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Temperature-related development and adult wasp longevity of three endoparasitic *Glyptapanteles* species (Hymenoptera: Braconidae) in their host *Lymantria dispar* (Lepidoptera: Lymantriidae)

Master Thesis

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Abstract

The present study analyzed the effects of temperature on development, survival and adult longevity of three endoparasitic wasp species, *Glyptapanteles liparidis*, *G. fulvipes* and *G. porthetriae* (Hymenoptera: Braconidae) parasitizing larvae of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), a serious forest pest in Europe, Asia and North America. The duration of the immature wasp stages (egg, first and second instar, pupa) and total development from oviposition to adult wasp emergence were tested in the laboratory at constant 15°C, 20°C and 25°C, respectively, under long day photoperiod. Additionally, *G. liparidis* and *G. fulvipes* wasp lifetimes were recorded at 15°C and 20°C. For all wasp species endoparasitic development (i.e. from oviposition until parasitoid emergence from the host) was longest at 15°C, intermediate at 20°C and shortest at 25°C. At the two lower temperature regimes, the solitary species *G. porthetriae* had shorter development times than the gregarious species *G. liparidis* and *G. fulvipes*; there were no differences among the wasps at 25°C. The lower developmental thresholds (LDTs) calculated from linear regression equations for the combined immature stages were 9.6°C (*G. liparidis*), 8.9°C (*G. fulvipes*) and 7.6°C (*G. porthetriae*), respectively. For the endoparasitic stages, the LDTs were 9.9°C (*G. liparidis*), 8.8°C (*G. fulvipes*) and 6.9°C (*G. porthetriae*). The thermal sums (°C) for the immature wasp development were 200, 228 and 235 degree-days (DD) for *G. liparidis*, *G. fulvipes* and *G. porthetriae*. For total immature development the thermal sums were 283, 310 and 325 DD for *G. liparidis*, *G. fulvipes* and *G. porthetriae*. Adult wasp longevity at 15°C was significantly longer than at 20°C (30 versus 10 days), and longer for males than for females; *G. liparidis* wasps lived longer than *G. fulvipes* wasps. The results are important for evaluating the potency of the wasp species as biological control agents of lepidopteran pest insects.

1 Introduction

1.1 Aims of the study

Growth and development of insects are influenced by abiotic factors such as temperature, light and moisture and biotic factors such as the quantity and quality of food and the vitality of the individual insect due to its phenotypic flexibility and genotypic diversity (Leonard 1981, Chown & Nicolson 2008). Because insects are ectothermic organisms, temperature has the greatest effect on insect development and development time decreases as temperature increases (Beck 1983). Within a certain range of physiologically suitable temperatures, the speed of development increases almost linearly with rising temperatures. At very low temperatures development is retarded and then ceases. As temperatures rise, development duration decreases up to an optimum temperature, above which development slows again and ceases at the temperature maximum. A common way to model temperature effects on insect development is to convert the times required to complete specified stages or instars to their reciprocals. This transformation allows to determine two important parameters of insect development, the lower development threshold (LDT) and the thermal constant (Damos & Savopoulou-Soultani 2012). The LDT for a species is the temperature below which development stops. The thermal constant is expressed as degree-days (DD) and refers to the sum of heat accumulated (°C) above the LDT that is required to complete a particular development stage. The definition of these parameters is a prerequisite for accurate prediction of the phenology of an insect. Under field conditions, a precise phenology description is important for successful timing of pest management practices. For parasitoids, understanding the effect of temperature on development and survival of parasitic wasps is of great importance for optimizing mass rearing in order to be used as biological agents in integrated management programs against insect pests.

In the present study I analyzed the effects of temperature on development, survival and adult longevity of three endoparasitic wasp species of the genus *Glyptapanteles* (Hymenoptera: Braconidae) parasitizing larvae of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). The purpose of this study was to obtain precise data about the duration of the endoparasitic development (egg, first, second instar), the pupal stage and the total immature development (egg to adult wasp eclosion) of *G. liparidis*, *G. fulvipes* and *G. porthetriae* under constant temperature regimes to calculate the LDT and thermal constants. Moreover, wasp lifetimes were followed at two temperature regimes. The

results are important for evaluating the potency of the wasp species as biological control agents of the gypsy moth.

1.2 Gypsy moth

The gypsy moth is a forest pest and a serious defoliator of broadleaf tree species in Europe, North America, Asia and North Africa (Alalouni et al. 2013, Doane et al. 1981, Elkinton & Liebhold 1990, Hoch & Schopf 1995, McManus & Csóka 2007, Nierhaus-Wunderwald 2001). The moth has only one generation per year, undergoes four developmental life stages (egg, caterpillar, pupa and adult) and overwinters in the egg stage on the bark of tree stems, where females lay between 500 to 1000 eggs in a single mass and cover them with body hair (Leonard 1981). Larvae hatch in spring when the new leaves emerge. Young instars feed during the day, older instars are active during the night. In early summer, the larvae pupate in bark crevices; the adult moths emerge in July/August. The moths do not feed and females are unable to fly. The moths usually die within a few days after mating and egg laying. During mass outbreaks, the feeding damage of the larvae limits photosynthetic assimilation and tree growth (Alalouni et al. 2013, Milanović et al. 2014). Defoliation over consecutive years reduces tree vigor and renders the trees susceptible to attack by secondary mortality-causing agents such as jewel beetles (McManus & Csóka 2007).

1.3 The braconid wasp genus *Glyptapanteles* (Hymenoptera: Braconidae)

Parasitic Hymenoptera help to maintain the ecological balance and provide greater stability of the ecosystems in which they occur (LaSalle 1993, Shaw & Hochberg 2001, Shaw 2006). Moreover, they are useful 'model organisms' to examine different fitness functions such as body size and development time (Harvey 2005). The gregarious braconid wasps *G. liparidis* and *G. fulvipes* and the solitary species *G. porthetriae* are larval parasitoids of the gypsy moth. As koinobionts, they are characterized by developing in a living, growing and molting host. The wasps are perfectly adapted to their host. Adult wasps are very effective in visually detecting a suitable host. In some wasp species, females are able to discriminate between parasitized and unparasitized hosts (Jervis et al. 1996). The movement and the size of the host are the most important stimuli eliciting host

grasping prior to oviposition (Matthews 1974). The high host finding capability of *G. liparidis* and *G. porthetriae* makes them especially effective in low-density gypsy moth populations (Hoch et al. 2001, Marktl et al., 2002). The host quality influences three main components of parasitoid fitness: survival to the adult stage, size and development time. Size and age are the most important measures of host quality (Strand 2002). In *G. liparidis* the molt of the parasitoid larvae to the second instar strongly depends on the nutritional status of the host (Schafellner 2004, Schopf & Nussbaumer 1996).

Endoparasitoids evolved physiological adaptations to prevent or resist the host's defense reactions, use the host's nutrients and influence its development. *G. liparidis* female wasps parasitize gypsy moth larvae during the 1st to 3rd instar and inject 5 to 30 eggs per host (Schopf and Steinberger 1996). However, more than 100 parasitoids can develop in a big host larva (Schafellner et al. 2007). During oviposition *Glyptapanteles* females introduce virus particles (polydnavirus) and venom to downregulate the host immune defenses such as encapsulation and melanization of the parasitoid egg (Schafellner et al. 2004, 2007). The parasitoid larvae hatch and pass through two endoparasitic instars. They do not damage the host tissue but feed on the host hemolymph. After two to three weeks the larvae emerge as newly molted third instars through the skin of the still living caterpillar. Close to the host's body, the parasitoid larvae immediately spin a white cocoon where the pupate (Schopf 2007). All *Glyptapanteles* species are bi- to multivolitine, i.e. they have two or more generations per year. Since the gypsy moth is a univolitine species, the wasps need alternate and/or overwintering host species. Larvae of the browntail moth, *Euproctis chrysorrhoea* (Lepidoptera: Erebidae), could serve as overwintering hosts (Marschnig 2013, Fromm 2014). *G. fulvipes* is a larval parasitoid of the square spot rustic moth, *Xestia xantographa* (Lepidoptera: Noctuidae) (Abbasipour 1996) and in Austria, the wasps can be retrieved from overwintering square spot rustic moth caterpillars (Connell, personal communication, Austrian Research Center for Forests, 2015).

Previous studies from Austria and Slovakia about the larval parasitoids of gypsy moth mention only *G. liparidis* and *G. porthetriae* (Hoch et al. 2001, Kahlbacher 2008). However, a publication from 1910 describes *G. fulvipes* as 'almost the first parasite of the gypsy moth' imported to help to restore the balance after the introduction of the gypsy moth to the United States in 1889 (Massachusetts, State forester 1910).

2 Material and methods

2.1 Insects

2.1.1 *Lymantria dispar*

Larvae of the gypsy moth, *L. dispar* L. (Lepidoptera: Lymantriidae), were obtained as egg masses from a laboratory culture (USDA/APHIS Otis Method Development Center Cape Cod) MA). Gypsy moth eggs were incubated in a climate chamber (Liebherr) at constant temperature 20±1°C under long day photoperiod (16L:8D). Young larvae were moved to the plastic boxes (Figure 1) with wheat germ diet (Table 1) (Bell et al. 1981) until parasitized (3rd instar for *G. liparidis* and *G. fulvipes* or 2nd instar for *G. porthetriae*).

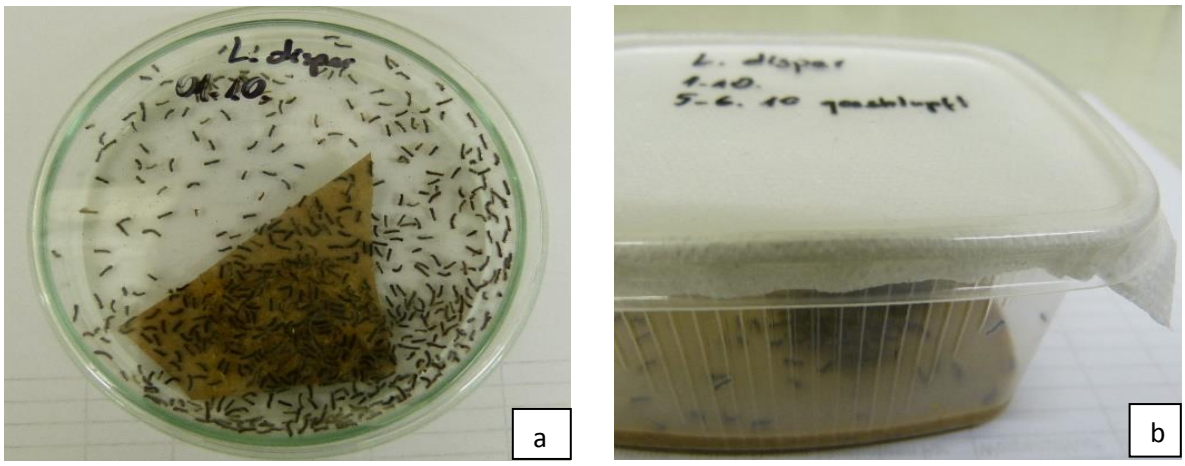

Figure 1: (a) Young larvae of the gypsy moth *Lymantria dispar*, newly hatched from egg mass, (b) larvae moved to a plastic box with wheat germ diet.

Table 1: Diet composition

Ingredients	Amounts	Ingredients	Amounts
Water	814 ml	Chlortetracycline	0.1 g
Agar	15.0 g	Ferric Citrate	0.1 g
Wheat Germ	120.0 g	Wesson Salt without iron Bio-serv	8.0 g
Ascorbic Acid (Vit. C)	5.0 g		
Casein	25.0 g	Sorbic Acid	2.0 g
Methyl Paraben	1.0 g	Vitamin Mixture Bio-serv	10.0 g

2.1.2 The braconid wasps *Glyptapanteles liparidis*, *Glyptapanteles porthetriae* and *Glyptapanteles fulvipes*

The gregarious wasp *G. liparidis* Bouché and the solitary *G. porthetriae* Muesebeck came from a laboratory colony originating from gypsy moth larvae collected in oak forests in Burgenland, Austria. A stock colony of gregarious *G. fulvipes* Haliday, originating from square-spot rustic larvae, *Xestia xantographa*, is maintained at the institute laboratory in larvae of the gypsy moth. Adult wasps were held at 15±1°C and a photoperiod of 14L:10D hrs and fed water and honey (Schopf 1991).

