolong their development or feeding period, but displayed a reduction in growth rate similar to early-parasitized hosts. The approximate digestibility (AD-value) and conversion efficiency of ingested and digested food (ECI- and ECD-values) was not influenced by parasitism.

No correlation was found between the number of parasitoid larvae per host and the duration of the endoparasitic development among most individuals of the three host types. The number of parasitoids per host, however, contributed markedly to the final gross weight, consumption, growth rate and biomass gained during the final host instar.

INTRODUCTION

*Glyptapanteles liparidis* (Hymenoptera: Braconidae) is the most abundant gregarious braconid species in Europe parasitizing the important oak pest, *Lymantria dispar*

(Lepidoptera: Lymantriidae) (Fuester et al., 1983; Hoch & Schopf, 1995). Successful development of the parasitoid larvae occurs when female wasps parasitize gypsy moth larvae during the 1st to the 3rd instar, during which about 5 to 30 eggs per host are laid (Schopf, 1991, 1993a). The 1st instar parasitoids hatch after 3 to 5 days of embryonic development. At 20°C, they grow slowly but moult into the 2nd instar after about two weeks. During the last 3 days of their endoparasitic development, 70 to 80% of their maximum body mass is gained. About 3 weeks after oviposition, they begin moulting into their 3rd instar and emerge from the host whilst shedding their exuviae. Immediately, they begin to spin their white cocoons and pupate near their host which remains alive for 5 to 7 days. During this period, metamorphosis of the parasitoids occurs and the adult wasps hatch; males first, followed 1 to 2 days later by females. Females begin oviposition 4 to 5 days after mating. Since *G. liparidis* is a multivoltine species, hibernating in the larval stage inside its host larva, alternate hosts, e.g., *Dendrolimus pini* in Europe or *D. spectabilis* in Japan (Lepidoptera: Lasiocampidae) (Burgess & Crossman, 1929), are required to complete its life cycle.

Endoparasitoids which spend most of their developmental period in the isolated living space of their host larvae, develop intimate physiological adaptations to resist the host's defense reactions, use the host's nutrients, and influence its development (Vinson & Iwantsch, 1980; Beckage, 1985; Slansky, 1986; Thompson, 1990; Beckage et al., 1993; Godfray, 1994). As the development of parasitoid larvae is extended over several host instars and the host larva itself must stay alive, the influence on the host's metabolism must be minimized. This is achieved by *G. liparidis*, as in other host-parasitoid systems (e.g. Führer, 1985; Beckage & Templeton, 1986), by shifting its main volume gain to late in the second instar, shortly before emerging from its host, and by avoiding any injuries to the host's organs throughout their endoparasitic period (Schopf, 1991).

The feeding of the parasitoid larvae is limited to the uptake of nutrients provided by the host's haemolymph. The amount of available nutrients depends on the relation of the host-body size to the number and volume of the parasitoid larvae. The development and emergence of the parasitoid larvae are relatively independent from the host's age at the time of its parasitization (Schopf, 1991, 1993a). Therefore, depending on when oviposition by the female wasp occurs, developing parasitoid larvae are faced with hosts containing widely varying volumes. This may affect the food supply during the final stages of endoparasitic development.

Parasitoids in general may overcome potential nutrient constraints by prolonging the host's development, stimulating the host's consumption, and influencing the host's utilization of food (Beckage & Riddiford, 1978; Führer & Keja, 1976; Slansky, 1986; Thompson, 1990). We investigated the effects that the parasitoid *G. liparidis* has on growth, food consumption, and food utilization of host larvae that differed in size due to alternate times of oviposition. Results demonstrate the strategies used by *G. liparidis* to influence its host's growth and nutrition in order to satisfy its own nutritional requirements.

#### MATERIALS AND METHODS

Test animals were obtained from laboratory cultures originating from oak forests in Burgenland (eastern Austria). Larvae of *Lymantria dispar* L. were reared in Petri dishes at 20°C and a 16L : 8D photoperiod, on an artificial diet (tobacco hornworm) modified after Bell et al. (1981). Rearing conditions used

for adult *Glyptapanteles liparidis* (Bouché) and the method of parasitization of gypsy moth larvae are described in Schopf (1991). The effect of host's age on growth and nutrition of the host-parasitoid system was studied with gypsy moth larvae parasitized at 3 stages of development:

A-larvae: parasitized as pharate 2nd instars during premoult into the 2nd instar,

B-larvae: parasitized during premoult into the 3rd instar.

C-larvae: parasitized on day 3 of the 3rd instar.

Unparasitized gypsy moth larvae originating from the same egg clusters were used as controls.

Test larvae were staged by selecting those larvae which hatched simultaneously in the main group from the egg masses and developed synchronously within 24 h into the 2nd or 3rd instar. During the 3rd instar, these larvae consume and egest little so we grouped 5 individuals in each Petri dish (90 mm in diameter); thereafter the larvae were held individually. We tested 15 of these groups of A-hosts and controls as well as 8 groups of B- and C-hosts for growth and consumption rates during the 3rd instar. After reaching the 4th instar a minimum of 35 individuals of each host type were tested. The duration of the feeding period of each test larva, as well as the length of the endoparasitic development of the *G. liparidis*-larvae, were recorded during the entire endoparasitic development with daily observations. As the host carcass was used to determine the final dry weight, the number of parasitoid larvae per host was determined by the number of emerged individuals. Dissections of comparable parasitized larvae revealed that no parasitoid larvae remained after emergence.

