Development of the braconid wasp *Glyptapanteles liparidis* (Hymenoptera: Braconidae) in larvae of the brown-tail moth, *Euproctis chrysorrhoea* (Lepidoptera, Noctuidae, Lymantriinae)

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Abstract

The gregarious braconid wasp *Glyptapanteles liparidis* is one of the most important natural enemies of gypsy moth, *Lymantria dispar* larvae, in Austria. The gypsy moth has only one generation per year and overwinters in the egg stage, while the wasp is multivoltine and needs alternate hosts to complete its life cycle. In my thesis, I tested the host suitability of larvae of the brown-tail moth *Euproctis chrysorrhoea*. This species overwinters in the larval stage and young instars suitable for oviposition are available from mid-July to early April. The main goal of the study was to gain basic information about the interactions in the host-parasitoid system *E. chrysorrhoea* - *G. liparidis*.

For a detailed overview of the endoparasitic development and growth of *G. liparidis* parasitoids in larvae of *E. chrysorrhoea* I determined the duration of the endoparasitic stages and the total development time from oviposition to adult wasp emergence. Furthermore, I focused on parameters like adult sex ratio, the number of parasitoids per host larva and successful parasitization rate as well as female wasp preference – if given a choice – to parasitize larvae of the gypsy moth or the brown-tail moth.

The results show that larvae of *E. chrysorrhoea*, when parasitized as third instars, were at least partially suitable hosts for the parasitic wasp. Parasitization experiments resulted in different outcomes, (i) in 23 % of parasitized hosts, parasitoids completed development and emerged successfully, (ii) 37 % of the parasitized hosts pupated successfully, no parasitoids emerged and (iii) 40 % of the parasitized hosts died before the parasitoids were able to emerge. The development time of the endoparasitoids was longer and parasitism rate was lower in brown-tail moth than in gypsy moth larvae. Female wasps preferred gypsy moth larvae for oviposition but host acceptance and performance in *E. chrysorrhoea* may be better in the field than under laboratory conditions because of different behavior of female wasps, e.g. when only brown-tail moth larvae are available for oviposition.
1. Introduction

1.1. The parasitoid Glyptapanteles liparidis

The gregarious braconid wasp Glyptapanteles liparidis (Hymenoptera: Braconidae) is one of the most important natural enemies of gypsy moth, Lymantria dispar, larvae (Lepidoptera: Lymantriidae) in Austria. Although gypsy moth is a serious pest of deciduous trees throughout the northern temperate regions of the world cyclic outbreaks are most pronounced in the Mediterranean and Balkan countries (Nierhaus-Wunderwald and Wermelinger, 2001).

Female wasps parasitize early-instar larvae of the gypsy moth and inject up to 30 eggs into the hemocoel of a single host larva (Schopf, 2007). Endoparasitic development comprises the egg stage, which lasts about 4 days post parasitization (Schopf, 2007), and two larval stages. The hatched first instars possess mandibles to kill other potential endoparasitic competitors (Marktl et al., 2002). The amandibulate second instars have a typical thin-walled vesicle at the end of the abdomen (anal vesicle), which is thought to be permeable to various molecules (Nussbaumer and Schopf, 2000). The anal vesicle is an endoparasitoid-specific structure for which various functional roles have been proposed, namely respiration, nutrition, excretion and interaction with the host, but the theme is very poorly analysed (Kaeslin et al. 2006). About three weeks after oviposition, G. liparidis larvae molt to third instars and emergence through their host’s cuticle, thereby leaving the old exuviae inside the host larva (Schopf and Steinberger, 1996). After emergence the parasitoid larvae spin a white cocoon next to their “empty” host and pupate. The host larva stays next to the cocoon masses and remains alive for five to seven days until it finally starves. During this period the parasitoids metamorphose and the adult wasps emerge, while the host larva acts like a “bodyguard” to protect the parasitoid pupae from predators, hyperparasitoids or other dangers (Poulin, 2010). If the moribund host larva is recognizing a disturbance it reacts with aggressive defence behavior and exhibits violent head-thrashing movements. The mechanisms responsible for this manipulation of host behavior were not identified yet but are probably thought to be induced by the parasitoid larvae, prior to or during emergence (Maure et al., 2013).

Female G. liparidis wasps emerge one to two days later than males and have a pool of mature eggs that can be fertilized by male wasps immediately but for further egg maturation females have to feed. After mating female wasps look for suitable host
larvae for oviposition (Schopf and Steinberger, 1996). The mode of sex determination is arrhenotokous parthenogenesis, in which diploid females develop from fertilized eggs and haploid males develop from unfertilized eggs (Heimpel and Boer, 2008).

The development time of *G. liparidis* from oviposition to adult wasp emergence takes about four weeks. The endoparasitic stages of *G. liparidis* feed exclusively on host hemolymph, while free-living adult wasps feed on nectar or pollen of flower plants. Unfed wasps die within two to three days, but honey-fed wasps live up to one month.

The gypsy moth has only one generation per year and overwinters in the egg stage, but the multivoltine *G. liparidis* wasp needs alternate hosts, especially for hibernation. Thus, the relation between the two species in the parasitoid-host system *G. liparidis* - *L. dispar* is probably not as close as in other host-parasitoid systems because each wasp generation emerging from gypsy moth larvae has to find a new suitable host that is in most cases a different species because *L. dispar* larvae are available in the field for only approximately two months.

*Glyptapanteles liparidis* is a koinobiont parasitoid characterized by endoparasitic stages that develop in a living and molting host larva. Hence, these species are perfectly adapted to their host species. Endoparasitoids evolved physiological adaptations to prevent or resist the host's defense reactions, use the host's nutrients and influence its development (Schopf and Steinberger, 1996). As early as the female wasps start to oviposit, the host's immune response has to be manipulated in order to avoid encapsulation of the injected eggs. Encapsulation is a primary cellular defensive mechanism used by lepidopteran larvae to eliminate objects that are too big to be phagocytized. It involves recognition of the invader as non-self by host hemocytes, subsequent recruitment of more hemocytes, and adherence of hemocytes to the invader's surface, eventually resulting in a multicellular capsule that kills the parasitoid (Alleyne and Wiedenmann, 2001). First instars of the parasitoid have a higher risk to get eliminated by this mechanism of immune defence. Moreover, it is important that endoparasitic larvae, which depend on the host's nutrients, act host-protecting to some degree especially at the beginning of endoparasitic development because a weak host could be an easy prey for predators or might be fall victim of pathogen attack. Thus, the host's immune defense must not be abrogated totally because infections of the host also affect the endoparasitoids and can cause early death of both. So it is important for the parasitoids that all
invaders, e.g. bacteria, viruses and fungi, are attacked except themselves. Host larvae need to grow and feed as undisturbed as possible and parasitoids must not affect the essential physiological process of host development. Similar to other parasitic wasps, *G. liparidis* has evolved different strategies of host regulation. For instance, the number and fertilization status of injected eggs during parasitization is regulated by the female wasp. Unfertilized eggs produce males while fertilized eggs become females. This phenomenon occurs in almost all Hymenopteran species. *Glyptapanteles liparidis* wasps tend to lay more eggs in larger fourth-instar gypsy moth larvae than in smaller second-instar gypsy moths. Another adaption is probably the low growth rates of the first-instar parasitoids and the enormous gain of body mass during the last 3 days of their endoparasitic development (Schopf, 2007). It is not known whether this developmental pattern is due to the limited amount of nutrients of young host larvae or behavior to reduce the pressure on the host by shifting the main volume gain towards the last period of their endophageous phase (Schopf and Steinberger, 1996). In case of premature pupation of their host, the parasitoid larvae might not be able to penetrate the thick cuticle of the pupae and would consequently die. Thus, second instar larvae of *G. liparidis* have to take over regulation of host development to ensure an easy emergence after completing their endoparasitic development (Schopf, 2007). The enormous increase of the juvenile hormone titer in the hemolymph of parasitized *L. dispar* larvae, which was shown to be released mainly by the parasitoid larvae, counteracts early pupation, prolongs the developmental stage of the host larvae and prevents host metamorphosis (Schafellner et al., 2004). This observation and the parasitoid’s ability to molt independently from their host indicate the hormonal self-reliance of *G. liparids* second instars (Schafellner et al., 2004).