2.2 Experimental set-up

2.2.1 Parasitization, development and growth of the host *L. dispar*

Before parasitization larvae were weighed on a micro balance to choose similar size insects (Figures 2 and 3). The 3rd instar larvae had between 20 and 30 mg, the 2nd instar larvae between 6 and 9 mg. Synchronously developing gypsy moth larvae in the 2nd instar were parasitized by *G. porthetriae*, and 3rd instars by *G. liparidis* and *G. fulvipes*, according to their preferred host size in the field (Cho et al. 2007, Schopf 1991). In order to be sure that the larvae were singly parasitized, they were offered individually to the wasps by forceps (Figure 4).

Figure 2: Larva of *L. dispar*, on day one of the 3rd instar; characteristically bright head capsule and long hair.

Figure 3: *L. dispar* larva on the micro balance on day one of the 3rd instar.

Parasitized larvae were kept either individually in glass Petri dishes (Ø 9cm) in climate chambers (Liebherr) at constant 15±1°C, 20±1°C and 25±1°C, under long day photoperiod (16L:8D) until the parasitoids emerged from their host (Figures 5, 6). For every wasp species and every temperature variant 40 *L. dispar* larvae were parasitized, 360 larvae in total. Wheat germ diet was exchanged as necessary and feces was removed regularly.

Figure 4: *L.dispar* larva provided by forceps to female wasp for oviposition.

Figure 5: Parasitized *L. dispar* larvae in a climate chamber.

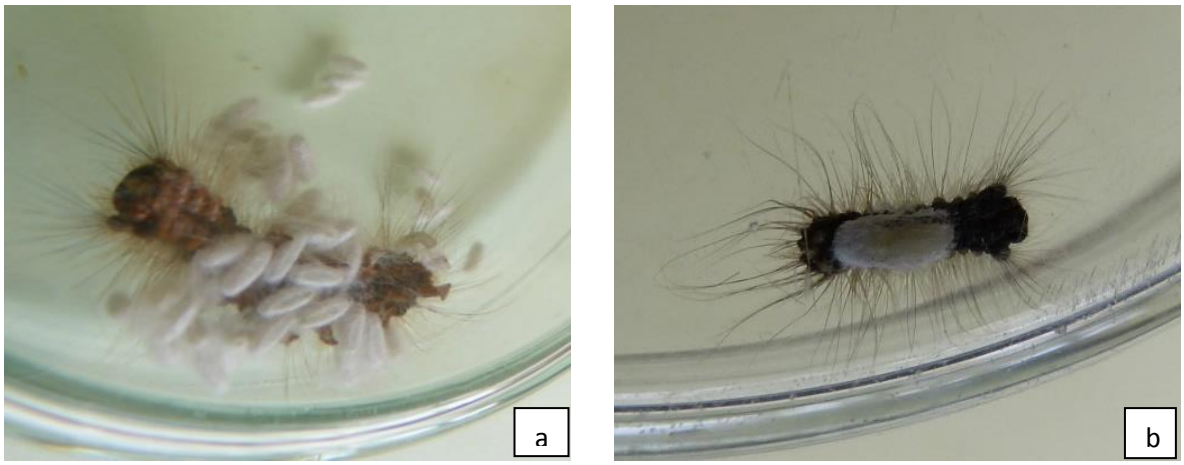

Figure 6: *L. dispar* larvae with parasitoids emerged from the host body, (a) *G. liparidis*, (b) *G. porthetriae.*

The development times of individuals at 15°C, 20°C and 25°C regimes were used to calculate linear regression equations, r^2 value, lower developmental thresholds and thermal sums for individual stages (e.g. egg, first, second instar, pupa) from oviposition to adult wasp eclosion from cocoons.

2.2.2 Endoparasitic development of the wasp species

To study the individual stages of endoparasitic development of the three parasitoid species at three different constant temperatures (15°C, 20°C and 25°C), larvae of the gypsy moth were offered to more than one wasp of the same species for oviposition, to be sure about successful parasitization. Twenty-five larvae were parasitized for every wasp species and temperature variant, 225 larvae in total.

Parasitized larvae were kept in groups of five in glass Petri dishes in climate chambers at the same conditions and with the same diet like singly parasitized larvae until dissected.

Every second day some larvae were dissected and examined under a dissecting microscope (Figure 7), starting 2 days after parasitization (dap) at 25°C, 5 dap at 20°C and 7 dap at 15°C. The pictures were taken by digital camera (Nikon COOLPIX P 6000). The development stages (egg, first and second instar) were recorded.

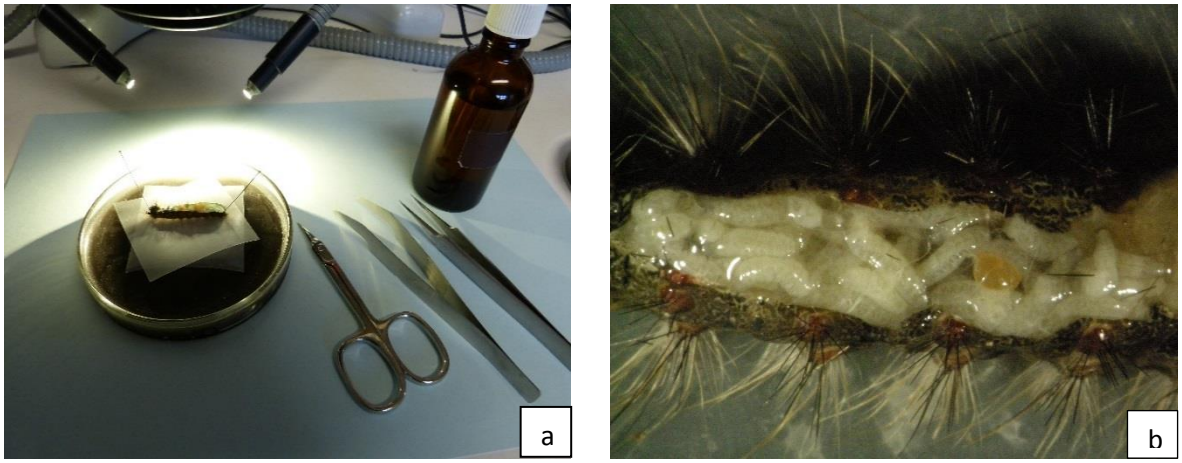

Figure 7: (a) Parasitized *L. dispar* larvae under the dissecting microscope, (b) parasitoid larvae present in the hemocoel of the host.

2.2.3 Wasp longevity

To investigate the longevity of adult wasps, cocoons of *G. liparidis and G. fulvipes* were kept in plastic boxes in a climate chamber (type LC 5000) under long day photoperiod (16L:8D) and at two temperature regimes, 15°C and 20°C. Due to initially high mortality of adult wasps in the new climate chambers, the boxes with adult wasps were moved to other climate chambers. Eventually, the new climate chambers emitted substances that caused wasp death after 2-3 days. However, it is important to state that the new climate chambers did not affect the gypsy moth larvae negatively. Adult wasps were fed water and honey. The wasps were controlled every day, mortality dates were recorded.

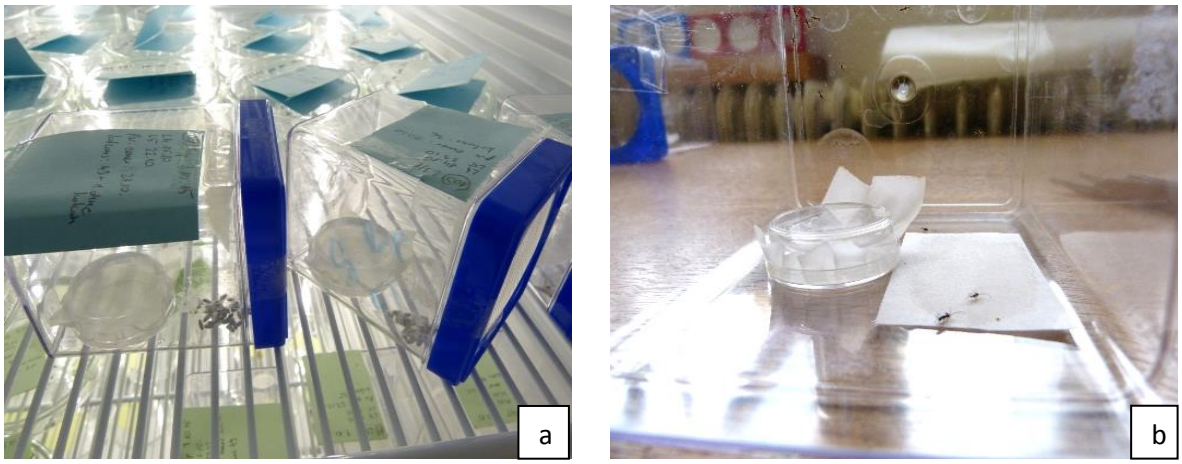

Figure 8: (a) *G. fulvipes* cocoons kept for wasp eclosion, (b) adult wasps in plastic box with water container and honey on filter paper.

2.3 Lower development threshold

Estimation of the lower development threshold LDT (developmental zero) was based on a linear model (Campbell et al. 1974, Gould & Elkinton 1990, Eliopoulos & Stathas 2003). Data obtained from the experiments were described by linear regression equations from:

$Y = a + bT$

Where y is the rate of development (day^{-1}) at temperature T (°C), and *a* = intercept, and *b* = slope.

Equation constants (*a, b*) were estimated with least squares method using excel statistical program. Estimation of constants was based on data obtained at 15°C, 20°C and 25°C. The regression line was extrapolated to meet the abscissa at development rate zero *t*, which was calculated from $t_0 = - a/b$.

2.4 Degree-days

Degree-days DD (thermal constant K) is one of the most commonly used indices of temperature, the total quantity of thermal energy above a threshold temperature, required to complete development (Campbell et al. 1974). Degree-day models have long been used to predict events in the life cycle of insects and therewith the timing of outbreaks of insect pests and their natural enemies (Hemerik 2016). DD is the accumulated heat in the range between the lower threshold temperature (t_{low}) and the upper threshold temperature (t_{high}) in which development takes place (Wu et al. 2015). DD was calculated as the sum of effective temperatures (i.e. number of DD above the lower developmental threshold necessary to complete development. DD measures the total amount of accumulated heat.

2.5 Statistical analyses

Statistical analyses were performed with SPSS version 21 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 Kolmogorov-Smirnov and Levene's test was applied to ensure equal variances. Data in the text represent means±SE (standard error).

3 Results

3.1 Parasitization success

Total parasitization success was higher for *G. liparidis* and *G. fulvipes* (88% and 85%, respectively) than for *G. porthetriae* (77%), regardless of the rearing temperature. Successful wasp development until wasp emergence from the host was also lowest for *G. porthetriae* (67%), followed by *G. liparidis* (76%) and *G. fulvipes* (77%). Moth eclosion of not successfully parasitized larvae was 13% in *G. porthetriae*, 7% in *G. liparidis* and 2% in *G. fulvipes* (Figure 1). Highest mortality of successfully parasitized larvae occurred in *G. liparidis* (9%), of not successfully parasitized larvae in *G. fulvipes* (11%).