From the newly moulted 3rd instar until the end of the 5th instar (or the date of emergence of the parasitoid larvae) the weight gain, food consumption and egestion by host animals were recorded. Fresh weight of each larva was measured each day during the moulting period and every second day otherwise. All uneaten food and faeces were collected and immediately deep frozen; this matter was later dried at 105°C and weighed using a micro-analytical balance (Mettler ME 22). Fresh food was accurately weighed and supplied in 2 g portions. To measure dry weight of the food supplied, and to avoid inaccuracy due to desiccation, every 7th food portion was retained and separately stored at 20°C for 2 days and then deep frozen. These pieces were later dried and used as a reference to determine the amount of food consumed.

Consumption rate (CR) was determined from the amount of total food ingested per feeding day during an instar (method after Waldbauer, 1968; modified by Slansky & Scriber, 1985). A growth rate (GR) was calculated from biomass gained per feeding day. Relative consumption and growth rates (RCR and RGR) were calculated from the CR or GR per mean body mass of the test larva (arithmetic mean value of its dry mass at the beginning and the end of each instar). Approximate digestibility [AD; 100·(food consumed–frass)/food consumed] and conversion efficiencies of ingested food (ECI; 100·biomass gained/food consumed) and digested food [ECD; 100·biomass gained/(food consumed–frass)] were measured according to Waldbauer (1968).

To calculate dry mass from fresh mass, a conversion factor was established for each type of test larvae: comparable parasitized and unparasitized larvae were weighed at the beginning of the 3rd instar and at the end of each instar during the premoult period. Dry weight of newly moulted 4th and 5th instar larvae were calculated by subtracting the dry weights of shed head-capsules and exuviae from the dry weight of the larvae during the former premoult period. Thus, the dry weight of parasitized larvae include the parasitoid larvae within. The weight of one post-emergent parasitoid larva, after spinning its cocoon, was calculated as the arithmetic mean value of the cocoon cluster per host.

Parameter distributions were tested for normality using Chi-square and Kolmogorov-Smirnov tests, mean values were compared by ANOVA and the Student-Newman Keuls (SNK) procedure. Relative frequencies were arcsine-transformed before testing (Sokal & Rohlf, 1981). Associations between variables were evaluated with Spearman's coefficient of rank correlation. Significant differences were tested at  $P < 0.05$ .

## RESULTS

### Developmental regimen of host larvae

The development of parasitized and unparasitized gypsy moth larvae differed with variations in the number of parasitoids per host. The majority of each parasitized host-type complex (90% of A-hosts, 77% of B-hosts, and 81% of C-hosts) as well as unparasitized