To down-regulate the immune response of their habitual hosts, parasitoids use different methods at different times after parasitization (Alleyne and Wiedenmann, 2001). Immunosuppressive mechanisms include venoms, polydnavirus and special types of cells (teratocytes) that are released from the serosa membrane that surround the parasitoid embryo when the first-instar parasitoids hatch in the hemocoel. In parasitized *L. dispar* larvae, teratocytes of *G. liparidis* increase extremely in size throughout the endoparasitic development and do not divide. Their exact function in the parasitoid-host system is not entirely understood and may probably differ from other species (Schopf, 2007). In general, teratocytes are thought
to create an environment favourable for the parasitoid’s development by manipulating host physiology and biochemistry. They appear to participate in preventing encapsulation, reducing hemolymph phenoloxidase activity and are supposed to serve as nutrient source for specific parasitoid species such as *Aphidius ervi* and *Dinocampus coccinellae* (Beckage and Gelman, 2004).

Venom secretions show very different functions between the analysed endoparasitic wasps, ranging from paralysing and antiseptic effects to a temporary protection of the parasitoid progeny from activated host immune response at oviposition (Asgari, 2006).

Polydnavirus (PDV) particles in the calyx fluid together with venom secretions and the parasitoid eggs are injected into the host during wasp oviposition. In addition to interfere with the host endocrine system, PDVs induce immunosuppression of the host by disrupting directly hemocyte function and effectiveness and preventing encapsulation of the parasitoid. PDVs are symbiotic viruses that provide long-term protection of the parasitoid and may act alone or together with venom components, which facilitate the action of PDV (Beckage and Gelman, 2004). Stoltz et al. (1988) observed that the venom of braconid wasps promoted the uptake of the virions by host larvae cells and enhanced their uncoating in the cell, so that the entry of nucleocapsid viral DNA into the nucleus of the host cell was faster than the rate observed in the absence of venom. Thus, venom enhanced the persistence of the virus in the host.

A study by Hoch et al. (2009) about the function of PDV/venom in the absence of a developing parasitoid showed that implantations of *G. liparidis* eggs or larvae were only successful when *L. dispers* host larvae were treated with PDV/venom. Without immune suppression by these associated factors, the parasitoids were immediately encapsulated by hemocytes. When the host contained PDV/venom the parasitoids survived. However, *G. liparidis* larvae implanted without teratocytes still suffered some attack from the host immune system and were unable to complete their development. Successful development of the parasitoids was only possible when all components (i.e., parasitoid larvae, teratocytes and PDV/venom) were present in the hemocoel.
To sum up, the ability of a koinobiont endoparasitoid to develop successfully in a host depends on the ability to overcome the immune system, ensure nutritional supply of the larval stages of the parasitoid by adjusting their own development and growth to that of their host, and to regulate the host’s organism to avoid premature pupation. Additionally, the suitability of hosts is provided when all or most parasitoids are able to mature and complete development. If a significant number of host individuals is able to debilitate the immature parasitoids and survive they are classified as “partially resistant” or “marginal” (Heimpel et al., 2003).

1.2. Alternate host *Euproctis chrysorrhoea*

Although *E. chrysorrhoea* is a univoltine species like *Lymantria dispar*, it could potentially serve as host for hibernation of *G. liparidis* parasitoids. While larvae of gypsy moth are available as hosts from April to June/July, larvae of the brown-tail moth are present from mid-July to early April.

*Euproctis chrysorrhoea* is distributed in Central and South Europe, the Baltic States, Belorussia, Ukraine, Transcaucasia, Moldova, Northwest Kazakhstan, Russia, Asia Minor and Northern Africa (Grichanov and Ovsyannikova, 2004). In North America it was introduced in 1897 (Elkinton et al., 2006) – 28 years after the invasion of *L. dispar*. The brown-tail moth is a highly polyphagous species which tends to eruptive population dynamics in forest and agricultural ecosystems; it may cause significant economic loss due to defoliation (Frago et al. 2012). Moreover, browntail-moth is responsible for sanitary problems because the larvae produce a type of urticating hairs which may cause severe skin rashes (Frago et al. 2010) and even death of people (Schaefer, 1974). Sensitive persons may develop dermatitis from direct contact with the caterpillar or indirectly from contact with airborne hairs. Hairs become airborne when they get dislodged from the living or dead caterpillar or from the old exuviae when larvae molt. The rash results from both a chemical reaction to a toxin in the setae (specialized hairs) (Figure 1, B) and a physical irritation as the barbed setae become embedded in the skin. Respiratory distress from inhaling the hairs has been reported from a health survey in the USA (Maine Forest Service - Forest Health and Monitoring Division, 2008).
In fall, colonies consisting of 25 to 400 individuals are building overwintering nests on branches. These nests are constructed from leaves which are wrapped tightly with white silk. As soon as the earliest leaf buds open in spring, the caterpillars emerge from their nests and start to walk around. The larval stages of *E. chrysorrhoea* last for nine months, from August through June and is followed by pupation. The emergence of moths from the cocoons takes place in July. Adults of *E. chrysorrhoea* have a short lifespan and do not feed. After mating, the female moth lays 200 to 400 eggs on the underside of leaves and covers them with brown hair from its abdomen. Brown-tail moth larvae usually hatch from eggs in mid-summer (August or early September) and feed gregariously (Frago et al., 2009) for a short time before they build their winter webs.

The number of larval instars before pupation in the brown-tail moth is not well known. Different authors suggest a total number of five to eight molts (Frago et al., 2009). This uncertainty may probably be explained by the occurrence of developmental polymorphism and the lack of knowledge about diapause in this insect. The importance and effects of diapause for *E. chrysorrhoea* was shown by Frago et al. (2009) who revealed that diapausing individuals had a better fitness than those reared under constant conditions (20 °C, long day photoperiod) from hatching to adulthood. Moreover, larvae reared under conditions that prevented diapause developed into heavier pupae and into females with increased reproductive output, i.e. higher fecundity and fertility. Larvae with a small size at hatching had additional instars compared to larger individuals.

**Figure 1:** Hairs of larvae of *Euproctis chrysorrhoea*. A: ordinary hairs, B: poisonous hairs (Kephart, 1914).
Populations of *E. chrysorrhoea* are phenologically adapted to their local host plants. In Spain winter larval feeding on the evergreen woody shrub strawberry tree, *Arbutus unedo*, has been reported despite the fact that young larvae usually diapause from early autumn to the beginning of spring (Frago et al., 2010). Crucial for this phenological shift is the presence of foliage. The study showed that brown-tail moth larvae doubled their size from October to March, whereas on deciduous *Ulmus minor* and *Quercus faginea* larval size did not change. Additionally, larvae feeding on *A. unedo* were arrested for 2 months and entered a true diapause in this period, while those feeding on deciduous trees remained in diapause for at least four more months (Frago et al. 2010).

The biotops of *E. chrysorrhoea* in Austria include avenue trees, parks, orchards, and mixed deciduous forests where the polyphagous larvae primarily feed on foliage of *Quercus sp.*, *Sorbus sp.*, *Prunus sp.*, *Malus sp.* and *Pyrus sp.*

Sixteen species of parasitoids are important in regulating the number of larvae or pupae of the brown-tail moth. The most widespread and effective ones are the braconids *Meteorus versicolor*, *Apanteles lacteicolor* and *A. laevigatus*; the ichneumonid *Scambus brevicornis* and the tachinid fly *Tachina praeceps* (Mamedov, 1988).

### 1.3. Aims of the study

The aim of this study is a detailed overview about the growth and development of *G. liparidis* parasitoids in larvae of the brown-tail moth, *E. chrysorrhoea*, to gain basic information about the compatibility of the two species as a host-parasitoid association. The experiments focused on parasitization success, duration of endoparasitic stages and total development time from oviposition to adult wasp emergence from cocoons. Moreover, to figure out the effects of parasitism on host development patterns and growth, parasitized brown-tail moth larvae were compared with unparasitized control larvae. Furthermore, parameters like parasitoid clutch size (number of parasitoids per host larva), adult sex ratio of parasitoids, growth and longevity of wasps as well as female wasp preference – if given a choice – to parasitize larvae of the gypsy moth or the brown-tail moth were analysed. The results
were compared with data from parasitized gypsy moth larvae to assess the host compatibility of *E. chrysorrhoea* larvae.