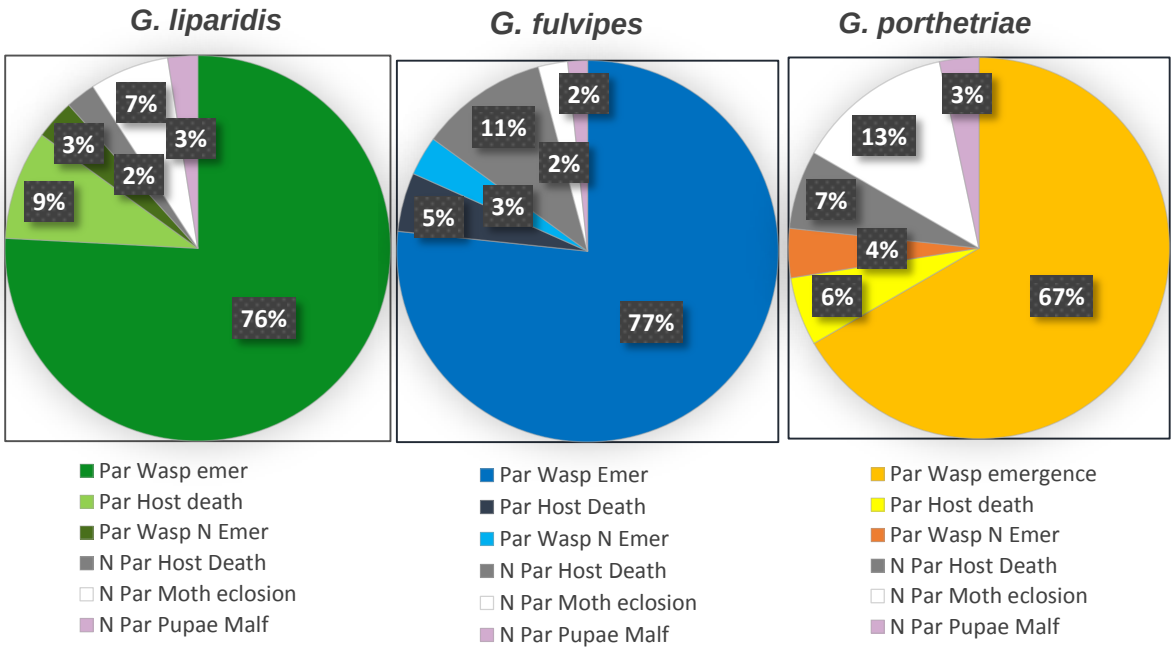

Figure 9: Result of parasitization (%) of *G. liparidis*, *G. fulvipes* and *G. porthetriae*, summarized for all three test temperatures (15°C, 20°C and 25°C). Number of test larvae was 120 per wasp species. Par = successful parasitization, N Par = non successful parasitization, Wasp Emer = wasp emergence, Wasp N Emer = wasp non emergence, Pupae Malf = pupae malformation.

In *G. liparidis* successful wasp emergence from the host was highest at 20°C (85%), followed by 25°C (75%) and 15°C (67%). The lowest host mortality occurred at 15°C (5%), followed by 20°C (7%) and 25°C (15%). Moth eclosion was observed in all temperature variants, malformation of pupae and mortality of not successfully parasitized host larvae were only found at 15°C (Figure 10).

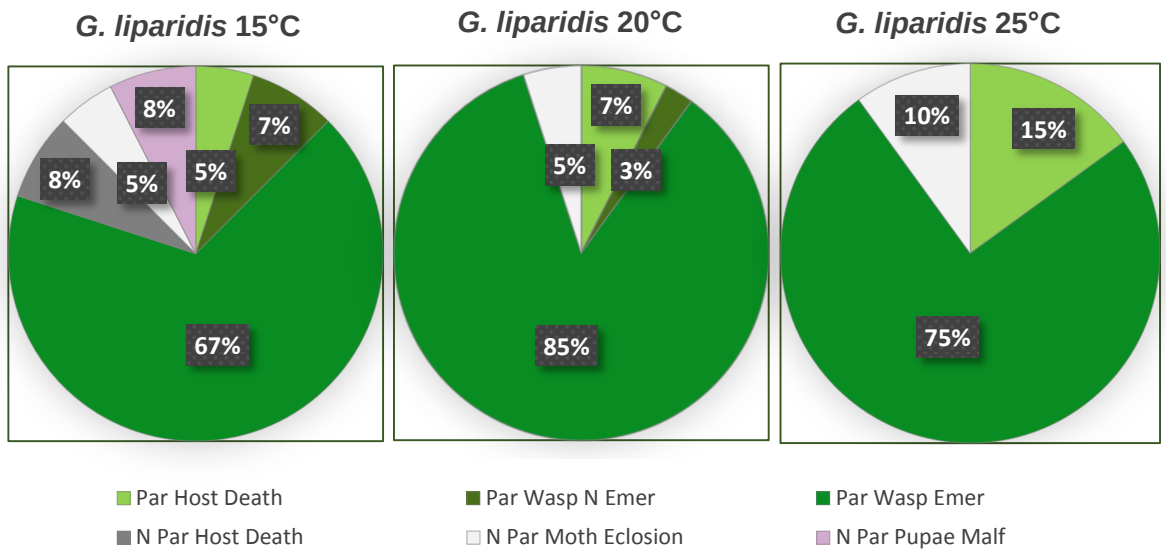

Figure 10: Result of parasitization (%) of *G. liparidis* at 15°C, 20°C and 25°C. Number of test larvae was 40 per temperature variant. Host larvae were parasitized on day one of the third instar. Par = successful parasitization, N Par = non successful parasitization, Wasp Emer = wasp emergence, Wasp N Emer = wasp non emergence, Pupae Malf = pupae malformation.

In *G. fulvipes* successful wasp emergence from the host was also highest at 20°C (95%), followed by 15°C (80%) and 25°C (55%). At 25°C the highest number of host mortality was recorded, both in successfully parasitized and not successfully parasitized larvae (10% and 18%, respectively) and only in this group malformation of pupae from not successfully parasitized host larvae occurred (Figure 11).

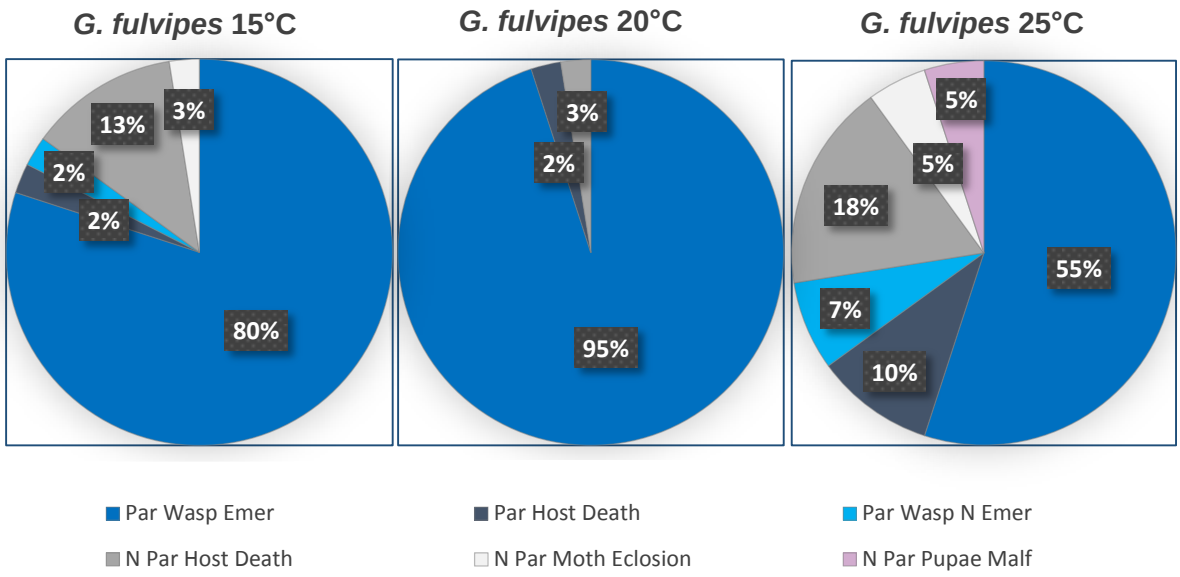

Figure 11: Result of parasitization (%) of *G. fulvipes* at 15°C, 20°C and 25°C. Number of test larvae was 40 per temperature variant. Host larvae were parasitized on day one of the third instar. Par = successful parasitization, N Par = non successful parasitization, Wasp Emer = wasp emergence, Wasp N Emer = wasp non emergence, Pupae Malf = pupae malformation.

In the solitary *G. porthetriae* successful wasp eclosion from the host was highest at 20°C (82%), followed by temperature conditions at 15°C (65%) and 25°C (52%). Host mortalities of successfully parasitized larvae at 15°C and 25°C were higher (both 7%) than at 20°C (2%). With not successfully parasitized larvae mortality of host larvae occurred at 15°C (18%) and 25°C (3%). Malformation of pupae from not successfully parasitized larvae was observed only at 15°C (10%) (Figure 12).

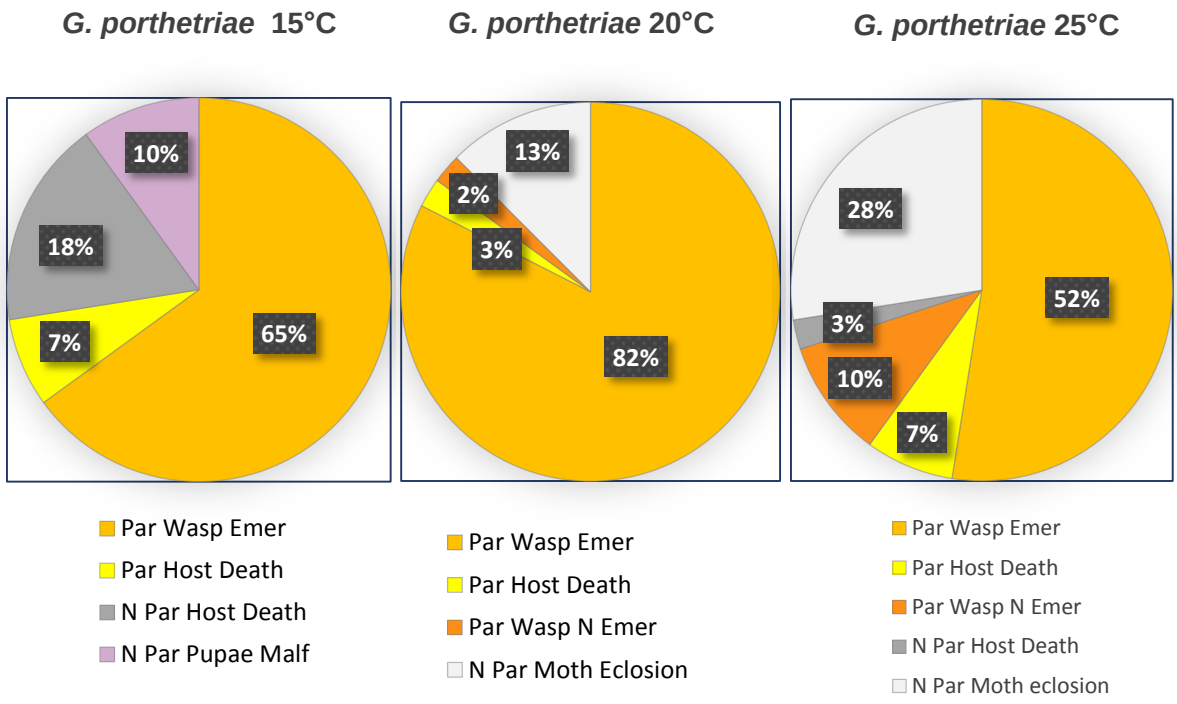

Figure 12: Result of parasitization (%) of *G. porthetriae* at 15°C, 20°C and 25°C. Number of test larvae was 40 per temperature variant. Host larvae were parasitized on day one of the second instar. Par = successful parasitization, N Par = non successful parasitization, Wasp Emer = wasp emergence, Wasp N Emer = wasp non emergence, Pupae Malf = pupae malformation.

Chi-square test revealed significant differences among the species regarding parasitization success (Table 2).

Table 2: Chi-Square test for *G. liparidis*, *G. fulvipes* and *G. porthetriae*, combined for the three temperature regimes. N = number of host larvae, Par = successfully parasitized, N_Par = not successfully parasitized.

Species	N	Par	N_Par	Chi-Square (Pearson)	Significance
G. liparidis	120	106	14	6.240	**0.044**
G. fulvipes	120	102	18		
G. porthetriae	120	92	28		

Temperature did not affect parasitization success in *G. liparidis* and *G. porthetriae*, but it affected parasitization success in *G. fulvipes* (Table 3).

Table 3: Chi-square test separately for *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15°C, 20°C and 25°C. N = number of host larvae, Par = successfully parasitized, N_Par = not successfully parasitized, T= temperature.