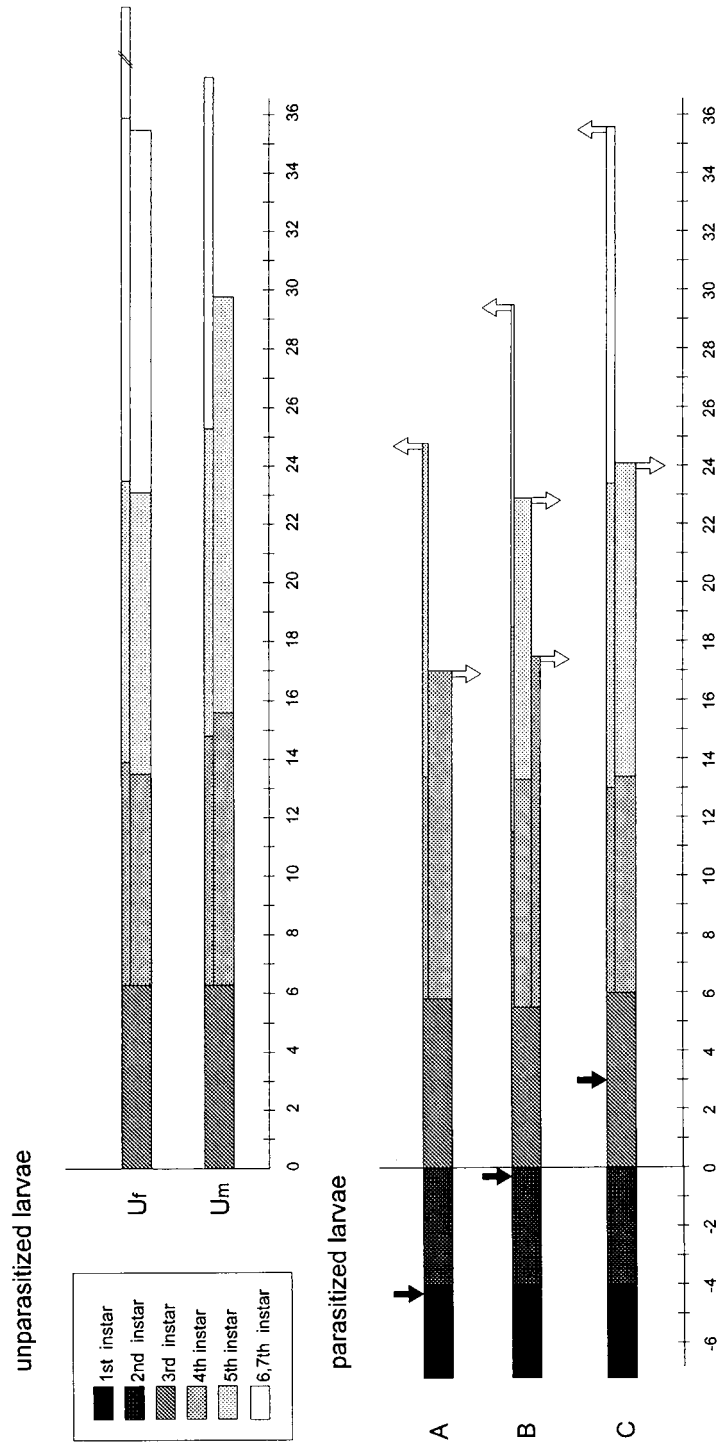


Fig. 1. Development of unparasitized female (Uf) and male larvae (Um) from the 3rd to their final instar in days at 20°C compared to the development of host larvae which were parasitized during premolt into the 2nd (A) or 3rd instar (B) or on day 3 of the 3rd instar (C); the width of the bars reflects the relative number of tested larvae of each developmental type, the dark arrows display the time of parasitization of the host, the light arrows the time of emergence of the parasitoid larvae from their host.

male (78%) and female larvae (72%), displayed a general developmental pattern which is described below (Fig. 1).

1. In A-hosts, which were parasitized during premoult into the 2nd instar, parasitoid larvae emerged at the end of the host's 4th instar.
2. In B- and C-hosts, which were parasitized either during premoult into the 3rd instar, or during the 3rd mid-instar stage, parasitoid larvae emerged during the host's 5th instar.
3. Unparasitized male larvae pupated after 5 instars.
4. Unparasitized female larvae pupated after 6 instars.

Heavily parasitized A- and B-hosts, demonstrated prolonged development through a supernumerary 5th or 6th instar (10% A-hosts and one B-host larva, respectively). This aberrant development was also seen in lightly parasitized B-hosts (20%) from which parasitoid larvae emerged precociously during host's 4th instar. Prolonged development with a supernumerary 6th instar was also displayed by 19% of the C-hosts; the number of parasitoid larvae within these hosts, however, was significantly smaller than in the majority of C-hosts, from which parasitoids emerged during the 5th instar. The majority of C-hosts showed also a significantly higher parasitoid density than both A- and B-hosts (Table 1 and 2).

TABLE 1. Duration of the 3rd to the 5th instar of unparasitized (U) and parasitized *L. dispar* which were oviposited into by *G. liparidis* females, either during premoult into the 2nd (A-host) and 3rd instar (B-host) or on day 3 of the 3rd instar (C-hosts); after moulting into the 4th stage the individually-held test larvae were differentiated by their final host instar, from which the parasitoid larvae emerged or by their final prepupal instar of the unparasitized female (f) or male (m) larvae;  $n_1$  = number of tested larvae during the 3rd instar,  $n_2$  = number of tested larvae during the 4th and 5th instar; means  $\pm$  SD followed by different letters within a column differ significantly,  $P < 0.05$ .

Host type	$n_1$	3rd instar	Final instar	$n_2$	4th instar	5th instar
A	72	$5.8 \pm 1.1b$	4th	28	$11.2 \pm 1.6a$	
			5th	3	$8.0 \pm 2.2b,c$	$11.0 \pm 2.2b$
B	34	$5.5 \pm 0.7b$	4th	6	$12.0 \pm 1.8a$	
			5th	23	$7.8 \pm 1.5b,c$	$9.5 \pm 1.3b$
			6th	1	6	7
C	35	$6.0 \pm 0.9a,b$	5th	21	$7.4 \pm 2.1c$	$10.7 \pm 2.0b$
			6th	5	$7.0 \pm 0.6c$	$10.4 \pm 0.8b$
U	71	$6.3 \pm 1.1a$	f 6th	13	$7.2 \pm 1.1c$	$9.6 \pm 1.1b$
			f 7th	5	$7.6 \pm 0.8b,c$	$9.6 \pm 1.4b$
			m 5th	14	$9.4 \pm 1.1b$	$14.1 \pm 2.5a$
			m 6th	4	$8.5 \pm 0.9b,c$	$10.5 \pm 1.1b$

In unparasitized gypsy moth larvae, 22% of males and 28% of females had a supernumerary 6th and 7th instar, respectively. Since these larvae showed the same difference between males and females in the mean duration of feeding period during their 4th instar, as was found for control larvae that followed the normal development pattern (males:  $7.4 \pm 1$  days, females:  $5.7 \pm 1.3$  days;  $t = 3.83$ ,  $P < 0.001$ ), an easy method for differentiating the sexes of 4th instar gypsy moth larvae of our strain was available.

Parasitism of *G. liparidis* significantly reduced the duration of the 3rd instar in both A- and B-hosts (Table 1). However, when parasitoids emerged in the 4th instar of these hosts,

this final instar was prolonged markedly. No such effects were observed in final 5th instar B- and C-hosts which followed the general developmental pattern.