A broader knowledge of interactions between the parasitoid and alternative hosts will lead to a better understanding of host–parasitoid complexes and a better implementation of biological control measures to ensure suitable habitats for potential alternate and overwintering hosts for adequate populations of antagonists which are able to keep pest populations more or less constant.
2. Materials and Methods

2.1. Insects

2.1.1. *Euproctis chrysorrhoea*

Nests of overwintering larvae of *E. chrysorrhoea* were collected in February 2012 from *Sorbus aria* trees near Traismauer, Lower Austria (15°46'00.28'' East, 48°20'52.34'' North, 191 m) and stored at 5°C in small cages until the beginning of the experiments in April.

To activate the larvae, they were put in a climate chamber (Sanyo Incubator, Model MIR-533) under long day photoperiod (16L: 8D) and a temperature of 20°C (day) and 10°C (night), respectively and fed with fresh leaves of *Crataegus monogyna* and *Rosa sp.*. Rearing the larvae on wheat germ diet (Table 1) (Bell et al., 1981) failed because they refused to feed and died of starvation. Accordingly, all *E. chrysorrhoea* larvae used in the following experiments were kept on fresh foliage in plastic boxes (25cm x 25cm x 15cm) with a fine-meshed lid in groups of 100 to 150 individuals (Figure 2 and 3) till they were used in experiments.

![Figure 2: Climate chamber with *E. chrysorrhoea* in plastic boxes.](image1)

![Figure 3: Nest with overwintering *E. chrysorrhoea* larvae from *S. aria* and fresh *Crataegus* branch; larvae start to leave their nest.](image2)
The *C. monogyna* consumed by the larvae were replaced as necessary and faeces was removed regularly. The transfer of the larvae from old to new twigs was carefully handled with forceps in order not to destroy the fragile webs of the larvae which they use to walk around.

To ensure the larval stages, approximately 20 head-capsules per instar were collected from molted *E. chrysorrhoea* larvae and the capsule widths were measured under a light microscope (Wild Heerbrugg). First instar larvae were obtained from overwintering nests; later instars were gathered from individuals kept in Petri dishes. The molting events were recorded regularly.

To establish a permanent colony of *E. chrysorrhoea* at the Institute of Forest Entomology, Forest Pathology and Forest Protection, eleven nests with overwintering larvae were put into a rearing cage filled with fresh *Crataegus* branches in a parafilm-covered glass jar with water and plant fertilizer (Figure 4). In May, the cages were located in a semi-shaded site of the institute garden. The larvae were provided with fresh foliage as necessary, however, the handling of the food-changes (Figure 5) turned out to be very difficult. As the larvae reached instar four, they produced poisonous hair which induced skin rages and increasingly allergic reactions with time.

**Figure 4:** Rearing Cage with overwintering nests of *E. chrysorrhoea* larvae and *Crataegus monogyna* branches as food for the growing larvae.
Figure 5: Food change in the rearing cage. The majority of the *E. chrysorrhoea* larvae moved from defoliated branches to fresh ones on their own. With time the poisonous hair from the larval exuviae accumulated inside the cage.

2.1.2. *Lymantria dispar*

Gypsy moth eggs were obtained from a laboratory culture (New Jersey strain) of the USDA/APHIS Otis Methods Development Center at Cape Cod, MA.

Upon molting to the third instar, larvae of *Lymantria dispar* were kept in plastic boxes in a climate chamber (Bosch, Kirsch) under long day conditions (16L:8D) at 20 °C (Figure 6). They were provided with wheat germ diet (Table 1) (Bell et al., 1981) ad libitum. Diet was exchanged as necessary and faeces was removed regularly.

Figure 6: Young larvae of *L. dispar* in plastic box with wheat germ diet.
<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amounts [g, ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, distilled</td>
<td>815</td>
</tr>
<tr>
<td>Agar, finely ground (Sigma-Aldrich)</td>
<td>15</td>
</tr>
<tr>
<td>Wheat Germ (Dr. Grandel GmbH)</td>
<td>120</td>
</tr>
<tr>
<td>Casein (Sigma)</td>
<td>25</td>
</tr>
<tr>
<td>Ascorbic acid (Vit. C) (Sigma)</td>
<td>5</td>
</tr>
<tr>
<td>Wesson Salt without iron (Bio-Serv)</td>
<td>8</td>
</tr>
<tr>
<td>Sorbic Acid (Sigma)</td>
<td>2</td>
</tr>
<tr>
<td>Methyl Paraben (Sigma-Aldrich)</td>
<td>1</td>
</tr>
<tr>
<td>Chlortetracycline, HCL (Appli Chem)</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin Mixture (Bio-Serv)</td>
<td>10</td>
</tr>
<tr>
<td>Ferric Citrate (Sigma)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

2.1.3. *Glyptapanteles liparidis*

Wasps of *G. liparidis* came from the labor colony of the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), University of Nature Resources and Life Sciences, Vienna. The wasps originated from parasitized *L. dispar* larvae collected in an oak forest in Klingenbach (Burgenland, Austria). The colony is maintained at the IFFF for almost 20 years and refreshed regularly with individuals from field populations. Larvae of *L. dispar* were used as hosts for *G. liparidis*. Adult wasps were fed water and honey. The wasps were kept in a climate chamber (Liebherr profiline) under long day photoperiod (16L:8D) at 15 °C (day) and 10 °C (night).

Both gypsy moth larvae and brown-tail moth larvae were used as hosts for *G. liparidis* oviposition. Before the onset of the experiments, the adult female wasps in the experiments had never been in contact with *E. chrysorrhoea* larvae.
2.2. Experimental set-up

2.2.1. Endoparasitic development of *Glyptapanteles liparidis*

To examine host suitability of *E. chrysorrhoea* larvae for *G. liparidis* wasps and to show host-specific differences between *E. chrysorrhoea* and *L. dispar*, 100 larvae of *E. chrysorrhoea* and 50 larvae of *L. dispar* were individually offered to female wasps for oviposition. In this experiment, one-day-old third instars (L3d1) were used. In order to prevent superparasitization, host larvae were offered by forceps and removed instantly when oviposition was observed. The body mass of the respective larva was recorded with a microbalance (accuracy 0.1 mg; Mettler Toledo) both before parasitization and dissection.

The parasitized larvae were kept individually in glass Petri dishes (Ø 9 cm) with fresh leaves (*E. chrysorrhoea*) (Figure 7) or wheat germ diet (*L. dispar*) (Figure 8).

![Figure 7: Glass Petri dish with twig of *Crataegus monogyna*. The leaves were kept fresh by submersing the petiole in tap water supplemented with plant fertilizer.](image1)

![Figure 8: *Lymantria dispar* larva (third instar) in glass Petri dish with wheat germ diet.](image2)

To document the stadium (egg, first and second instar) and the number of parasitoids per host, larvae were dissected at regular intervals under a stereo microscope (Reichert Biovar) (Figures 9 and 10). Parasitoid numbers were counted and pictures were taken by a digital camera (Nikon COOLPIX P 6000).
2.2.2. Growth and development of parasitized and unparasitized *Euproctis chrysorrhoea* larvae

The effect of parasitization on weight gain and larval development were observed from third instar to pupation in two groups of *E. chrysorrhoea* larvae (unparasitized and parasitized). Thirty larvae were parasitized on the first day after molting to the third instar; individuals were offered to female wasps until a single sting was observed. The larvae were kept in groups of five in Petri dishes until molting to the fourth instar and then transferred individually to Petri dishes. From instar four, larval weights were recorded daily with a microbalance (accuracy 0.1 mg; Mettler Toledo). From parasitized and unparasitized larvae, the dates of molting, the instar number, the date of pupation and the weight of the pupae on the third day after pupation were documented for every single individual. For parasitoids, the date when *G. liparidis* larvae emerged from the host and the number of parasitoids per host larva were recorded.

The pupae were sexed according to morphological differences of the first abdominal segments of males and females (Figure 11). Female and male pupae had comparable sizes.
Parasitism of *E. chrysorrhoea* by *G. liparidis* produced three different outcomes (Table 2): (i) the parasitoids emerged successfully from the host and the host larva died; (ii) parasitization was not successful, but the host larvae developed successfully to pupae and moths; and (iii) both host larvae and parasitoids died in the course of parasitization.