Species	T (°C)	N	Par	N_Par	Chi-Quadrat (Pearson)	Significance
G. liparidis	15	40	32	8	4.528	0.104
	20	40	38	2		
	25	40	36	4		
G. fulvipes	15	40	34	6	9.804	0.007
	20	40	39	1		
	25	40	29	11		
G. porthetriae	15	40	29	11	4.006	0.135
	20	40	35	5		
	25	40	28	12		

3.1.1 Wasp cocoons

G. liparidis wasps produced the highest number of offspring per host at 25°C, *G. fulvipes* produced most offspring per host at 20°C (Table 4).

Table 4: Total number of emerged parasitoids that spun or did not spin cocoons for *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15°C, 20°C and 25°C. T= temperature, N= number of host larvae, Par= successfully parasitized host larvae, N_cocoons= number of cocoons, N_without cocoons= number of parasitoids emerged without cocoons.

Wasp species	T	N	Par	N_cocoons	N_without cocoons	Sum	Par/Host
G. liparidis	15°C	40	32	556	32	588	18
G. liparidis	20°C	40	38	722	32	754	20
G. liparidis	25°C	40	36	906	18	924	26
Total G. liparidis		**120**	**106**	**2184**	**82**	**2266**	**21**
G. fulvipes	15°C	40	34	573	21	594	17
G. fulvipes	20°C	40	39	831	53	884	23
G. fulvipes	25°C	40	29	401	28	429	15
Total G. fulvipes		**120**	**102**	**1805**	**102**	**1907**	**19**
G. porthetriae	15°C	40	29	26	0	26	0.9
G. porthetriae	20°C	40	35	33	1	34	1
G. porthetriae	25°C	40	28	24	1	25	0.9
Total G. porthetriae		**120**	**92**	**83**	**2**	**85**	**0.9**

3.2 Host mortality and development disorders

3.2.1 Host mortality

Mortality of host larvae was observed in all wasp species and at all temperature variants, both in successfully parasitized and not successfully parasitized larvae. Three test groups were without any host mortality; not successfully parasitized larvae of *G. liparidis* and *G. porthetriae* at 20°C and *G. liparidis* at 25°C. Lowest mortality occurred at 20°C in all wasp species, both successfully parasitized and not successfully parasitized hosts. Host mortality was highest in not successfully parasitized larvae of *G. fulvipes* at 15°C and 25°C in the successfully parasitized group (Tables 5-6 and Figures 10-12).

Table 5: Total larval mortality (%) of hosts parasitized by *G. liparidis*, *G. fulvipes* and *G. porthetriae*, at 15°C, 20°C and 25°C. Par = Successfully parasitized, N_Par = not successfully parasitized (see also figures 10-12).

T (°C)	***G. liparidis***			***G. fulvipes***			***G. porthetriae***		
	Par	N_Par	Sum	Par	N_Par	Sum	Par	N_Par	Sum
15 (n=40)	5	8	**13**	2	13	**15**	7	18	**25**
20 (n=40)	7	0	**7**	2	3	**5**	2	0	**2**
25 (n=40)	15	0	**15**	10	18	**28**	7	3	**10**
Total Mort (n=120)	9	3	**12**	5	11	**16**	6	7	**13**

Table 6: Host mortality recorded as premature death of host larvae. T= temperature, N= number of host larvae, Par = successfully parasitized, Par-Mort = number of dead, but successfully parasitized larvae, N_Par-Mort = number of dead, but not successfully parasitized larvae.

Wasp species	T (°C)	N	Par	Par-Mort	N_Par-Mort	Total mortality
1	2	3	4	5	6	5 + 6
G. liparidis	15	40	32	2	3	5
	20	40	38	3	0	3
	25	40	36	6	0	6
***G. liparidis* total**		**120**	**106**	**11**	**3**	**14**
G. fulvipes	15	40	34	1	5	6
	20	40	39	1	1	2
	25	40	29	4	7	11
***G. fulvipes* total**		**120**	**102**	**6**	**13**	**19**
G. porthetriae	15	40	29	3	7	10
	20	40	35	1	0	1
	25	40	28	3	1	4
***G. porthetriae* total**		**120**	**92**	**7**	**8**	**15**

In the group of successfully parasitized larvae, highest mortality was observed in larvae parasitized by *G. liparidis* (11) followed by *G. porthetriae* (7) and *G. fulvipes* (6). Thus, the highest total mortality (successfully parasitized larvae plus not successfully parasitized larvae) was recorded in larvae parasitized by *G. fulvipes* (19). Total mortality for *G. liparidis* and *G. porthetriae* was 14 and 15, respectively. Lowest mortality for the three parasitoids occurred at 20°C (Table 6).

3.2.2 Pupal malformation

Malformation of pupae (Figure 13) occurred in three of nine groups: *G. liparidis* and *G. porthetriae* at 15ºC and *G. fulvipes* at 25ºC. In the *G. liparidis* and *G. porthetriae* groups, pupal malformation war 38% and 36%, respectively, from larvae considered as not successfully parasitized, and 18% in the *G. fulvipes* group.

Figure 13: Malformation of *L. dispar* pupae.

3.2.3 Molting disorders of host larvae

Molting problems of host larvae (Figure 14) occurred in both successfully parasitized larvae and not successfully parasitized larvae, specifically with larvae kept at 15°C (see Table 7). No molting problems were seen in parasitized larvae kept at 25°C.

Table 7: Number of host larvae with molting disorders in *G. liparidis*, *G. fulvipes* and *G. porthetriae* of successfully and not successfully parasitized hosts. T = temperature, Par = successfully parasitized, N_Par = not successfully parasitized larvae.

T	Species	Par	N_Par
15°C	*G. liparidis*	-	2
	G. fulvipes	1	2
	G. porthetriae	1	3
20°C	*G. liparidis*	-	-
	G. fulvipes	-	1
	G. porthetriae	-	-
25°C	*G. liparidis*	-	-
	G. fulvipes	-	-
	G. porthetriae	-	-

Figure 14: Parasitized *L. dispar* larva with molting disorders.

3.2.4 Unequal development of host larvae

Before being parasitized, all gypsy moth larvae were weighed to choose individuals with similar body masses. Nevertheless the larvae did not grow equally upon parasitization and some were several times bigger than others even when they were parasitized on the same day and kept under the same conditions.

Figure 15: Differences in growth of *L. dispar* larvae parasitized on the same day, (a) both larvae were successfully parasitized by *G. fulvipes*, (b) the larva on the left was successfully parasitized by *G. porthetriae*, the larva on the right was not successfully parasitized. All insects shown were kept at 25°C.

3.2.5 Parasitoid larvae without cocoons

In every wasp species examined some of the emerged parasitoid larvae did not spin cocoons (Figure 16). Only in the *G. porthetriae* group at 15°C there wasn't any larva without cocoon.

Figure 16: *L. dispar* host larva with egressed parasitoid larvae with and without cocoon.

In the *G. liparidis* and *G. fulvipes* groups at 20°C, from 47% and 49% of the parasitized hosts parasitoids did not spin cocoons upon egression, at 15°C it was 41% and 35% and at 25°C it was 22% and 45%, respectively. In the *G. porthetriae* groups at 20°C and 25°C parasitoids from only one larva in each group did not spin cocoons (Figure 16).

3.2.6 Encapsulated parasitoid larvae

Host larvae that died prematurely or showed development problems were dissected. In 20 cases the parasitoids were encapsulated or melanized in different stages (first or second instar) (Table 8 and Figures 17-18).

Table 8: Number of hosts in which melanized or encapsulated parasitoid larvae were found upon dissection.

Temperature	Species	Melanized	Encapsulated
15°C	*G. liparidis*	2	
	G. fulvipes	0	
	G. porthetriae	2	1
20°C	*G. liparidis*	4	
	G. fulvipes	1	
	G. porthetriae	0	
25°C	*G. liparidis*	3	
	G. fulvipes	4	
	G. porthetriae	3	

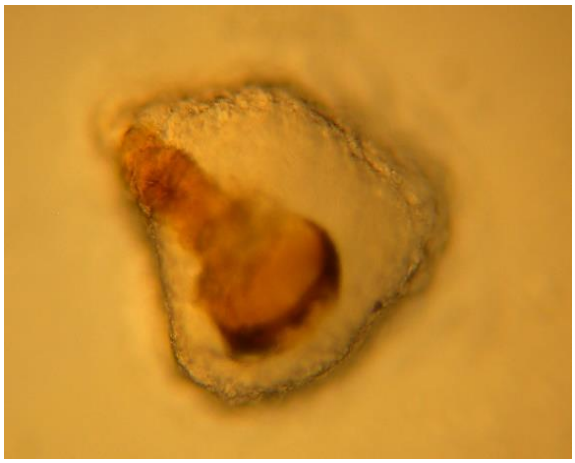

Figure 17: *G. porthetriae* first-instar parasitoid melanized and encapsulated by host hemocytes.

Figure 18: *G. fulvipes* second-instar parasitoids heavily melanized in the host that died prematurely.

3.3 Parasitoid loads

For *G. liparidis* and *G. fulvipes,* the number of parasitoids per host larva were not significantly different (t-Test, P>0.05). *G. liparidis* injected 22.8±1.6 eggs (min 1, max 85), *G. fulvipes* 18.9±1.3 (min 1, max 53) (Figure19).

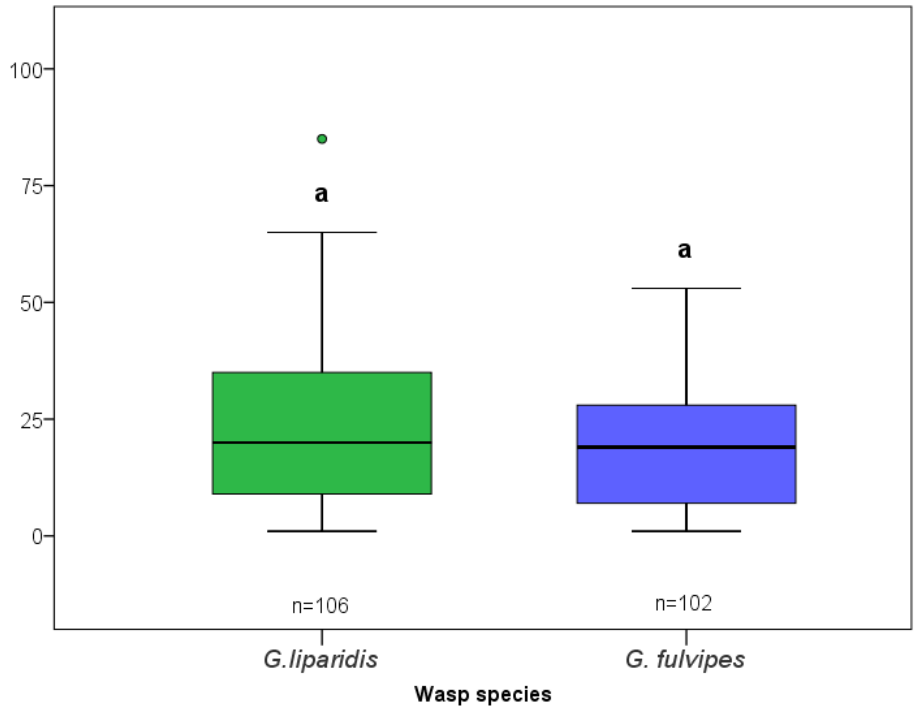

Figure 19: Number of parasitoids per host larva of *G. liparidis* and *G. fulvipes*. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75^{th} percentile – 25^{th} percentile). Error bars indicate minimum and maximum values. Significant differences are indicated by different letters above the boxplots (t-Test, P<0.05), n= number of host larvae.

3.4 Parasitoid development

For a detailed overview of the development and growth of the three *Glyptapanteles* species in larvae of the gypsy moth I determined the duration of the endoparasitic stages, the pupal stage, and the total development time from oviposition to adult wasp eclosion.

Development time was calculated as the number of days from parasitization (first day of L3 for *G. liparidis* and *G. fulvipes*, first day of L2 for *G. porthetriae*) until parasitoid emergence from the host (i.e. endoparasitic development) or until wasp eclosion (i.e. total development of the immature stages) (Table 9).