The size of the prospective host larva at the time of parasitization appeared to influence the number of eggs laid by the female wasp. There is a clear trend toward a higher number of parasitoids in later-parasitized host larvae, with regard to all three host types of individuals that exhibited the general developmental pattern. The total duration of endoparasitic development was shorter in lightly parasitized 4th-instar B-hosts and in heavily parasitized 5th-instar C-hosts, than in the majority of B-hosts as well as in the A- and C-hosts which exhibited a supernumerary instar. Nevertheless, this rapid development had no influence on the final weight of the emerged parasitoid larvae. Interestingly, from the time of the host's last moult into its final instar, and with the exception of 5th-instar B-hosts, all parasitoid larvae remained in their hosts for an equal amount of time (Table 2).

TABLE 2. Number of *G. liparidis* larvae (number/host) which emerged from the various host instars (final instar) of *L. dispar* parasitized at different ages (A-, B-, and C-host), duration of the endoparasitic development in total (endoparas. develop.) and during the final host instars in days, and mean dry weight of a single parasitoid larva inside its cocoon one day after eclosion from the host in mg; n = number of tested larvae; means  $\pm$  SD followed by different letters within a column differ significantly,  $P < 0.05$ .

Host type	Final instar	n	Number/host	Endoparas. develop.	Days in final host instar	Dry weight (mg)
A	4th	28	17.2 $\pm$ 10.3b	22.3 $\pm$ 2.1b,c	11.2 $\pm$ 1.6a	0.80 $\pm$ 0.46
	5th	3	34.3 $\pm$ 12.9a,b	32.3 $\pm$ 5.4a	11.0 $\pm$ 2.9	0.94 $\pm$ 0.11
B	4th	6	12.3 $\pm$ 3.0b	18.8 $\pm$ 1.2c	12.0 $\pm$ 1.8	0.86 $\pm$ 0.12
	5th	23	23.3 $\pm$ 13.9b	24.0 $\pm$ 2.9b	9.5 $\pm$ 1.3b	0.90 $\pm$ 0.30
	6th	1	35	31	12	0.60
C	5th	21	36.9 $\pm$ 21.2a	21.3 $\pm$ 3.7c	10.7 $\pm$ 2.0	0.75 $\pm$ 0.16
	6th	5	22.6 $\pm$ 10.5b	29.6 $\pm$ 3.9a	12.2 $\pm$ 2.7	0.98 $\pm$ 0.19

No correlation was found between the number of parasitoids per host and either the duration of the final host instar, or the duration of endoparasitic development of the parasitoid larvae. Furthermore, the number of parasitoids per host did not influence the weights of emergent parasitoid larvae (weighed inside their cocoons).

#### Consumption of host larvae

During the 3rd instar, A-hosts showed a significantly higher consumption rate (CR) than B- and C-hosts as well as unparasitized controls (Table 3). During their final 4th instar, A-hosts consumed nearly twice as much total food as all other larvae, but this was not reflected in their consumption rate. Because of their markedly prolonged feeding period and their higher mean body weight, the mean RCR in the 4th instar was actually lower than in 4th-instar B- and C-hosts or unparasitized female larvae.

During the final 5th instar, the consumption of unparasitized male larvae exceeded that of all remaining hosts. The consumption rate (absolute and relative) of male controls in fact exceeded that of all other tested larvae. C-hosts displayed the lowest RCR, due to their relatively high weight during the 5th instar (40% more than B-hosts).

TABLE 3. Total consumption (consumpt.) in mg dry weight, consumption rate (CR) in mg dry weight per day, and relative consumption rate (RCR) in mg dry weight per day and mean body mass from the 3rd to the 5th instar of hosts (A, B, C), from which the parasitoids emerged during the 4th (A-host) or 5th instar (B- and C-hosts), and of unparasitized larvae (U), which were differentiated after moulting into the 4th instar between unparasitized female (Uf) pupating after the 6th and unparasitized male larvae (Um) pupating after the 5th instar; N = number of tested grouped larvae during the 3rd instar; n = number of test larvae during the 4th to the 5th instar; means  $\pm$  SD followed by different letters differ significantly within a column, means  $\pm$  SD of RCR values followed by stars (\*) differ significantly within a row,  $P < 0.05$ .

Host	N	3rd instar			n	4th instar			5th instar		
		Consumpt. (mg)	CR (mg/d)	RCR (mg/d-mg <sup>-1</sup> )		Consumpt. (mg)	CR (mg/d)	RCR (mg/d-mg <sup>-1</sup> )	Consumpt. (mg)	CR (mg/d)	RCR (mg/d-mg <sup>-1</sup> )
A	15	16.30 $\pm$ 5.97	3.61 $\pm$ 1.09a	0.66 $\pm$ 0.21	28	88.03 $\pm$ 33.90a	7.99 $\pm$ 3.20	0.50 $\pm$ 0.15b*			
B	8	12.50 $\pm$ 2.62	2.85 $\pm$ 0.52b	0.63 $\pm$ 0.11	23	49.68 $\pm$ 18.20b	8.81 $\pm$ 3.45	0.72 $\pm$ 0.26a	119.25 $\pm$ 54.22b	12.49 $\pm$ 5.13b	0.44 $\pm$ 0.13b*
C	8	12.60 $\pm$ 3.75	2.65 $\pm$ 0.61b	0.60 $\pm$ 0.14	21	48.80 $\pm$ 17.45b	8.57 $\pm$ 2.90	0.70 $\pm$ 0.18a	141.39 $\pm$ 73.40b	13.07 $\pm$ 5.46b	0.37 $\pm$ 0.08c*
U (f)	15	12.20 $\pm$ 4.42	2.48 $\pm$ 0.75b	0.52 $\pm$ 0.15	13	45.79 $\pm$ 12.99b	8.67 $\pm$ 4.18	0.74 $\pm$ 0.31a*	132.59 $\pm$ 37.08b	16.54 $\pm$ 4.56b	0.47 $\pm$ 0.08b
U (m)					14	57.72 $\pm$ 15.26b	7.84 $\pm$ 1.85	0.59 $\pm$ 0.14 a,b	274.16 $\pm$ 68.45a	26.73 $\pm$ 7.00a	0.61 $\pm$ 0.15a