**Table 2:** Outcome of parasitization of *E. chrysorrhoea* larvae by *G. liparidis*.

<table>
<thead>
<tr>
<th><em>E. chrysorrhoea</em></th>
<th><em>G. liparidis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>host larva dead</td>
<td>parasitoids emerge from host larva</td>
</tr>
<tr>
<td>host pupates</td>
<td>parasitoids dead inside host larva</td>
</tr>
<tr>
<td>host larva dead</td>
<td>parasitoids dead inside host larva</td>
</tr>
</tbody>
</table>

**Figure 11:** Abdominal segments of male (left) and female (right) *E. chrysorrhoea* pupae (view from ventral side). A: Anal area, B: Genital area.
2.2.3. Host preferences of female *Glyptapanteles liparidis* wasps

To know how female *G. liparidis* wasps react and behave in terms of oviposition if confronted with a different host than *L. dispar* larvae, an experiment about host preference of female wasps was implemented.

To analyze the host preferences of *G. liparidis* adult female wasps, 5 larvae of *E. chrysorrhoea* and 5 larvae of *L. dispar* were put into a small transparent box with a fine meshed-lid together with 3 female wasps for 20 minutes (Figure 12). As potential hosts, third instars of *E. chrysorrhoea* and second instars of *L. dispar* were used so that both host insects were of similar size and weight. The experiment was repeated 10 times. The parameters observed included the time from exposure to observed oviposition, the species which was chosen as host by the parasitoid and the parasitoid’s behavior when being confronted with the two species at the same time.

Larvae which were observed to be parasitized by female wasps were not removed until the end of the experiment (20 min). After the experiment the larvae were separated according to species, put in Petri dishes and fed with leaves of *Rosa sp.* (*E. chrysorrhoea*) and wheat germ diet (*L. dispar*), respectively.

*Figure 12*: Plastic box with female *G. liparidis* wasps and larvae of *E. chrysorrhoea* and *L. dispar* provided simultaneously for oviposition.
2.3. Statistical analyses

All statistical analyses were performed with SPSS (17.0) for Windows.

The means of two groups were compared by independent-samples t-test with a level of significance of $P \leq 0.05$.

The means of more than two variants were tested with one-way ANOVA and post-hoc Scheffé-test.

Data were tested for normality and Levene’s test was applied to ensure equal variances.

A chi-2 test was used to analyze differences of host preference of *G. liparidis* for *E. chrysorrhoea* and *L. dispar*, respectively.

Data in the text represent means ± SE (standard error).
3. Results

3.1. Separation of *Euproctis chrysorrhoea* instars

To determine each instar and the total number of larval stages of brown-tail moths, head capsule widths were analyzed (Figure 13).

The head capsule width of first instar larvae were 0.84 ± 0.02 mm and doubled when larvae molted to second instars (Table 3). Third-instar larvae of *E. chrysorrhoea* had a head capsule width of 1.36 ± 0.02 mm and fourth instars 2.17 ± 0.04 mm. When larvae molted to L5, the cephalic capsule width was 6 times higher than the initial one.

The increase of head capsule width from the lower to the upper larval stage was highest from instar three to instar four with approximately 0.8 mm, while the lowest was observed between L1 and L2.

In total, *E. chrysorrhoea* larvae had five instars form hatching until pupation under long day photoperiod (16L:8D) and temperatures of 20°C (day) and 10°C (night), respectively.

Head capsule widths differed significantly among the instars.
Figure 13: Head capsule widths (mm) of first to fifth instars of *E. chrysorrhoea*. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate the minimum and maximum values. Significant differences in head capsule width between the instars are indicated by different letters above the boxplots (P < 0.05). *n* = number of tested head capsules.

Table 3: Head capsule widths (mm, means ± SE) of *E. chrysorrhoea* larvae. Means were tested with one-way ANOVA and post-hoc Scheffé-test; values followed by different letters differ significantly between the instars (P < 0.05). Δ - values show the difference between the upper to the lower instar.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Head capsule width</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.48 ± 0.02a</td>
<td>0.36</td>
</tr>
<tr>
<td>L2</td>
<td>0.84 ± 0.00b</td>
<td>0.52</td>
</tr>
<tr>
<td>L3</td>
<td>1.36 ± 0.02c</td>
<td>0.81</td>
</tr>
<tr>
<td>L4</td>
<td>2.17 ± 0.04d</td>
<td>0.61</td>
</tr>
<tr>
<td>L5</td>
<td>2.82 ± 0.05e</td>
<td></td>
</tr>
</tbody>
</table>
3.2. Parasitoid development in *Euproctis chrysorrhoea* and *Lymantria dispar* host larvae

Parasitoid eggs (length about 400 µm) have an oval, elongate form with a tiny tail-like structure on the smaller end, that eventually serves to adhere at host organs (Figure 14, A). First instars (length about 500 µm) have sickle-shaped mandibles (Figure 14, B) with which they fight competitors, but not siblings, while second instars (length about 8 mm) have sucking mouthparts and a characteristic anal vesicle (Figure 14, C). Upon molting from second to third instars, the parasitoid larvae emerge from the host (Figure 14, D).

*Figure 14: Glyptapanteles liparidis*. A: Egg with embryonic band (arrow); B: First instar with sclerotized mandibles (arrow); C: Second instar with anal vesicle (white arrow), the white structures inside the parasitoid are spinning glands (black arrow); D: Third instars emerged from the host larva, spinning cocoons.
3.2.1. Development time of *G. liparidis*

Endoparasitic development of the parasitoids from oviposition into the host until parasitoid emergence from the host larva was 29.7 ± 2.3 days in *E. chrysorrhoea* and 22.7 ± 1.0 days in *L. dispar*, respectively (*t*<sub>d.f.8</sub> = 1.947; *P* = 0.087<sub>two-tailed</sub>) (Figure 15). Immediately after emerging from the host larva, the third-instar parasitoids started to spin a white cocoon for pupation, which took 4 to 6 hours. There were no differences observed in this behavior between the parasitoids from the two host species.

In brown-tail moth larvae, the parasitoids hatched 3.6 ± 0.4 days and in gypsy moth larvae 3.7 ± 0.6 days post oviposition (*t*<sub>d.f.14</sub> = 0.130, *P* = 0.898<sub>two-tailed</sub>). In larvae of *E. chrysorrhoea* the parasitoid larvae molted to second instars 9.2 ± 0.6 days after oviposition (Figure 16, D). Similarly, in gypsy moth larvae the parasitoids ecdysed to second instars 8.8 ± 0.88 days after parasitization. These differences were not significant (*t*<sub>d.f.33</sub> = 1.953, *P* = 0.062<sub>two-tailed</sub>).

While the duration of the egg stage (Figure 16, A) and the first instar (Figure 16, B and C) was unaffected by the host species (Table 4), the second instar of *G. liparidis* lasted about 7 days longer in brown-tail moth host larvae than in *L. dispar* hosts. However, statistically, there were no significant differences between the development times of *G. liparidis* in the two parasitized hosts.

![Figure 15](image-url): Endoparasitic development (days) of *G. liparidis* in *L. dispar* (L.d.; *n* = 34) and *E. chrysorrhoea* (E.c.; *n* = 60) host larvae from oviposition to parasitoid emergence (black arrows) under long day photoperiod (16L:8D) at 20°C (day) and 10°C (night), respectively. The insects were kept in glass Petri dishes and fed with *C. monogyna* and *Rosa sp.* (E.c.) foliage and with wheat germ diet (L.d.). Host larvae were parasitized on day one of the third instar.
Table 4: Comparison of time span (days) of endoparasitic stages (egg, first instar L1, second instar L2, emergence of third instars L3) of *G. liparidis* (G.l.) in the two different hosts *E. chrysorrhoea* (E.c.) and *L. dispar* (L.d.) larvae. Values indicate first and last observation of the stage in the parasitized host larva post parasitization. n = number of tested host larvae.