Table 9: Duration (days, mean±SE) of endoparasitic development, pupal stage and total development until wasp eclosion from cocoons, n= number of test insects

	G. liparidis			***G. fulvipes***			***G. porthetriae***		
	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
Endoparasitic development	37.23 ± 0.94 n=30	21.14 ± 0.47 n=35	13,00 ± 0.26 n=30	35.88 ± 1.06 n=33	21.29 ± 0.55 n=38	1.,84 ± 0.30 n=30	31.46 ± 1.25 n=26	16.18 ± 0.33 n=34	13.16 ± 0.33 n=25
Pupal stage	14.81 ± 0.13 n=27	7.47 ± 0.09 n=34	5.33 ± 0.10 n=30	13.91 ± 0.22 n=32	7.82 ± 0.11 n=38	5.45 ± 0.11 n=24	15.12 ± 0.30 n=26	8.12 ± 0.11 n=33	6.09 ± 0.09 n=21
All immature stages (total)	51.22 ± 0.80 n=27	28.41 ± 0.41 n=34	18.33 ± 0.28 n=30	49.37 ± 1.17 n=32	29.11 ± 0.58 n=38	18.91 ± 0.32 n=24	46.58 ± 1.41 n=26	24.27 ± 0.35 n=33	18.90 ± 0.32 n=21

3.4.1 Endoparasitic development

Endoparasitic development at 15°C and 20°C was significantly longer for *G. liparidis* and *G. fulvipes* in comparison to *G. porthetriae* (ANOVA, post hoc Scheffé P<0.05). There was no difference at 25°C (P>0.05) (Figure 20).

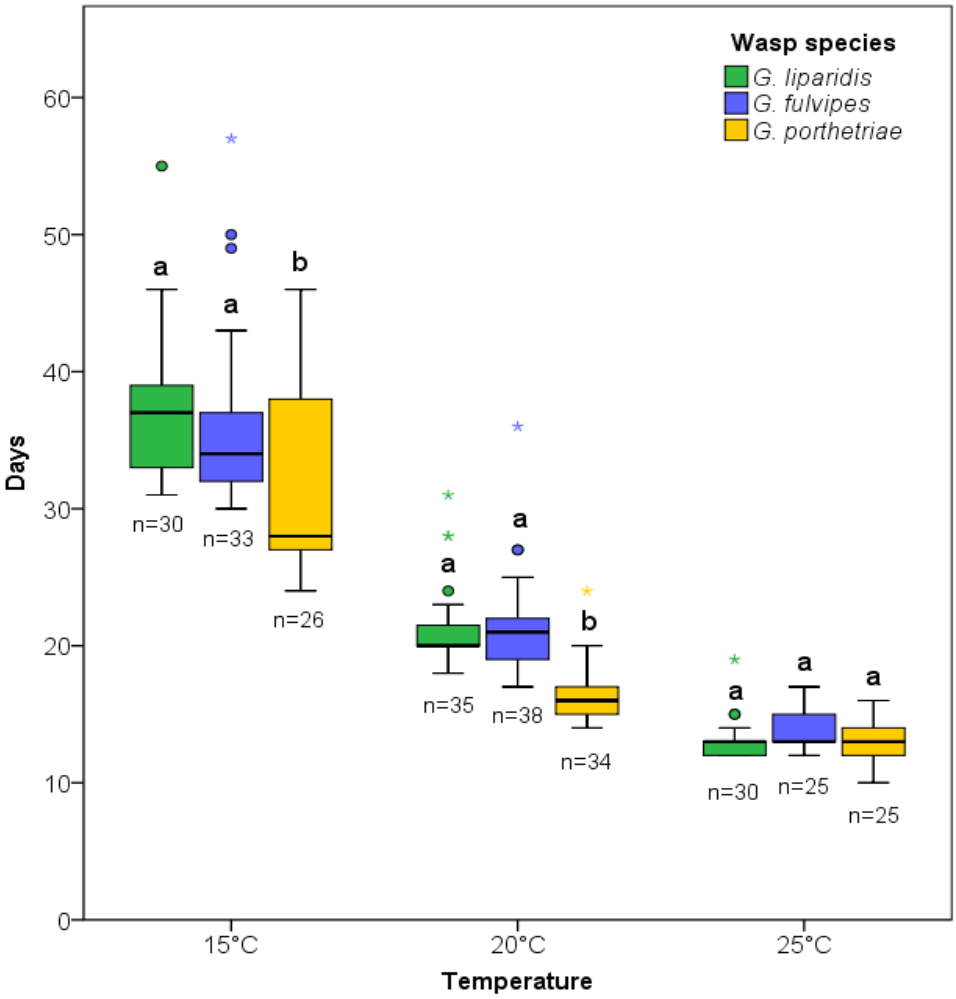

Figure 20: Endoparasitic development (days) of *G. liparidis*, *G. fulvipes*, and *G. porthetriae* at 15°C, 20°C and 25°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in duration among the wasp species are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé P<0.05), n = number of host larvae.

3.4.2 Total development

Total immature development at 15°C was significantly different between *G. liparidis* and *G. porthetriae*. No significant differences were observed between *G. liparidis* and *G. fulvipes* and between *G. fulvipes* and *G. porthetriae* (ANOVA, post hoc Scheffé P>0.05) (Figure 21).

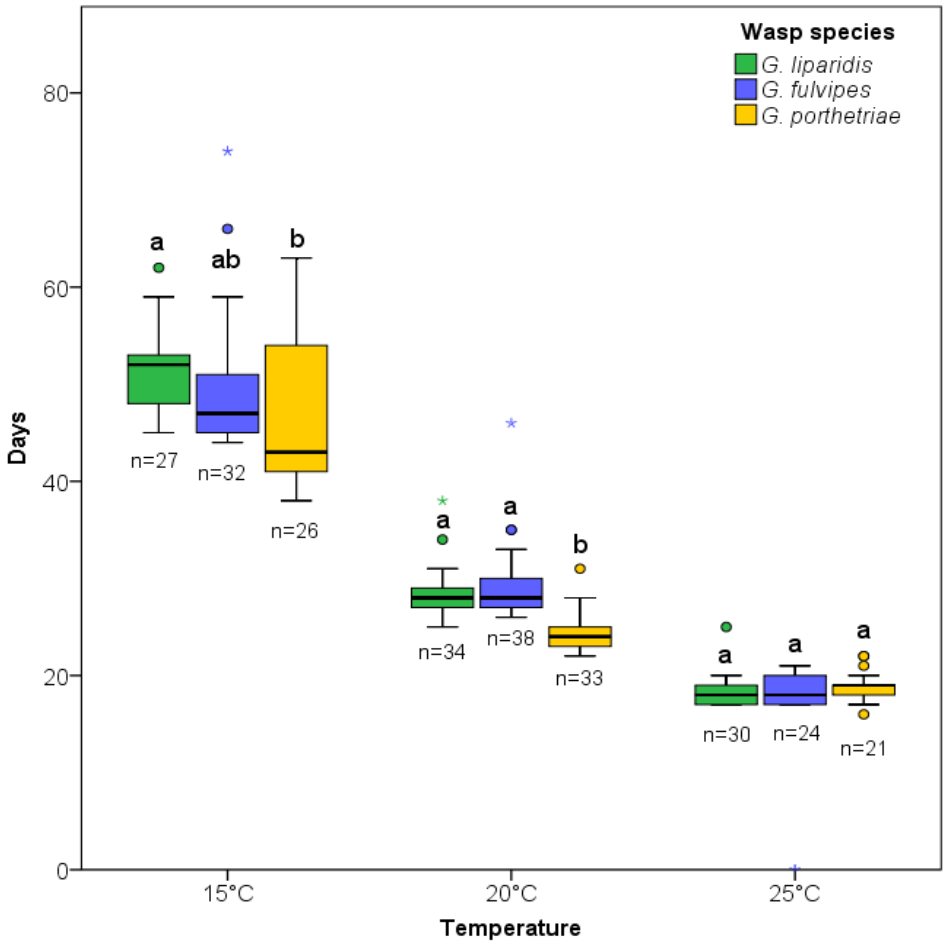

Figure 21: Total development (days) from oviposition until wasp eclosion from cocoons, by *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15°C, 20°C and 25°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in developmental duration are indicated by different letter above the boxplots (P<0.05), n = number of host larvae.

At 20°C, development of *G. porthetriae* was significantly shorter than development of *G. liparidis* and *G. fulvipes*. At 25°C, no significant differences occurred among the wasps (Figure 21).

3.4.3 Duration of immature parasitoid stages (egg, L1, L2, cocoon)

Of all wasp species, the egg stage was longest for *G. fulvipes* at all temperatures. At 15°C the egg stadium was shortest for *G. porthetriae* (5 days) followed by *G. liparidis* and *G. fulvipes* (7 and 11 days, respectively). At 20°C, the egg stage was also shortest for *G. porthetriae* (2 days) while it lasted 5 and 8 days for *G. liparidis* and *G. fulvipes*, respectively. At 25°C, eggs from *G. liparidis* and *G. porthetriae* hatched after one day, in *G. fulvipes* after days (Table 10, Figure 22).

Table 10: Duration of parasitoid immature stages (days) for *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15°C, 20°C and 25°C. *G. lip=G. liparidis*, *G. ful=G. fulvipes*, *G. por=G. porthetriae*, T= temperature. For egg stadium and first and second instars only 2-3 larvae per wasp species and temperature regime were dissected. For the pupal stage data were obtained from the main experiment (i.e. 40 host larvae per wasp species and temperature regime).

T	Egg			L1			L2			Pupa		
	G. lip	*G. ful*	*G. por*	*G. lip*	*G. ful*	*G. por*	*G. lip*	*G. ful*	*G. por*	*G. lip*	*G. ful*	*G. por*
15°C	7	11	5	12	10	15	18	15	11	15	14	15
20°C	5	8	2	6	4	2	10	9	12	7	8	8
25°C	1	4	1	4	3	5	8	7	7	5	5	6

At 15°C, the first instar was shortest for *G. fulvipes* (10 days), followed by *G. liparidis* (12 days) and *G. porthetriae* (15 days). At 20°C, this instar lasted 4 and 6 days for *G. fulvipes* and *G. liparidis*, but only 2 days for *G. porthetriae*. At 25°C, the first instar lasted 3, 4 and 5 days for *G. fulvipes*, *G. liparidis* and *G. porthetriae*, respectively.

At 15°C, the second instar was shortest for *G. porthetriae* (11 days), followed by *G. fulvipes* (15 days) and *G. liparidis* (18 days). At 20°C, the second instar was longest for *G. porthetriae* (12 days) followed by *G. liparidis* (10 days) and *G. fulvipes* (9 days). At 25°C, the duration of the second instar was equal in *G. fulvipes* and *G. porthetriae* (7 days), in *G. liparidis* it lasted 8 days (Table 10, Figure 23).