Parasitized larvae displayed a significant decrease in RCR between the penultimate and final larval stages. For female controls the RCR in the 4th instar was higher than in the sexually undifferentiated 3rd instar, but lower than in the 5th instar. However, male larvae displayed the same RCR from the 3rd to the final 5th instar.

#### Growth of host larvae

Only A-hosts showed a significantly higher biomass gain than the controls during their penultimate 3rd, and final 4th instar. This difference was also reflected in the absolute growth rate (GR) during the 3rd instar, but was equalized by the prolonged duration of the A-host's 4th stage.

Neither B- nor C-hosts differed from controls in biomass gained or GR during their penultimate 4th instar. In their final 5th instar, B-hosts had the lowest biomass gain, while C-hosts did not differ from unparasitized female larvae. However, both hosts had a significantly lower GR than 5th-instar controls. Generally, parasitism reduced significantly the relative growth rates (RGR) of gypsy moth larvae in their final stadium. Thus, 4th-instar A-hosts, and final 5th-instar B- and C-hosts, had a lower RGR than the corresponding controls, as well as their own penultimate instar.

Unparasitized male larvae which, during the 4th instar, already differed in their RGR from females, showed clearly the highest increase in biomass during the 5th instar but, again, a significantly lower RGR than corresponding females (Table 4). As with 5th-instar females, the RGR of penultimate 4th-instar males differed from that in the 3rd instar.

#### Food utilization by host larvae

Approximate digestibility (AD) provides an estimate of the percentage of ingested food that is assimilated. Our AD values did not differ between parasitized hosts and controls

during the instars analyzed. These values were generally high with an average maximum of about 71% in the 4th instar (Table 5). However, the efficiencies with which ingested or digested food is converted to biomass (ECI and ECD) were highest during the 3rd instar. Due to high variances in the data, especially observed with unparasitized larvae of the 3rd instar, this declining trend between the 3rd and 4th instar was significant only for the ECD value of A-hosts. These high variances might be due to an accumulation of measuring errors on the small amounts of food consumed and egested.

TABLE 4. Biomass gain (biomass) in mg dry weight, growth rate (GR) in mg dry weight per day, and relative growth rate (RGR) in mg dry weight per day and mean body mass from the 3rd to the 5th instar of hosts (A, B, C) and unparasitized larvae (U): further explanations in Table 3. Means  $\pm$  SD followed by different letters differ significantly within a column, means  $\pm$  SD of RGR values followed by stars (\*) differ significantly within a row,  $P < 0.05$ .

Host	N	3rd instar			n	4th instar			5th instar		
		Biomass (mg)	GR (mg/day)	RGR (mg/day·mg <sup>-1</sup> )		Biomass (mg)	GR (mg/day)	RGR (mg/day·mg <sup>-1</sup> )	Biomass (mg)	GR (mg/day)	RGR (mg/day·mg <sup>-1</sup> )
A	15	5.42 $\pm$ 1.54a	1.26 $\pm$ 0.45a	0.22 $\pm$ 0.06	28	18.16 $\pm$ 11.69a	1.67 $\pm$ 1.12	0.09 $\pm$ 0.03c*			
B	8	3.91 $\pm$ 1.27b	0.91 $\pm$ 0.35b	0.19 $\pm$ 0.05	23	13.62 $\pm$ 7.37b	2.34 $\pm$ 0.95	0.18 $\pm$ 0.04a	25.30 $\pm$ 16.15c	2.66 $\pm$ 1.72b	0.08 $\pm$ 0.03c*
C	8	3.76 $\pm$ 1.68b	0.80 $\pm$ 0.38b	0.17 $\pm$ 0.06	21	12.30 $\pm$ 3.35b	2.21 $\pm$ 0.84	0.18 $\pm$ 0.05a	35.49 $\pm$ 18.26b	3.32 $\pm$ 1.58b	0.09 $\pm$ 0.02c*
U (f)	15	4.23 $\pm$ 1.32b	0.89 $\pm$ 0.35b	0.18 $\pm$ 0.05*	13	10.68 $\pm$ 3.15b	1.96 $\pm$ 0.75	0.17 $\pm$ 0.05a <sup>1)</sup>	38.32 $\pm$ 11.08b	4.79 $\pm$ 1.36a	0.14 $\pm$ 0.02a
U (m)					14	12.84 $\pm$ 3.12b	1.79 $\pm$ 0.54	0.13 $\pm$ 0.03b	49.38 $\pm$ 12.81a	4.81 $\pm$ 1.20a	0.11 $\pm$ 0.02b

<sup>1)</sup> Mean value does not differ from the mean RGR values of unparasitized 3rd and female larvae of the 5th instar.