<table>
<thead>
<tr>
<th>Stadium</th>
<th>G.l.</th>
<th>E.c.</th>
<th>L.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>days</td>
<td>n</td>
</tr>
<tr>
<td>Egg</td>
<td>10</td>
<td>1 - 5</td>
<td>6</td>
</tr>
<tr>
<td>L1</td>
<td>30</td>
<td>6 - 14</td>
<td>8</td>
</tr>
<tr>
<td>L2</td>
<td>18</td>
<td>15 - 29</td>
<td>17</td>
</tr>
<tr>
<td>L3 emergence</td>
<td>7</td>
<td>23 - 39</td>
<td>3</td>
</tr>
</tbody>
</table>

Hosts were offered for oviposition on day one of their third instar. During the parasitoid’s egg stage, 100 % of the host larvae of both species remained in instar 3 (Table 5). When the parasitoids hatched, 56 % of brown-tail moth larvae were in L4 while all *L. dispar* larvae stayed in the L3. When *G. liparidis* larvae molted to second instars, 17 % of *E. chrysorrhoea* larvae were still in L3, 67 % were in L4 and 16 % had already molted to L5, whereas only one third of the gypsy moth larvae had molted to the fourth instar and two thirds were still in L3. Fifty-seven percent of *E. chrysorrhoea* larvae were in instar five when the parasitoids emerged, while 43 % were in instar four. In contrast, 67 % of the *L. dispar* hosts remained in third instar until parasitoid emergence and only 33 % of the host larvae were in L4 when the parasitoids emerged.

The majority of parasitized *E. chrysorrhoea* larvae stayed in the third instar until the parasitoids had completed the first instar. When the parasitoids molted to second instars, the host molted from the fourth to the final instar (L5). One day after the emergence of the parasitoids the host larvae died.

Most of *L. dispar* host larvae remained in the third instar during the endoparasitic development of *G. liparidis*. About one third of the host larvae molted to the fourth instar until the emergence of the parasitoids.

There was no correlation between the molting events of the parasitoids and their host larva.
**Table 5:** Percentage of *E. chrysorrhoea* (E.c.) and *L. dispar* (L.d.) host larvae in different instars in comparison to the parasitoid’s stadium.

<table>
<thead>
<tr>
<th>Stadium</th>
<th>G.I.</th>
<th>E.c. [%]</th>
<th>L.d. [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L3</td>
<td>L4</td>
</tr>
<tr>
<td>Egg</td>
<td>100</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>L1</td>
<td>44</td>
<td>56</td>
<td>---</td>
</tr>
<tr>
<td>L2</td>
<td>17</td>
<td>67</td>
<td>16</td>
</tr>
<tr>
<td>L3 emergence</td>
<td>---</td>
<td>43</td>
<td>57</td>
</tr>
</tbody>
</table>

**Figure 16:** Endoparasitic stages of *G. liparidis*. A: Egg 4 days after oviposition; B: Early first-instar larva; C: Late first-instar larva; D: Second-instar larva.

Successful parasitization of *E. chrysorrhoea* larvae was significantly lower than that of *L. dispar*. Of 97 brown-tail moth larvae where oviposition by *G. liparidis* was observed, 59 larvae (i.e. 61 %) were successfully parasitized, i.e. parasitoids were
found inside the host hemocoel. In contrast, 36 out of 47 gypsy moth larvae (i.e. 77 %) contained parasitoids.

3.2.2. Host body mass

The body mass of parasitized *E. chrysorrhoea* larvae increased six-fold from the time of oviposition until the last endoparasitic instar (L2) of the parasitoid; in *L. dispar* larvae host body mass increased ten-fold.

At the time of oviposition (first day of the third instar – L3d1), *L. dispar* larvae were significantly bigger and heavier than *E. chrysorrhoea* larvae (L.d. 28.2 ± 0.9 and E.c. 11.6 ± 0.8 mg) (Table 6).

Unlike brown-tail moth larvae, which hardly gained weight during the egg stage of the parasitoid, gypsy moth larvae reached twice their initial body mass. During the parasitoids first instar, body mass of *L. dispar* was almost four times higher than that of *E. chrysorrhoea* (L.d. 120.2 ± 17.7 and E.c. 32.1 ± 3.3 mg) (Figure 17). When parasitoids molted from the first to the second instar, gypsy moth larvae gained 163.5 mg and brown-tail moth larvae 44.8 mg, respectively. However, the relative increase of body mass of the two species during the last endoparasitic instar (L2) of *G. liparidis* was nearly the same for both hosts.

<table>
<thead>
<tr>
<th>Parasitoid stadium</th>
<th>E.c.</th>
<th>L.d.</th>
<th>T-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mg</td>
<td>increase</td>
<td>n</td>
</tr>
<tr>
<td>Oviposition</td>
<td>58</td>
<td>11.6 ± 0.8</td>
<td>---</td>
<td>31</td>
</tr>
<tr>
<td>Egg</td>
<td>10</td>
<td>12.9 ± 1.7</td>
<td>1.1x</td>
<td>6</td>
</tr>
<tr>
<td>L1</td>
<td>30</td>
<td>32.1 ± 3.3</td>
<td>2.8x</td>
<td>8</td>
</tr>
<tr>
<td>L2</td>
<td>18</td>
<td>76.9 ± 9.9</td>
<td>6.6x</td>
<td>17</td>
</tr>
</tbody>
</table>
Figure 17: Host body mass (mg, means ± SE) of L. dispar and E. chrysorrhoea larvae during parasitoid development. Grouping of the host larvae (E.c. and L.d.) were done according to the developmental stage of the parasitoids (oviposition, egg, first instar L1, second instar L2) regardless of the host instar. Values at the bottom of the boxes represent the number of tested host larvae. Significant differences in body mass within a host species is given by different letters above the columns (P < 0.05). Significant differences in host body mass between the two host species during a given parasitoid stadium is indicated by P-values (t-Test).

3.2.3. Parasitoid loads

The number of parasitoids per E. chrysorrhoea host larva showed no significant differences in clutch size compared to the number of parasitoids per L. dispar larva ($t_{df.92} = 0.717, P = 0.474_{\text{two-tailed}}$). On average, 22.1 ± 1.3 eggs were injected in brown-tail moths and 23.7 ± 2.0 in gypsy moths; minimal numbers were 4 in
*E. chrysorrhoea* and 3 in *L. dispar*, maximum numbers were 51 and 52, respectively (Figure 18).

No correlation was found between the number of parasitoids per host larva and the host body mass of the host larva at the time of oviposition.

![Figure 18](image-url)

**Figure 18**: Number of parasitoids per host larva in *Lymantria dispar* (L.d.) and *Euproctis chrysorrhoea* (E.c.). The horizontal line inside the box represents the median. The length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate the minimum and maximum value. *n* = number of tested host larvae.

### 3.3. Growth and development of parasitized and unparasitized *E. chrysorrhoea* larvae

#### 3.3.1. Development time

Development time of parasitized larvae was calculated as the number of days from parasitization (L3d1) until emergence of the parasitoids; for unparasitized larvae, the number of days between L3d1 and the completion of pupation was 44.8 ± 0.9 days (Figure 19) to reach the pupal stage. Pupation usually started after 40.1 ± 0.9 days.

While parasitized host larva molted after 10.1 ± 0.4 days to the fourth and after 22.0 ± 0.7 days to the fifth instar, control larvae (up) needed 9.7 ± 0.3 days to molt into L4 and 21.3 ± 0.7 days to reach instar five. However, there were no significant differences in development time between parasitized and unparasitized L3 and L4 brown-tail moths, respectively (L3: *t*~d.f.29~ = 1.214, *P* = 0.235 two-tailed; L4: *t*~d.f.26~ = 0.576,
P = 0.570\textsubscript{two-tailed}. Parasitism of *G. liparidis* did not reduce or prolong the duration of the third and fourth instar of *E. chrysorrhoea*.

Due to the fact that parasitoids emerged 30.6 \pm 2.4 days post oviposition from parasitized *E. chrysorrhoea* larvae, host development was interrupted in the fourth or fifth instar (see Table 5). All parasitized larvae died one day after the emergence of the parasitoids. All unparasitized brown-tail moth larvae pupated after five instars, i.e., 44.8 \pm 0.9 days after L3d1. It took four to five days until they fully pupated under the given experimental conditions (20 °C day, 10 °C night).