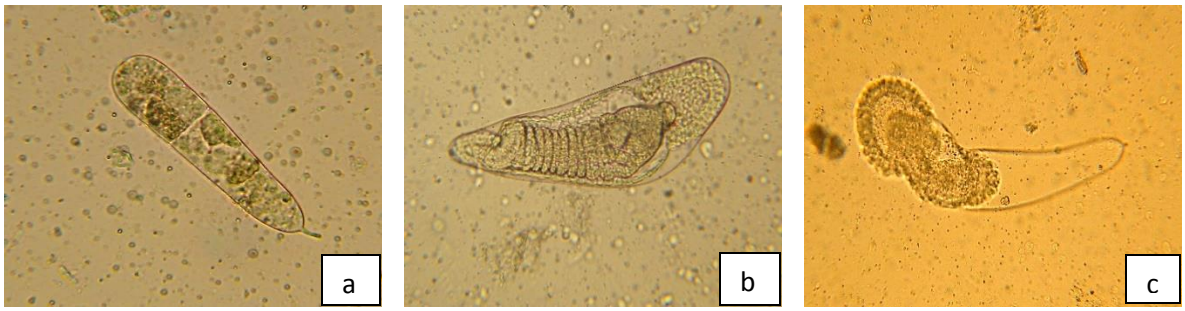

Figure 22: *G. fulvipes*, (a) early egg stadium, (b) larva prior to hatching, (c) larva hatching

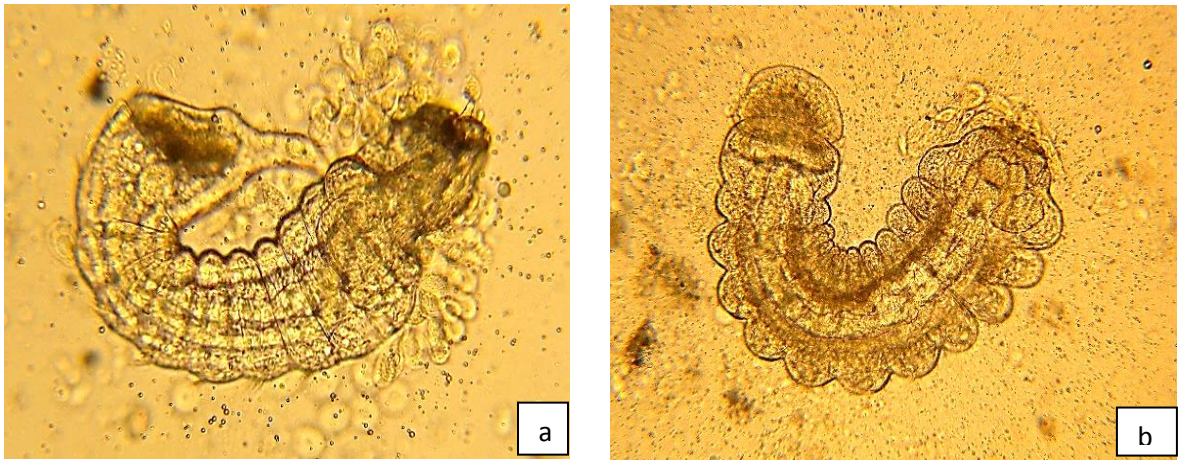

Figure 23: (a) *G. porthetriae* first instar L1, (b) *G. fulvipes* second instar L2

In all *Glyptapanteles* species, third instars emerged from the host and immediately spun a cocoon for pupation, therefore L3 was not indicated separately. At 15°C, first egression of parasitoid larvae from the host was observed for *G. liparidis* 31 days post parasitization (dpp), for *G. fulvipes* 30 dpp and for *G. porthetriae* 24 dpp. The pupal stage was 14.8±0.13 days for *G. liparidis*, 13.9±0.22 days for *G. fulvipes* and 15.12±0.29 days for *G. porthetriae*.

At 20°C, larvae of *G. liparidis* and *G. fulvipes* started to egress 18 and 17 dpp, respectively. The first parasitoids of *G. porthetriae* emerged 14 dpp. Pupal stage of *G. liparidis* lasted 7.47±0.09 days, the pupal stages of *G. fulvipes* and *G. porthetriae* 7.82±0.11 and 8.12±0.11 days, respectively.

At 25°C, parasitoid larvae of *G. liparidis* and *G. fulvipes* started to emerge 12 dpp and first parasitoid larvae of *G. porthetriae* egressed 10 dpp. At 25°C, the pupal stage lasted

5.33±0.09 days for *G. liparidis*, 5.45±0.11 days for *G. fulvipes* and 6.1±0.09 days for *G. porthetriae* (Table 10).

At 15°C, adult wasp eclosion started 11 days after cocoon spinning (mean 14 days, n=32) in *G. fulvipes,* 12 days (mean 15 days, n=26) in *G. porthetriae*, and 14 days (mean 15 days, n=27) in *G. liparidis*. At 20°C, first wasp eclosion occurred in *G. liparidis* 6 days after cocoon spinning (mean 7 days, n=34), followed by *G. fulvipes* and *G. porthetriae* 7 days after cocoon spinning (mean 8 days for both species, n=38 and n=33, respectively). At 25°C, wasps from all three species started to emerge 5 days after cocoon spinning.

3.5 Lower development thresholds of the wasp species

3.5.1 Lower development thresholds of the endoparasitic stages

The lower development threshold (LDT) of the endoparasitic stages (from oviposition until parasitoid emergence from the host) was lowest for *G. porthetriae* (LDT= 6.9°C), followed by *G. fulvipes* (LDT= 8.8°C) and *G. liparidis* (LDT= 9.9°C) (Figures 24-26).

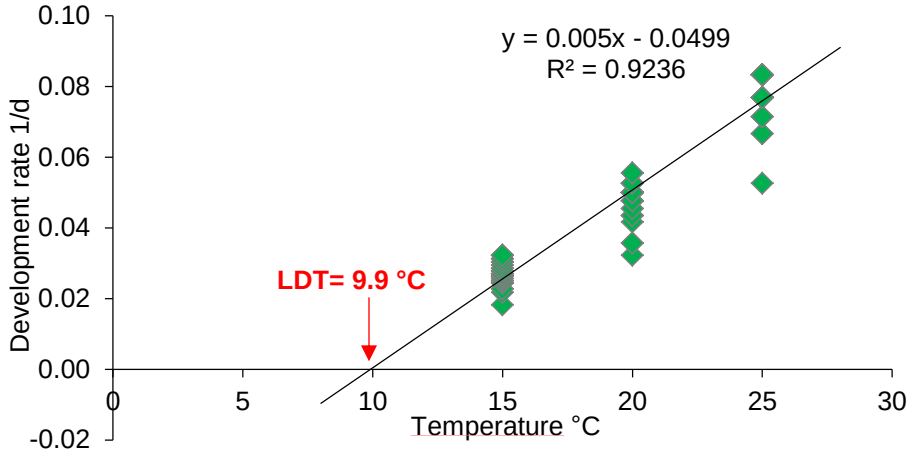

Figure 24: Endoparasitic development rate (1/days) of *G. liparidis* in *L. dispar* larvae at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C).

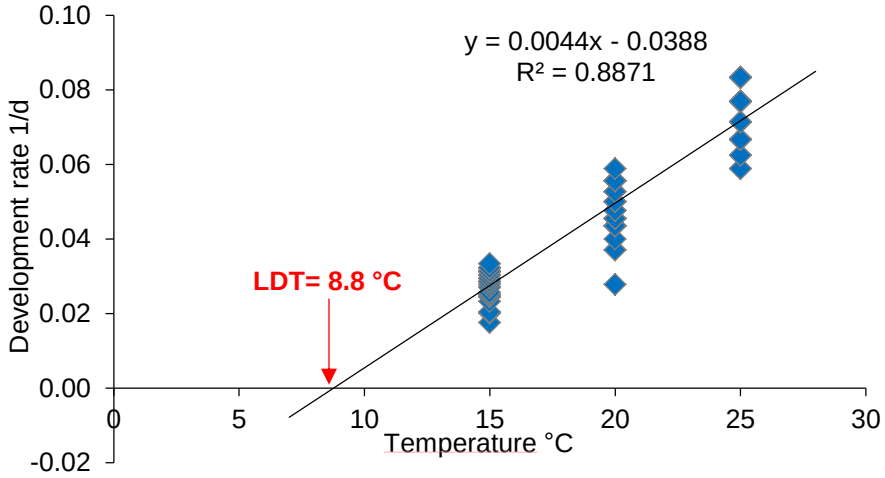

Figure 25: Endoparasitic development rate (1/days) of *G. fulvipes* in *L. dispar* larvae at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C).

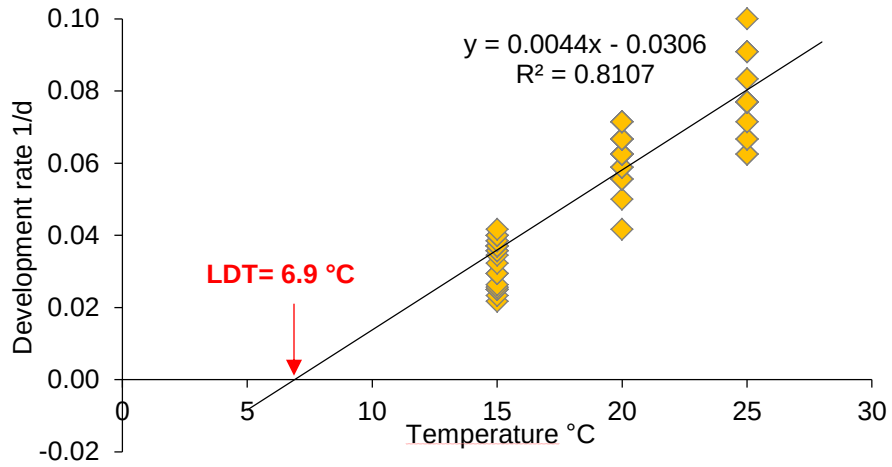

Figure 26: Endoparasitic development rate (1/days) of *G. porthetriae* in *L. dispar* larvae at 15°C, 20°C and 25°C. The red arrow shows the lower developmental threshold (°C).

3.5.2 Lower developmental threshold of the pupal stage

The lower developmental threshold of the pupal stage was lowest for *G. porthetriae* (LDT = 7.94°C) followed by *G. fulvipes* (LDT = 8.54°C) and *G. liparidis* (LDT = 9.22°C) (Figures 27-29).

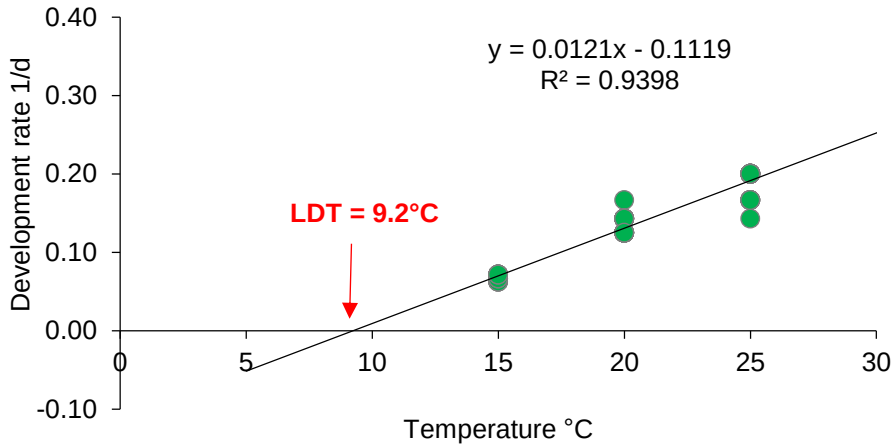

Figure 27: Development rate (1/days) of *G. liparidis* pupae at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C) of the pupal stage.

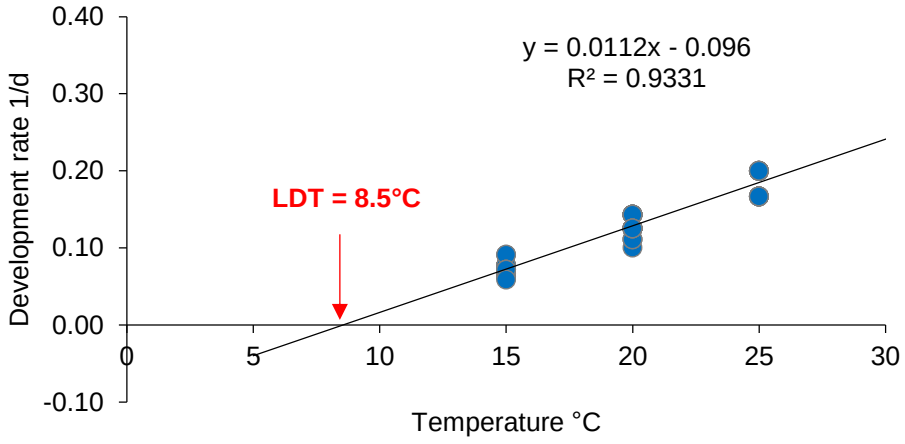

Figure 28: Development rate (1/days) of *G. fulvipes* pupae at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C) of the pupal stage.