TABLE 5. Approximate digestibility (AD) and efficiencies of conversion of ingested food (ECI) and digested food (ECD) in % from the 3rd to the 5th instar of hosts (A, B, C) and unparasitized larvae (U); further explanations in Table 3; only means  $\pm$  SD followed by a star (\*) differ significantly within a row,  $P < 0.05$ .

Host	N	3rd instar			n	4th instar			5th instar		
		AD	ECI	ECD		AD	ECI	ECD	AD	ECI	ECD
A	15	64 $\pm$ 10	38 $\pm$ 17	63 $\pm$ 36*	28	72 $\pm$ 8	21 $\pm$ 10	31 $\pm$ 19			
B	8	59 $\pm$ 7	32 $\pm$ 11	56 $\pm$ 20	23	70 $\pm$ 10	28 $\pm$ 9	42 $\pm$ 20	66 $\pm$ 8	21 $\pm$ 10	33 $\pm$ 20
C	8	57 $\pm$ 10	31 $\pm$ 16	57 $\pm$ 34	21	69 $\pm$ 10	26 $\pm$ 6	40 $\pm$ 14	65 $\pm$ 8	25 $\pm$ 6	40 $\pm$ 12
U (f)	15	58 $\pm$ 17	39 $\pm$ 19	57 <sup>1)</sup>	13	73 $\pm$ 6	24 $\pm$ 6	33 $\pm$ 10	64 $\pm$ 8	29 $\pm$ 5	47 $\pm$ 10
U (m)					14	69 $\pm$ 10	24 $\pm$ 9	37 $\pm$ 21	67 $\pm$ 4	18 $\pm$ 4	28 $\pm$ 7

<sup>1)</sup> Value was calculated by the means of the dry weights of biomass gain and assimilated food.

The mean efficiency values of parasitized and unparasitized 4th and 5th instars did not differ significantly. Compared with unparasitized female larvae, final parasitized host larvae and male larvae tended to have lower efficiencies for converting ingested and digested food to body mass.

### Number of parasitoids per host

Again, we found no clear influence of parasitoid density on the final weight of individual parasitoid larva from A- and B-hosts or on the host's growth, until they had reached their final instar. However, during this final host instar, the number of the parasitoid larvae contributed significantly to the weight of the host-parasitoid complex, weakly at the onset but markedly at the end of the endoparasitic development (Table 6). With the exception of B-hosts, this effect was also found on the host's carcass weight.

TABLE 6. Spearman's coefficient of rank correlation between: a) the number of parasitoid larvae per host (n/-host) of the three host types (A, B, C) and the dry weight of the host-parasitoid complex at the onset (weight day<sub>0</sub>) and at the end of the final host instar (weight day<sub>n</sub>; gross weight = weight of host carcass plus the emerged parasitoid larvae inside their cocoons, net weight = weight of carcass alone), as well as the biomass gain (biomass), food consumption, and growth rate (GR) of the host larva during the final host stadium; b) the mean dry weight of a parasitoid larva per cluster of the three host types (dw/-host) and the above mentioned parameters; all weight data based on dry weight measurements; number of tested host larvae: A = 28, B = 23, C = 21; asterisks symbolize the degree of significance: \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001.

	Parasitoids	Weight day <sub>0</sub>	Weight day <sub>n</sub>		Biomass	Consumption	GR
			Gross weight	Net weight			
a)	n/A-host	0.509 **	0.705 ***	0.547 **	0.694 ***	0.710 ***	0.710 ***
	n/B-host	0.419 *	0.563 **	0.118	0.445 *	0.546 **	0.571 **
	n/C-host	0.630 **	0.888 ***	0.773 ***	0.849 ***	0.794 ***	0.825 ***
b)	dw/A-host	0.095	0.486 *	0.254	0.492 **	0.040	0.477 *
	dw/B-host	0.432 *	0.489 *	0.709 ***	0.420 *	0.419 *	0.367
	dw/C-host	-0.157	0.062	0.040	0.118	-0.086	0.165

Furthermore, the host's parasite load correlated well with consumption, biomass gain, and growth rate of the final A- and C-host instars. However, only in B-hosts was there a highly significant correlation between the mean dry weight of the emerged parasitoid larvae and the weight of the host's carcass.

### DISCUSSION

Growth and nutrition of the host-parasitoid system of *L. dispar* and *G. liparidis* was mainly influenced by the ratio of host size to the volume of its parasitoid larval complex, as reported in several other host-parasitoid associations (Führer & Keja, 1976; Führer, 1985; Beckage & Riddiford, 1978, 1983; Tagawa et al., 1982; Sato & Tanaka, 1984). While the host's size depends on the age of the host larva at the time of its parasitization by the female wasp and further gains in biomass made after being parasitized, the volume of the parasitoid larvae is fixed by the number of eggs laid by the wasp and the dynamics of their growth. As with several other gregarious braconids, female wasps of *G. liparidis* also tend to lay more eggs in larger hosts (Vinson & Iwantsch, 1980; Beckage & Riddiford, 1983; Führer, 1985).