**Figure 19**: Duration of larval stages (days) of parasitized (p) *E. chrysorrhoea* host larvae compared to unparasitized (up) larvae from the first day of the third instar (L3d1) until parasitoid emergence (parasitized larvae) or pupation (control larvae).

### 3.3.2. Larval and pupal body mass

Test larvae were weighed on day one of the third instar before the experiment started and then divided into two groups, each consisting of 30 *E. chrysorrhoea* larvae. Larvae intended for parasitization had 8.89 \pm 0.50 mg and the control group 10.61 \pm 0.51 mg body mass (Figure 20).

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Figure 20: Body mass (mg) of *E. chrysorrhoea* larvae before parasitization on day one of the third instar (L3d1). Individuals were divided into two groups – p (intended for parasitization) and up (unparasitized – control group). The horizontal line inside the box represents the median. The length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate the minimum and maximum value. n = number of tested larvae.

Analyses of the maximum weights per larva in the fourth instar showed similar results for the two groups – 115.43 ± 17.03 mg for parasitized and 125.70 ± 4.26 mg for unparasitized larvae – while body mass in the fifth instar was 154.51 ± 32.73 mg for parasitized individuals and 267.45 ± 8.67 for the control group (Figure 21).

Due to the high variability in maximum weights of individual larvae of the parasitized group, differences between parasitized and unparasitized host larvae were not significant in L4 (*t*<sub>d.f.29</sub> = 0.864, *P* = 0.395<sup>two-tailed</sup>) but highly significant in L5 (*t*<sub>d.f.26</sub> = 4.572, *P* = < 0.001).
Eighty percent (n = 24) of the control larvae reached the pupal stage, but also 37 % (n = 11) of the parasitized larvae pupated successfully.

In some cases, mortality was observed at the beginning of pupation of fifth-instar larvae of the brown-tail (Figure 22). The larvae attached their bodies to the glass surface of the Petri dish but were unable to proceed with pupation and instead died. This mortality rates were similarly for both groups – 7.7 % (2 out of 26) for unparasitized and 8.3 % (1 out of 12) for parasitized individuals.

**Figure 21:** Body mass (mg, mean ± SE) of parasitized (p) and unparasitized (up) larvae of *E. chrysorrhoea* in the fourth (L4) and fifth (L5) instar. Values within the boxes are the numbers of tested larvae.

**Figure 22:** Death of *E. chrysorrhoea* larva at the onset of pupation. Arrow indicates silk threads.
Pupal body mass was recorded on the third day after pupation. Pupae of parasitized *E. chrysorrhoea* larvae weighed 173.46 ± 10.35 mg and pupae of the control group 177.61 ± 6.36 mg (Figure 23). The differences between the groups were not statistically significant ($t_{d.f.32} = 0.349$, $P = 0.489_{\text{two-tailed}}$).

![Figure 23: Body mass (mg) of *E. chrysorrhoea* pupae on the third day after pupation. *p* = pupae from parasitized larvae; *up* = pupae from unparasitized larvae; *n* = number of tested larvae. The horizontal line inside the box represents the media, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate the minimum and maximum value.](image)

### 3.3.3. Mortality rates

Compared to unparasitized brown-tail moth larvae, parasitized individuals showed a higher mortality (Figure 24). Of a total number of 30 larvae parasitized by *G. liparidis* wasps in L3d1, 13 % died in the third instar ($n = 4$) and 10 % in the fourth instar ($n = 3$). Highest mortality occurred in the fifth instar (17 %; $n = 5$). In contrast, only one out of 30 unparasitized larvae died in the third instar (3 %) and three in the fifth instar (10 %); no mortality was observed in the fourth instar. In total, 40 % ($n = 12$) of the parasitized *E. chrysorrhoea* larvae died, whereas only 13 % ($n = 4$) of the control group (unparasitized) perished.

All host larvae of the group of parasitized larvae were dissected post mortem; analyses showed that none of the dead larvae contained parasitoids.
3.3.4. Successful wasp development

Utilization of *E. chrysorrhoea* larvae as hosts produced different outcomes for the parasitoid. Thirty-seven % (n = 11) of brown-tail moth larvae parasitized by *G. liparidis* developed into normal pupae and moths, suggesting that the host larvae were able to mount significant defence reactions to kill the parasitoid eggs or that the wasps did not oviposit into the larvae (parasitoids dead/host pupate). Twenty-three % (n= 7) of the host larvae were successfully parasitized and *G. liparidis* larvae (L3) emerged; the host larvae died one day after parasitoid emergence (parasitoids emerge/host dead). In most cases (40 %; n = 12), however, both the parasitoids and their host died (Figure 25).

*Figure 24:* Mortality (%) of parasitized (p; n = 30) and unparasitized (up; n = 30) *E. chrysorrhoea* larvae. Larvae were parasitized by *G. liparidis* wasps as L3d1.
3.3.5. Offspring sex ratio

In total, parasitoid larvae emerged from seven brown-tail moth larvae and started immediately to spin cocoons. Adult wasps emerged successfully of all cocoons from six host larvae. No wasps emerged of the cocoons (n = 9) from the seventh host larvae. The reason why no wasps emerged is unknown.

On average, the parasitoid offspring sex ratio from *E. chrysorrhoea* was clearly male-biased (2.3:1). Seventy percent (n = 46) of adult wasps were males, 30 % females (n = 20). This ratio was identical to the sex ratio of parasitoids which developed in *L. dispar* larvae (males, n = 233; females, n = 101) (Figure 27). From a single *E. chrysorrhoea* host larva more females than males emerged (Figure 26).

The sex ratio of parasitoids from gypsy moth host larvae showed high variations between the individual larvae. In almost 50 % (n = 7) of *L. dispar* host larvae the sex ratio was 1:1, while from the rest of the host larvae considerably more male than female wasps emerged.

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**Figure 25:** Survival and mortality (%) of host and parasitoids in the system *E. chrysorrhoea* and *G. liparidis*. The host larvae were parasitized in L3d1.
Figure 26: Number of male and female *G. liparidis* wasps emerging from individual *E. chrysorrhoea* (E.c.) host larvae (1-7). In the case of host larva number 5 no female wasps were found; from host larvae number 7 no wasps emerged from the cocoons (n = 9).

Figure 27: Number of male and female *G. liparidis* wasps emerging from individual *L. dispar* (L.d.) host larvae (1-13).
3.4. Wasp oviposition choice

To test the preference of the parasitic wasps for one or the other host, three adult females were put into a small box together with 5 larvae of *E. chrysorrhoea* and 5 larvae of *L. dispar* for 20 minutes. Third instars of brown-tail moths and second instars of gypsy moths were used so that the potential hosts were of similar size. The experiment was repeated 10 times. Larvae which were observed to be parasitized were not removed until the end of the experiment (20 min).

Female *G. liparidis* wasps were not very eager to attack and oviposit in *E. chrysorrhoea* larvae. The wasps inspected the brown-tail moth larvae and were obviously attracted by them, however, they significantly preferred gypsy moth larvae for oviposition (Chi-2 = 15.5, P < 0.001). From all larvae (n = 14) parasitized in the experiment, 93 % (n = 13) were *L. dispar* larvae while only 7 % (n = 1) were *E. chrysorrhoea* larvae (Figure 28).

In total, 14 % (n = 14) of 100 host larvae offered were parasitized in this experiment (Figure 29). This means that only 2 % (1 out of 50) of brown-tail moth larvae and 26 % (13 out of 50) of gypsy moth larvae were used for oviposition.

On average it took 2.1 ± 0.7 sec. between wasp release into the box with potential host larvae and wasp oviposition.

![Wasp oviposition choice](image)

**Figure 28:** Wasp oviposition choice between *E. chrysorrhoea* (E.c.; n = 50) and *L. dispar* (L.d.; n = 50). Wasp oviposition choice differed significantly between the two hosts.
Figure 29: Observed parasitization (%) of host larvae (E.c. and L.d.) presented to G. liparidis wasps. p = parasitized (n = 14); up = unparasitized (n = 86).
4. Discussion

4.1. Parasitoid development in *E. chrysorrhoea* and *L. dispar* host larvae

The reproductive success of female parasitoids depends on their ability to find and select suitable hosts in a changing and diversified environment. The host selection process depends on environmental and host factors and the parasitoid is “guided” to a host habitat and to the host itself by chemical and physical cues (Rehman and Powell, 2010). Successful endoparasitic development occurs if the progeny is able to overcome the host immune reaction and if it finds optimal nutritional conditions to feed and grow properly. Therefore, parasitoid larvae have to adjust their own development to their host, but on the other hand they have to regulate the host’s organism to meet their own requirements.