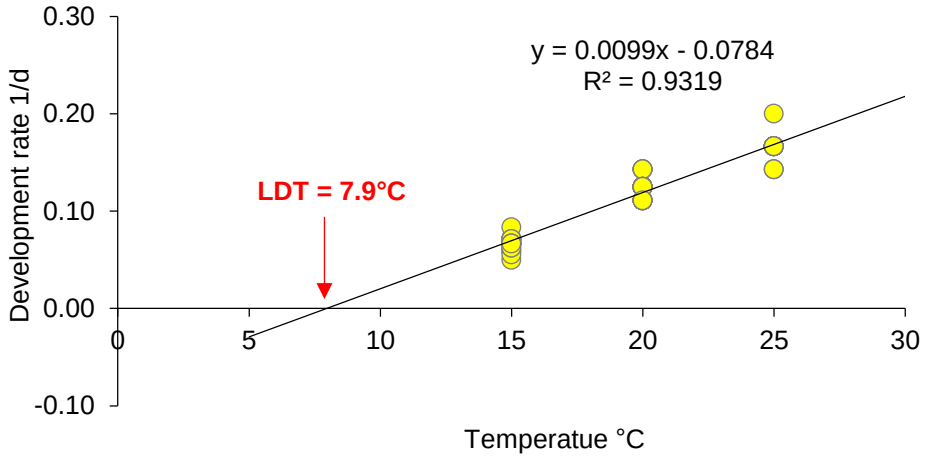

Figure 29: Development rate (1/days) of *G. porthetriae* pupae at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C) of the pupal stage.

3.5.3 Lower development threshold of all immature stages

The lower development threshold of all immature stages (from oviposition until wasps eclosion from cocoons) was lowest for *G. porthetriae* (LDT= 7.6) followed by *G. fulvipes* (LDT= 8.9) and *G. liparidis* (LDT= 9.6) (Figures 30-32). The differences between LDT of the endoparasitic development and the LDT for all immature stages was 0.3°C for *G. liparidis,* 0.1°C for *G. fulvipes* and 0.7°C for *G. porthetriae*.

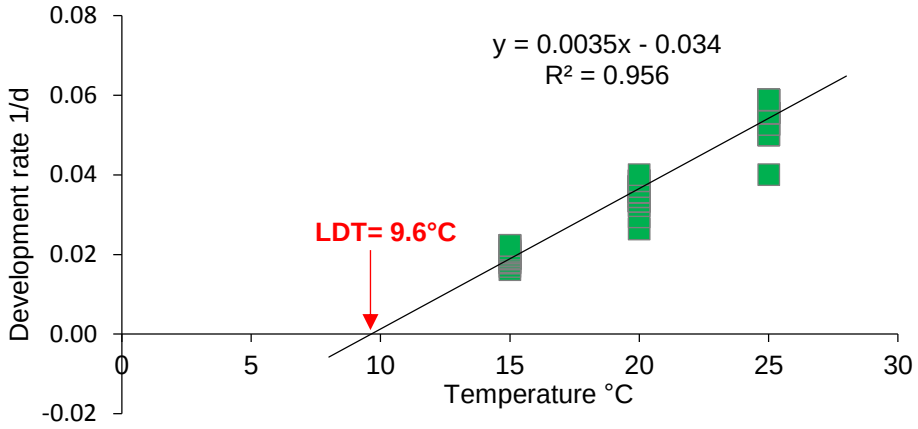

Figure 30: Total immature development rate (1/days) of *G. liparidis* in *L. dispar* larvae at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C) of all immature stages.

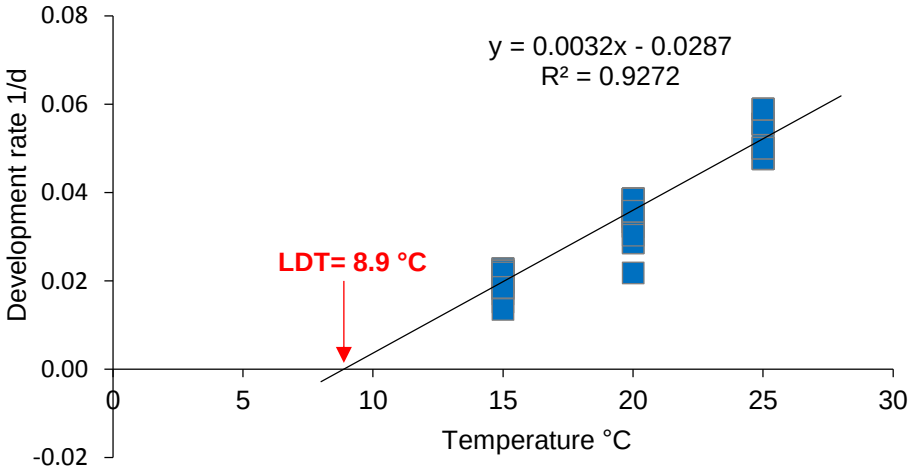

Figure 31: Total immature development rate (1/days) of *G. fulvipes* at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C) of all immature stages.

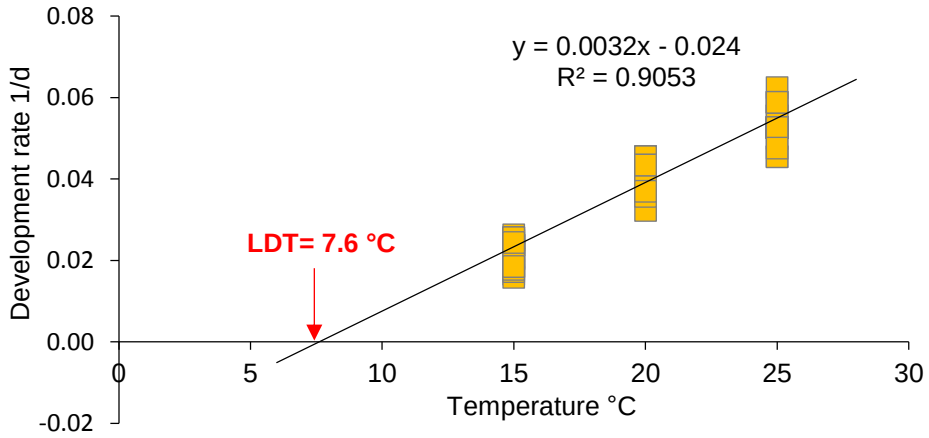

Figure 32: Total immature development rate (1/days) of *G. porthetriae* at 15°C, 20°C and 25°C. The red arrow shows the lower development threshold (°C) of all immature stages.

3.6 Accumulation of degree-days

3.6.1 Accumulation of degree days for the endoparasitic development

At 15°C, the accumulation of degree-days (DD) for the endoparasitic development was significantly different among *G. liparidis*, *G. fulvipes* and *G. porthetriae*. At 20°C, there was no significant difference between *G. liparidis* and *G. porthetriae*, but DD were significantly higher for *G. fulvipes* in comparison to the two other species. At 25°C, DD for *G. fulvipes* and *G. porthetriae* did not differ significantly, but DD for *G. liparidis* were significantly lower than DD for the two other species (Figure 33).

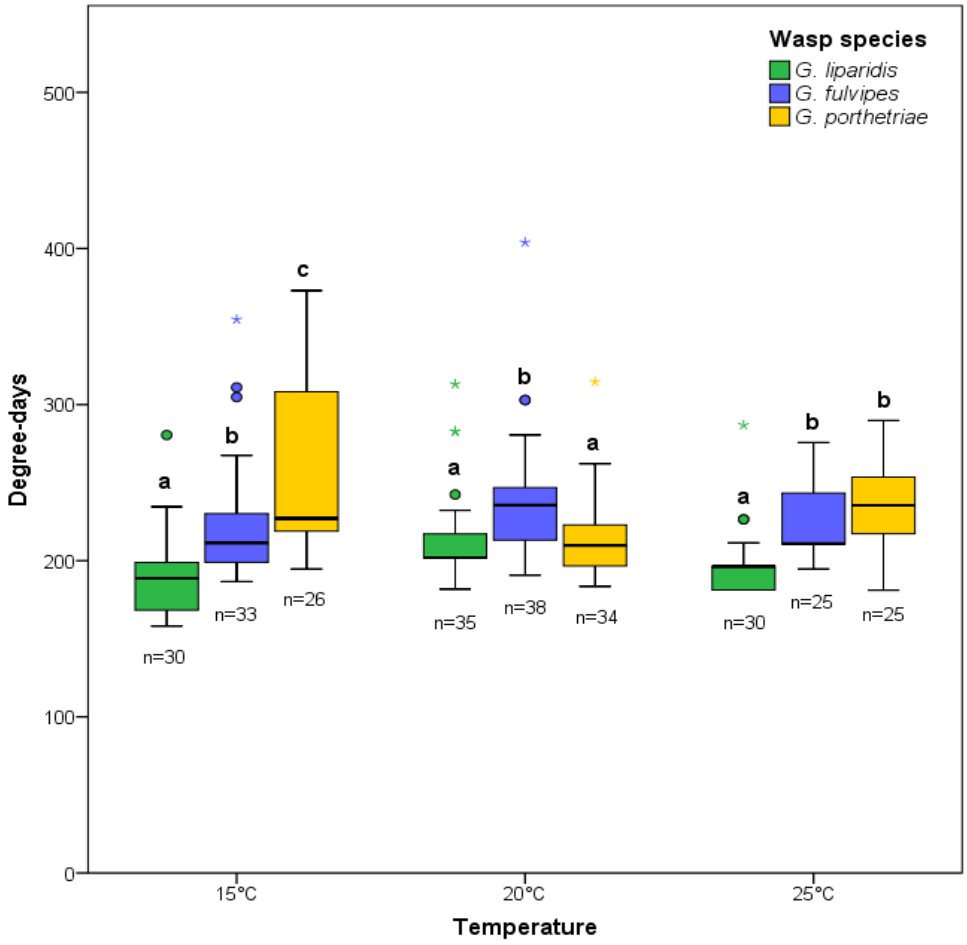

Figure 33: Accumulation of degree-days of *G. liparidis*, *G. fulvipes* and *G. porthetriae* during the endoparasitic development at 15°C, 20°C and 25°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75^{th} percentile – 25^{th} percentile). Error bars indicate minimum and maximum values. Significant differences in DD are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé $P<0.05$), n = number of hosts.

The thermal constants for the endoparasitic development of *G. liparidis* were not significantly different at 15°C and 25°C. At 20°C, DD were significantly higher than those of the other temperature regimes. For *G. fulvipes*, the differences in DD at the three temperatures were not significant. For *G. porthetriae*, the thermal constant at 25°C was significantly higher than that at the two other temperatures (Figure 34).

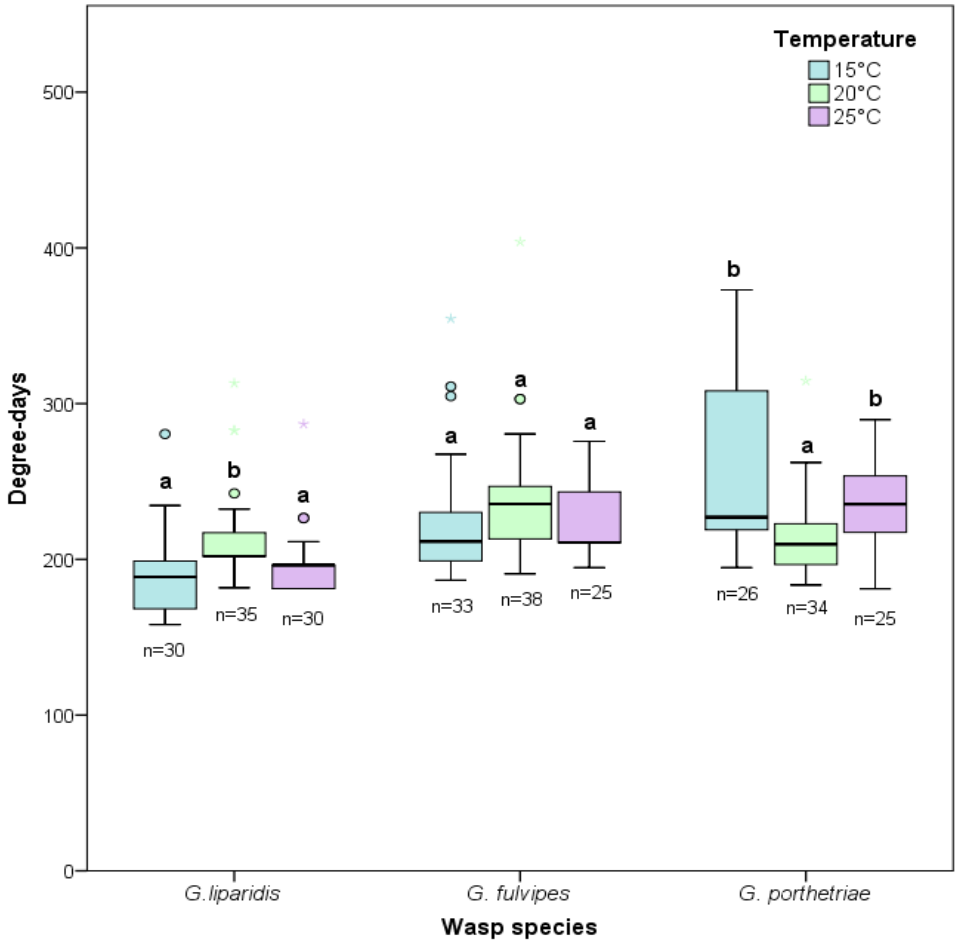

Figure 34: Accumulation of degree-days of *G. liparidis*, *G. fulvipes* and *G. porthetriae* during the endoparasitic development at 15^0C, 20^0C and 25^0C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75^{th} percentile – 25^{th} percentile). Error bars indicate minimum and maximum values. Significant differences in DD are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé $P<0.05$), n = number of hosts.