Parasitoids emerged from 77% to 90% of the tested host larvae during either the 4th or 5th instar, depending on the time of parasitization. The remaining host larvae deviated from this standard developmental scheme, by a shortened or prolonged development,

depending on the parasitoids' density. This was particularly noticeable with B-hosts, which were parasitized during the premoult period into the 3rd instar: when lightly parasitized the parasitoid larvae emerged precociously during the 4th instar whereas, with heavy parasitization, hosts moulted to a supernumerary 6th instar, from which the parasitoids emerged finally (in our study this occurred only once).

A considerably different situation was observed in C-hosts parasitized in the middle of the 3rd instar; a relatively low number of parasitoids per host prolonged the development to a supernumerary 6th host instar, whilst heavily parasitized hosts followed the normal developmental pattern, with parasitoid larvae emerging during the 5th instar. Parasitoid larvae appear to benefit from higher densities inside this host type, as has been observed in a previous study on the volume gain of the parasitoid larvae (Schopf, 1993a). The apparent developmental disadvantage for parasitoid larvae inside hosts of group C, with relatively small parasitoid loads, may be due to insufficient conditioning of the host by the parasitoids. This conditioning may involve regulating the host's physiology. As in other host-parasitoid systems, the development of the parasitoid larvae may be retarded and individual weights may vary widely in lightly parasitized hosts, in comparison with heavily parasitized hosts (reviewed by Vinson & Iwantsch, 1980). However, there also might be an increased resistance to parasitoid conditioning in hosts parasitized during a mid-instar stage in comparison with those parasitized during the premoult period.

Dose-related effects of parasitism on the developmental variability of their host larva were also found in the host-parasitoid system *Manduca sexta* (Lepidoptera: Sphingidae)-*Cotesia congregata* (Hymenoptera: Braconidae), when the hosts were parasitized during their first instar (Beckage & Riddiford, 1978, 1983), but are normally not existent in parasitized larvae of *Pieris brassicae* (Lepidoptera: Pieridae), from which *Cotesia glomerata* always emerges during the host's 5th instar (Führer & Keja, 1976, Führer, 1985). There are exceptional cases in which parasitoid larvae are only able to emerge from extremely lowly-loaded hosts during the host's 4th instar (Führer, pers. com.). However, heavy parasitization only prolonged the development of *Pieris* larvae in their final instar, but did not induce an additional larval instar (Führer, 1981).

The relatively independent duration of the endoparasitic period in hosts following the normal developmental pattern suggests that *G. liparidis* larvae do not depend directly on their host's endocrine activities. Parasitism induces an extreme accumulation of juvenile hormone in the late host instar that might be necessary to prolong the host's development and overcome its endocrine system, in order to avoid pupation (Schopf, 1993b). However, the relatively constant period between the host's moult to its final instar and the emergence of the parasitoid larvae, which was observed in all host types except B-hosts, suggests that there might be an influence by the host's endocrine conditions on parasitoid larval development. Further studies are needed to test this hypothesis.

The relatively constant time required by *G. liparidis* for its endoparasitic development also causes varying nutritional conditions for the host-parasitoid complex, depending on the age and size of the host larva. It is not known whether the delayed growth of parasitoids during their first instar (Schopf, 1991) is due to the limited availability of nutrients in young host larvae or whether this reveals an adaptive behaviour to avoid stress by shifting the main volume gain of the parasitoid larvae towards the last days of their endophagous period. Similar developmental patterns were found in other gregarious host-parasitoid

systems (Sato, 1980; Beckage, 1985; Slansky, 1986). However, even the slow continuous growth of 1st-instar *G. liparidis* larvae requires elevated nutritional supply in young, small A-host larvae, since penultimate 3rd-instar A-hosts showed a significantly elevated gain in biomass and a higher absolute consumption and growth rate in comparison with both control larvae and other host types. But due to the higher mean weight of the host-parasitoid complex, these differences were not reflected in their relative growth and consumption values.

During their final 4th instar, the increased demand for nutrients by the rapidly growing 2nd-instar parasitoids was compensated for, by primarily, significantly prolonged feeding by the A-hosts; more than twice as much food was consumed and absorbed by these hosts as in unparasitized female larvae or later-parasitized host types. Because neither the AD values nor the efficiencies of food utilization (ECI and ECD values) differed significantly between the parasitized and control larvae, most of the consumed food might be used for metabolic costs of both the host and the parasitoid larvae. This is confirmed by the significant decrease of the ECD value in A-hosts from the 3rd to the 4th instar, which was markedly stronger than in the other tested larvae. Reduced ECD values in final A-host instar suggest that a higher proportion of nutrients is used for the rapid growth of parasitoids during this stage, when about 50% of the total dry weight of the host-parasitoid complex was converted to the parasitoids final biomass. Furthermore, the potential biomass gain might be affected by loss of energy caused by the conversion of nutrients from the lower trophic level of the herbivores to the higher level of the parasitoid larvae. Reduced food conversion efficiencies were also reported from the host-parasitoid associations of *M. sexta*-*C. congregata* (Beckage & Templeton, 1986; Bentz & Barbosa, 1990) and *P. brassicae*-*C. glomerata* (Führer, 1985).