4.1.1. Development time of *G. liparidis*

Sequeira and Mackauer (1992) describe the ultimate goal of a parasitoid’s strategy as the maximum biomass acquisition in the shortest possible developmental time. This implies that faster development will lead to earlier sexual maturation and reproduction of the offspring.

*Glyptapanteles liparidis* parasitoids that developed in *E. chrysorrhoea* and *L. dispar* host larvae had similar development times in the egg stage and only marginal differences were noticeable during the parasitoid’s first instar which took about one day longer in brown-tail moth larvae than in gypsy moth larvae. Total development time from oviposition until the emergence of the fully developed parasitoids from the host was 29 to 30 days in brown-tail moth and 22 to 23 days in gypsy moth, i. e., development lasted one week longer in the comparatively smaller brown-tail moth larvae.

It is known that host size of *L. dispar* larvae at the time of oviposition influences the endoparasitic development time of *G. liparidis*. The development time of *G. liparidis* larvae lasted about two to three days longer when hosts were parasitized in L2/3 than in the bigger fifths instar (Schafellner, pers. communication). The more parasitoids develop in a single host the less nutrients are available for the single individuum. This implies that the higher need for nutrients of the gregarious
parasitoids is more difficult to meet for smaller host larvae than for bigger ones and results in a prolonged development time of the parasitoids.

Studies showed that the development time of *G. liparidis* larvae is also influenced by the diet the host larvae feed. *Lymantria dispar* larvae reared on a leaf powder diet made from lyophilized young leaves of *Quercus petraea* and *Q. cerris* developed faster and achieved higher pupal weights than on mature leaves because of the lower nutritional quality. When host larvae were fed less suitable diets, the development of *G. liparidis* wasps was delayed but adult wasp weight and longevity were hardly affected. Hence, parasitoids seem to be able to adjust to unfavourable nutritional conditions mediated by their host by prolonging the duration of development to overcome nutrient constraints (Krämer et al., 2005). The development of the parasitoid larvae can even come to a halt when host larvae are badly nourished. Dissections of dead *L. dispar* host larvae that were fed on a diet of apricot-leaves showed that the parasitoids were alive but unable to molt from the first to the second instar (Schafellner and Schopf, 2003).

The prolonged period of development of *G. liparidis* in brown-tail moths occurred during the parasitoids’ second instar. This elongation was probably the reaction of the parasitoid larvae to suboptimal nutrient quality and/or quantity. As a consequence, the parasitoids needed longer to finish their endoparasitic development. This assumption is corroborated by the observation that *G. liparidis* parasitoids gain 80% of their maximum body mass during the last three days before they emerge from the host (Schopf, 2007); by contrast, earlier parasitoid stages are characterized by slow growth rates.

Another explanation for the prolonged development time of the parasitoids in brown-tail moth larvae is the observed difference in the growth patterns of the two host species, not only with respect to the host’s final size, but also to the development time. While *E. chrysorrhoea* larvae developed faster and a large part of the parasitized larvae reached the fifth instar, gypsy moth gained more body mass from oviposition until parasitoid emergence but the majority of the larvae remained in the third instar. These results are influenced by the experimental conditions the larvae were exposed to (long-day photoperiod at a temperature of 20°C day and 10°C night, larvae were parasitized in L3d1) thus, general conclusions have to be made carefully.
In addition to host size and nutritional conditions, development time of parasitoids is influenced by temperature and photoperiod. The period between parasitization and parasitoid emergence decreases with increasing temperature (Nealis and Fraser, 1988; Gould and Elkinton, 1990; Allen and Keller, 1991; Tillman and Powel, 1991; Oliveira et al., 1998).

Larvae of *G. liparidis* need a host larva for overwintering. In a current study, Fromm (pers. communication) investigates the overwintering behavior of *E. chrysorrhoea* larvae parasitized by *G. liparidis*. Brown-tail moth larvae exhibit obligatory diapause during winter. Dissections showed that the parasitoids remained as first instars inside the host hemocoel regardless of the temperature or photoperiod (long/short day conditions, 20 °C constant). This implies that the first instar of the parasitoids is the overwintering larval stage and development stops until the host completes diapause and starts feeding on plants and the nutritional requirements of the endoparasitoids are met.

### 4.1.2. Rate of parasitization

Successful parasitization of host larvae represents the ability of the parasitoid to avoid or overcome host immune responses, colonize and use its host for successful development throughout their parasitic stages. For *G. liparidis*, Schopf and Steinberger (1996) proved that the wasps prefer younger host instars for oviposition; the parasitoids emerged successfully from 77% to 90 % of gypsy moth larvae parasitized in L1 to L3. In my study, the host larvae were offered on the first day of their third instar to female wasps for oviposition. Parasitization success was higher in *L. dispar* larvae than in brown-tail moth larvae but statistically the differences were not significant. These results prove that *G. liparidis* is able to develop successfully in *E. chrysorrhoea* as well as in gypsy moth larvae. It may be assumed that parasitization success will increase when younger brown-tail moth host larvae are parasitized. In gypsy moth, Schopf and Steinberger (1996) observed a parasitization success of 90 % when larvae were parasitized during the premolt of the second instar. Host larvae stung in L3/4 reached a lower parasitization success of 76 % (Schafellner and Schläger, 2009)
4.1.3. Parasitoid load

To optimize the fitness of their progeny, female wasps adjust the number of eggs to a host depending on the host’s quality (Häckermann et al., 2007). In general, large hosts are expected to be more advantageous in terms of offspring fitness than small hosts because they contain a greater quantity of resources (Harvey et al., 2004). Contrary to the assumption that a lower number of parasitoid larvae develop in smaller host larvae, the comparison of clutch size revealed that *G. liparidis* females deposited an equal number of eggs in the comparatively smaller *E. chrysorrhoea* larvae as in the larger *L. dispar* larvae. Dorn et al. (2007) came to the same result when smaller *Cydia molesta* and larger *C. pomonella* host larvae were parasitized by *Hyssopus pallidus*. Hence, it appears that not only host size but also the nutritional and endocrinological status of the host at oviposition could have an impact on clutch size. Therefore, host quality comprises not only the host’s body mass but also the amount and quality of nutrients available (Sequeria and Mackauer, 1992). The current results support this assumption: no correlation was found between clutch size and host body mass of both gypsy moth and brown-tail moth larvae.

4.1.4. Offspring sex ratio

In hymenopterans, females determine the sex of their offspring by controlling fertilization of their eggs (Flanders, 1965). Their haplodiploid system, which means that males develop from unfertilized eggs and females from fertilized eggs, the female mother to adapt the offspring sex ratio to environmental conditions (e. g. local host availability and quality) (Nussbaumer and Schopf, 2000). Offspring sex allocation theory predicts that more fertilized eggs (yielding female offspring) are deposited into hosts of superior quality, and unfertilized eggs (yielding males) are injected into lower-quality hosts (Godfray, 1994). Therefore, male biased sex ratios suggest low host quality (Van Driesche and Murray, 2004) and hinder efforts to mass release of parasitic Hymenoptera by making the production of females costly (Fuester et al., 2007). However, it is assumed that inbreeding of parasitoids in laboratory colonies has negative effects on the offspring ratio of female progeny. Probably this is the reason why the sex ratio of *G. liparidis* in the laboratory differs from that in the field. Due to the fact that the offspring sex ratio was 3:7 females to
males for parasitoids developing in *L. dispar* and *E. chrysorrhoea* larvae, it appears that the sex ratio was not influenced by the host species but is a result of the wasp behavior.

4.2. Development of parasitized and unparasitized *E. chrysorrhoea* larvae

4.2.1. Development time

Host larvae parasitized by koinobiont species continue to grow and develop, but larval endoparasitoids can alter growth and development and even the behavior of their hosts to meet their own nutritional requirements (Gauld, 1988).