The high variation of the ECD values, particularly those of the unparasitized 3rd instar larvae, might be caused by the limited precision of the gravimetric method when only small portions of food were eaten. Generally, ECD is subject to an accumulation of errors as Schmidt & Reese (1986) have demonstrated. However, our data on unparasitized larvae agree well with those of the control larvae reported by Nussbaumer (1992) and Stockhoff (1993). The discrepancy between the AD-values in Stockhoff's and the present study may be caused by different diets, temperatures and different strains of insects. In contrast with the present results, Führer (1985) found a distinct increase in the AD value during the first 3 days of the final instar of parasitized *Pieris*. Our recorded AD levels, using an artificial diet, showed a maximum of 70 to 75 % during the 4th instar, which might correspond to the upper limit of digestibility that cannot be further increased by parasitism.

In contrast with the host-parasitoid association *P. brassicae*-*C. glomerata* (Führer, 1985, Karowe & Schoonhoven, 1992), no significant influence on the food utilization values was found by parasitism in gypsy moth larvae. Compensation of food intake to overcome nutrient constraints, by increasing the consumption rate and prolonging development and feeding time, was not only observed with host-parasitoid systems (Slansky, 1978; Slansky & Scriber, 1985; Sato, 1980; Führer, 1985; Tanaka, 1987 etc.), but also in studies evaluating the reduction of dietary nutrient concentrations (Slansky & Wheeler, 1992; Timmins et al., 1988). Reduced growth rates of terminal host instars, which might be due to reduced consumption during the late parasitoid larval development, were also observed in the later-parasitized gypsy moth larvae and have been reported for other host-parasitoid

associations (Vinson & Barras, 1970; Beckage & Riddiford, 1978, 1983; Führer, 1981, 1985; Thompson, 1982; Bentz & Barbosa, 1990).

The nutritional constraints in the larger B- and C-hosts were clearly lower than in A-hosts. This is characterized by the lack of differences in biomass gain during the 3rd to 5th instar (C-hosts) in comparison with unparasitized female larvae, the similar duration of the hosts' penultimate and ultimate instars, and finally by the same absolute and relative growth and consumption rates during the 3rd and 4th instar of the host-parasitoid complexes. However, both absolute and, as in A-hosts, relative growth rates were significantly reduced in final-instar B- and C-hosts, indicating the increased nutritional demands for the rapid growth of the parasitoid larvae shortly before their emergence (Schopf, 1991). The nutritional shortage in B-hosts, which might be higher than in later-parasitized C-hosts, apparently was compensated for by their higher relative consumption rate and their lower biomass gain in this stadium. Both parasitized host types consumed as much food as unparasitized 5th-instar females and their metabolic costs (about 65% of the absorbed food) did not differ statistically from that of the female controls (53%). The highest metabolic losses were recorded in final-instar male control larvae which only used 28% for their anabolic metabolism.

In addition to a host's age at the time of parasitization, the number of parasitoid larvae per host also had a significant influence on the nutrition of the host-parasitoid system. This is indicated by the positive correlation between consumption and growth rate during the final instar of A- and C-hosts, as reported for several other host-parasitoid systems (Führer & Keja, 1976; Beckage & Riddiford, 1983; Sato & Tanaka, 1984). The higher collective impact of parasitoids on their hosts might be due to the increased nutritional stress of the host-parasitoid system, in the case of heavily parasitized A-hosts, or to the stronger conditioning effect of a large number of parasitoids in the case of C-hosts, whose net carcass weight was also related to the parasitoids' density. Although host's parasite load had a marked influence on the duration of the endoparasitic development, by increasing or reducing the number of host instars, the small number of A- and B-hosts with a supernumerary host instar demonstrates that such prolonged development is avoided primarily. The high energy costs for an additional moult by the host might deprive the system of appropriate nutrients and the prolongation of the host's development might increase the danger of a longer exposure to predators or other parasites.

Unparasitized male larvae differed significantly in their penultimate 4th instar from unparasitized female and parasitized B- and C-hosts by prolonging the duration of both feeding and development. In particular, the difference in the feeding period between unparasitized 4th-instar males and females might be a criterion for sex differentiation, which would be much easier to use than the developmental characters of 5th-instar gypsy moths described by Tanaka et al. (1989). Furthermore, the combination of measurements on the feeding duration and final weight allowed the differentiation between all developmental types in this strain of gypsy moth larvae at the end of the 4th instar. Due to their higher intake of food, penultimate male control larvae showed an absolute growth and consumption rate similar to corresponding female and host larvae. However, their RGR was reduced markedly due to their elevated mean body weight. This effect was more pronounced during males' ultimate 5th instar, when they consumed twice as much as females or parasitized larvae, resulting in the highest biomass gain as well as mean CR and RCR.

However, their RGR was higher than that of parasitized larvae, but due to their 2-day longer feeding period and their high mean body weight, it was markedly lower in comparison with female controls. Due to these discrepancies between the sexes in control larvae, the existing differences between parasitized larvae, which were not sexually distinct, and unparasitized control larvae might be more pronounced.

From these data it may be concluded that the nutritional demand for the multivoltine, gregarious haemolymph feeder *G. liparidis* was supplied, primarily, by influencing the host's food consumption, growth and development. The effect of parasitism depended on host's size and to some degree on its parasitoid load. The lack of special adaptations to the host's efficiency to utilize food may well be explained by the need to use alternate host species for hibernation.

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