Schopf and Steinberger (1996) mentioned that the influence of parasitism by *G. liparidis* on its host *L. dispar* could prolong the duration of the instar of their host from which the parasitoids emerged and induced extended or additional host instars. This is a very common phenomenon in host-parasitoid systems, because slowing down host larval development, especially the last host instar (i.e., the instar from which the parasitoids emerge), is necessary for the parasitoids to finish their development and prevent further energy-intensive molts of the host (Nussbaumer and Schopf, 2000). Besides, the host is developmentally arrested as a larva and lives for several days in a post-emergence nonfeeding state and acts as a “bodyguard” of the parasitoid progeny before it dies (Beckage and Gelman, 2004). In my studies, I couldn’t observe such behavior in the host *E. chrysorrhoea*. Parasitized brown-tail moth larvae followed the same developmental pattern like unparasitized ones from the first day of the third instar till molting to the fifth instar. Differences in development time were given in L5, due to the fact that parasitoids emerged from parasitized individuals, which consequently died, while unparasitized ones developed continuously, pupated and eclosed as adult moths.

In the field, it is conceivable that *E. chrysorrhoea* larvae parasitized in later instars, follow different developmental patterns, i.e., there are probably prolonged or additional host instars in reaction to parasitism. Similarly, parasitoids themselves will eventually change their behavior if developing in younger host larva, e.g. first or second instars. Since the parasitoids depend on their host’s nutritional condition, which correlates with age and size of the host, they may remain in the egg stage or
as first instars till they find better nutritional conditions for further development. Further studies are needed to test this hypothesis.

4.2.2. Larval and pupal body mass

Comparisons of the body mass of parasitized and unparasitized *E. chrysorrhoea* larvae did not show any differences in L4. The hemolymph-feeding larvae of *G. liparidis* seem to pursue their comprehensible strategy not to harm the host body because they depend on host resources. The disturbance of the host metabolism and behavior is kept low as long as possible. However, the effect of parasitism was clearly visible when the host larvae reached their final (i.e., fifth) instar. Unparasitized L5 larvae were significantly heavier (267.45 ± 8.67 mg) than parasitized ones (154.51 ± 32.73 mg). Nevertheless, the differences in body mass of L5 were not statistically significant because of the small sample size and the broad range in individual body masses of parasitized host larvae. In response to parasitism by gregarious parasitoids, it is reported that many host species consume more food to compensate for the nutrients that are directed towards the parasitoid (Parker and Pinnell, 1973; Sato et al., 1986; Schopf and Steinberger, 1996; Nakamatsu et al., 2001). Thus, increased food consumption, which was not evaluated in this study, does not imply that the host gains higher body mass because of the parasitoid’s nutritional requirements.

Several studies have demonstrated that the effect of parasitism on growth and development of the host depends on the number of parasitoids per host (Cloutier and Mackauer, 1980; Schopf and Steinberger, 1996; Alleyne and Beckage, 1997; Harvey, 2000). In my study, results did not show a correlation between the clutch size and the host’s body mass at the time of oviposition.

In contrast to idiobionts, for koinobionts the host size at oviposition is less important (Reudler Talsma et al., 2007). If the host is too small at parasitization, there may be not enough resources for proper development of the parasitoid larvae. Otherwise, if the host is too big, its defenses may kill the parasitoid eggs injected.

No significant differences were observed between the body mass of parasitized *E. chrysorrhoea* pupae and pupae of the unparasitized control group. This
observation together with the fact that there was an unexpected high number of parasitized larvae that developed into pupae moths (37 %), indicates “pseudoparasitization”. This psuedoparasitism includes several possibilities why the parasitoids were not able to emerge: (i) the female wasps did not inject eggs at oviposition, (ii) the wasps injected eggs, but the parasitoids were unable to develop, e. g. they were killed by the host’s immune reaction.

Similarly, parasitization effects were not reflected in the rates of malformation of pupating larvae, which was generally very low and almost equal for both groups (parasitized and unparasitized).

4.2.3. Mortality and parasitization rates

A major factor for successful parasitoid development is host suitability. Twenty-three percent of *G. liparidis* developed successfully in larvae of the brown-tail moth and were able to overcome the host’s cellular encapsulation reaction and humoral defences. Approximately one third of the parasitized *E. chrysorrhoea* larvae pupated and molted into moths. In this case the parasitoid larvae probably died at some time because of host immune responses (e.g. the parasitoid-eggs were encapsulated) or the host larvae were pseudoparasitized, i. e., no eggs were injected.

A pathogenic infection of the parasitized host may result in early death of both the host and the parasitoid. Results from dissection of the dead larvae did not give any hint of pathogen infections as a possible reason for the early death. Eventually, venom and calyx protein amounts or activity were too severe for the host larvae to survive. Similar mortality rates of host larvae were observed when unsuitable hosts of *G. liparidis* (e. g. *Lymantria monacha*, nunmoth) were parasitized or when young gypsy moth larvae were superparasitized (i. e., when more than one female wasp oviposited into a single larva).

In the present study, 40 % of the parasitized hosts died prematurely, i. e., neither the host nor the parasitoids reached the reproductive stage. During wasp oviposition not only the parasitoid eggs are injected into the host hemocoel but also polydnavirus particles, venom and calyx fluids (Alleyne and Wiedenmann, 2001). These wasps-associated maternal factors may harm the host larvae and lead to intoxication.
followed by premature host death. Mortality of unparasitized *E. chrysorrhoea* larvae was low (13.3 %) compared to parasitized ones (40 %) and all instars (L3, L4, L5) where affected. Larvae which died in L4 or L5 were dissected. The result that no parasitoids were found in the host hemocoel suggests that either eggs were encapsulated and eliminated so that they could not be found or that the female wasps did not inject eggs at all. If *G. liparidis* wasps injected only polydnavirus particles, venom and calyx fluid mortality may be attributed to these maternal factors.

### 4.3. Wasp oviposition choice

Host selection in parasitoids often involves a hierarchy of several behavioral steps like habitat location, host location and host examination. Parasitoids use a variety of chemical and physical cues in this process. Several parasitoid species respond to stimuli associated with the host or the host’s food plants before the host itself is encountered (Rehman and Powell, 2010). Parasitoids search non-randomly but learn cues from different trophic levels during foraging and alter their decisions accordingly (Vet, 1996) to improve their chances of finding suitable hosts by changing their searching behavior in reaction to chemical stimuli (Rehman and Powell, 2010).

Successful parasitism of a host encountered by a female wasp depends largely on host quality (Gu et al, 2003). Host preference may either be a rationalized attitude of the female that determines whether to accept or reject a host (Rehman and Powell, 2010). In my study, only a single brown-tail moth larva was parasitized by a wasp compared to 13 gypsy moth larvae. To exclude the influence of host size on wasp oviposition choice, the hosts presented to the wasps were of similar size (i.e., second instars of *L. dispar* and third instars of *E. chrysorrhoea*). The main reason for the observed results was probably not host quality but the fact that female wasps used for oviposition were no naïve individuals but had oviposition experience with gypsy moth larvae before used in this experiment. However, in the field we must assume that *G. liparidis* wasps are able to accept host species that are different from the host where the wasps developed because a switch to alternate hosts is necessary for the wasp population to survive.
5. Summary

When parasitized as third instars and reared under long day photoperiod, larvae of *E. chrysorrhoea* proved to be at least partially suitable hosts for the gregarious wasp *G. liparidis*. Development time of the endoparasitoids lasted about one week longer in brow-tail moth than in gypsy moth larvae but the number of parasitoids per host larva and the offspring sex ratio were identical for both hosts. However, parasitism rates of *E. chrysorrhoea* were lower compared to *L. dispers* larvae. Forty percent of brown-tail moth larvae died after parasitization, from seven host larvae parasitoids emerged and a significant part of parasitized *E. chrysorrhoea* larvae pupated successfully, which means that parasitoids were not able to develop. When given a choice female wasps preferred gypsy moth larvae for oviposition, which is thought to be mainly influenced by the use of experienced and not naïve wasps. In the field host, acceptance and performance in *E. chrysorrhoea* may be better than under the given laboratory conditions because of different behavior of female wasps, e.g. when they are in need for host larvae or when no other host larvae are available.
6. References


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