3.6.2 Accumulation of degree days for the pupal stage

Accumulation of degree-days (DD) for the pupal stage was not significantly different in *G. liparidis* and *G. fulvipes* at 15°C and 25°C, while DD in solitary *G. porthetriae* were significantly higher. At 20°C, DD differed significantly among the wasp species (Figure 35).

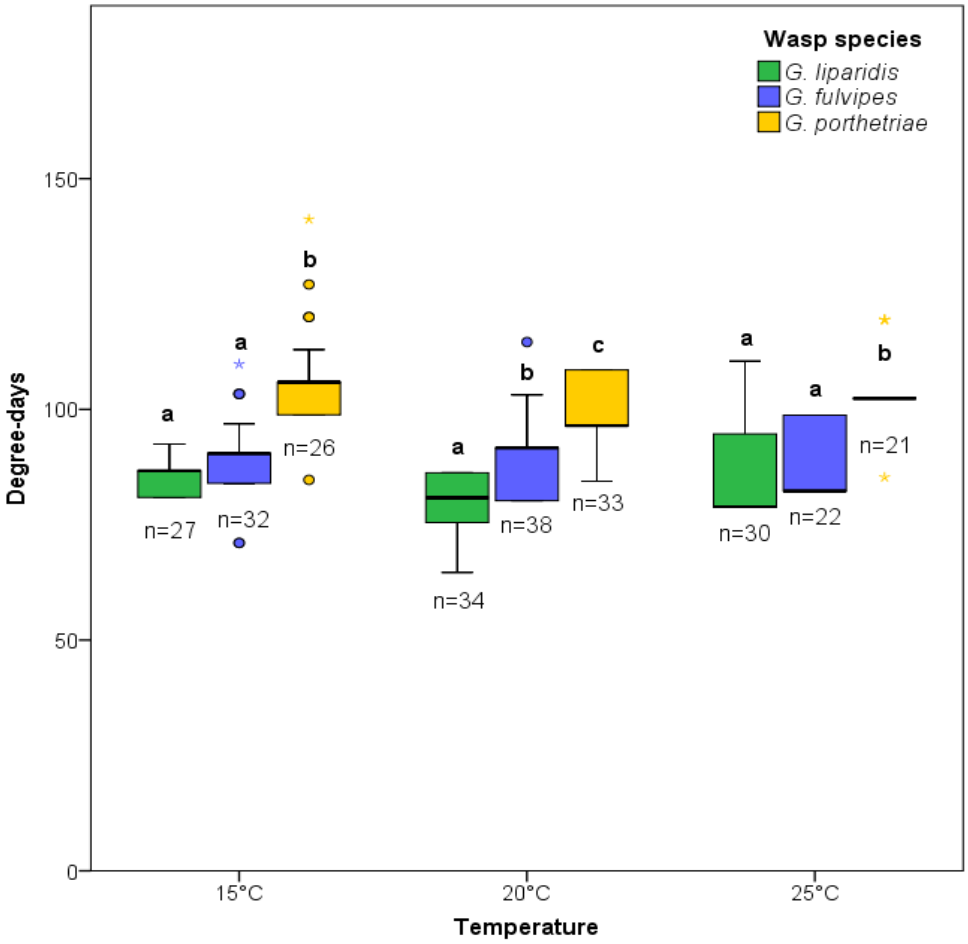

Figure 35: Accumulation of degree-days of *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15^{0}C, 20^{0}C and 25^{0}C during the cocoon stages. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in DD are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé P<0.05), n = number of hosts.

During the pupal stage, thermal constants at 15°C, 20°C and 25°C in *G. fulvipes* were not significantly different. In *G. liparidis* and *G. porthetriae,* DD at 15°C were significantly higher than at 20°C. At 25°C, DD differed not significantly in comparison to 15°C and 20°C (Figure 36).

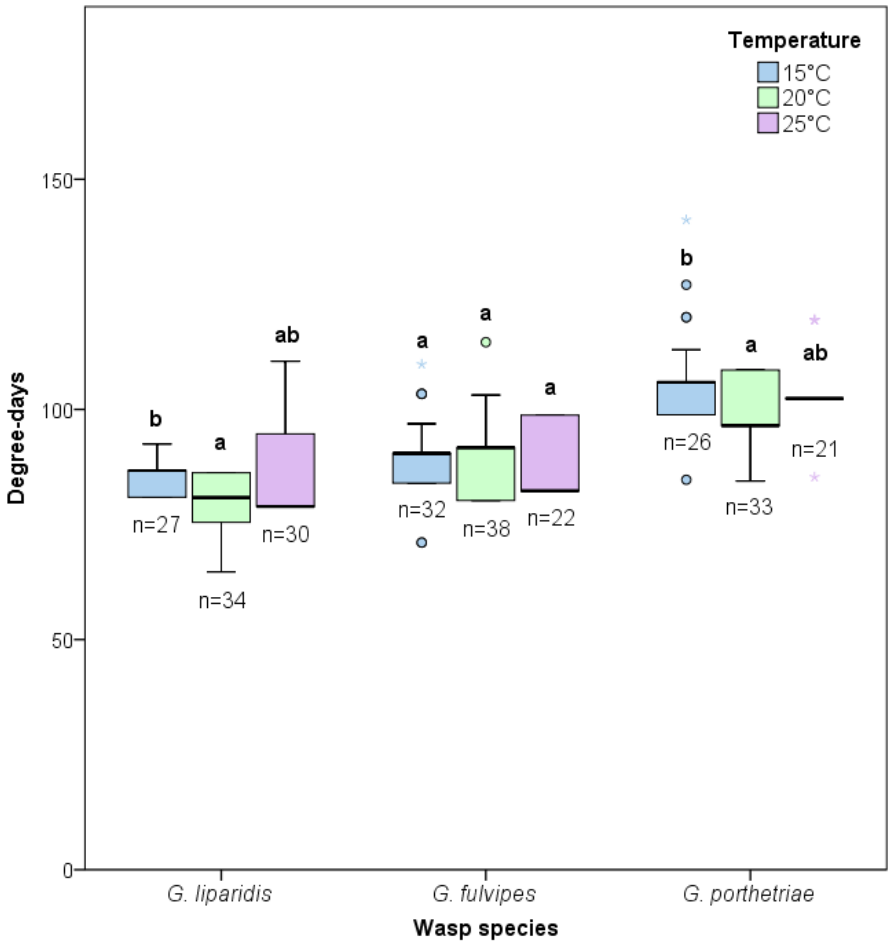

Figure 36: Accumulation of degree-days of *G. liparidis*, *G. fulvipes* and *G. porthetriae* during the pupal stage at 15^0C, 20^0C and 25^0C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75^{th} percentile – 25^{th} percentile). Error bars indicate minimum and maximum values. Significant differences in DD are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé $P<0.05$), n = number of hosts.

3.6.3 Accumulation of degree days for the total development

At 15°C and 25°C, the accumulation of degree-days (DD) for the total development (from oviposition until wasp eclosion from cocoons) was significantly different among the wasp species. At 20°C, DD were significantly higher in *G. fulvipes* than in *G. liparidis* and *G. porthetriae* (ANOVA, post hoc Scheffé p<0.05) (Figure 37).

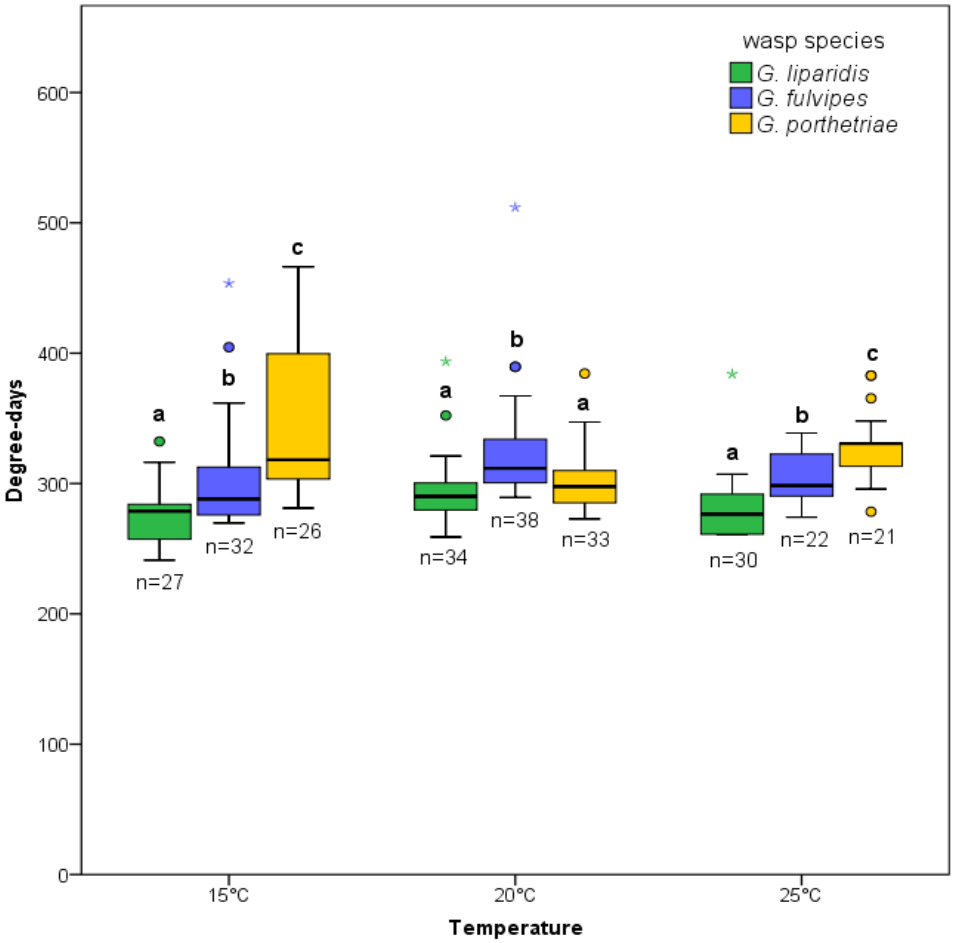

Figure 37: Accumulation of degree-days of *G. liparidis*, *G. fulvipes* and *G. porthetriae* during the total development at 15°C, 20°C and 25°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in DD are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé P<0.05), n = number of hosts.

At 15°C, 20°C and 25°C, the accumulation of DD for total development did not differ significantly in *G. fulvipes*. In *G. liparidis*, DD at 20°C were significantly higher than at

15°C, while the thermal constant at 25°C did not differ significantly from both other temperature regimes. In *G. porthetriae*, DD at 20°C were significantly lower than at 15°C and 25°C (Figure 38).

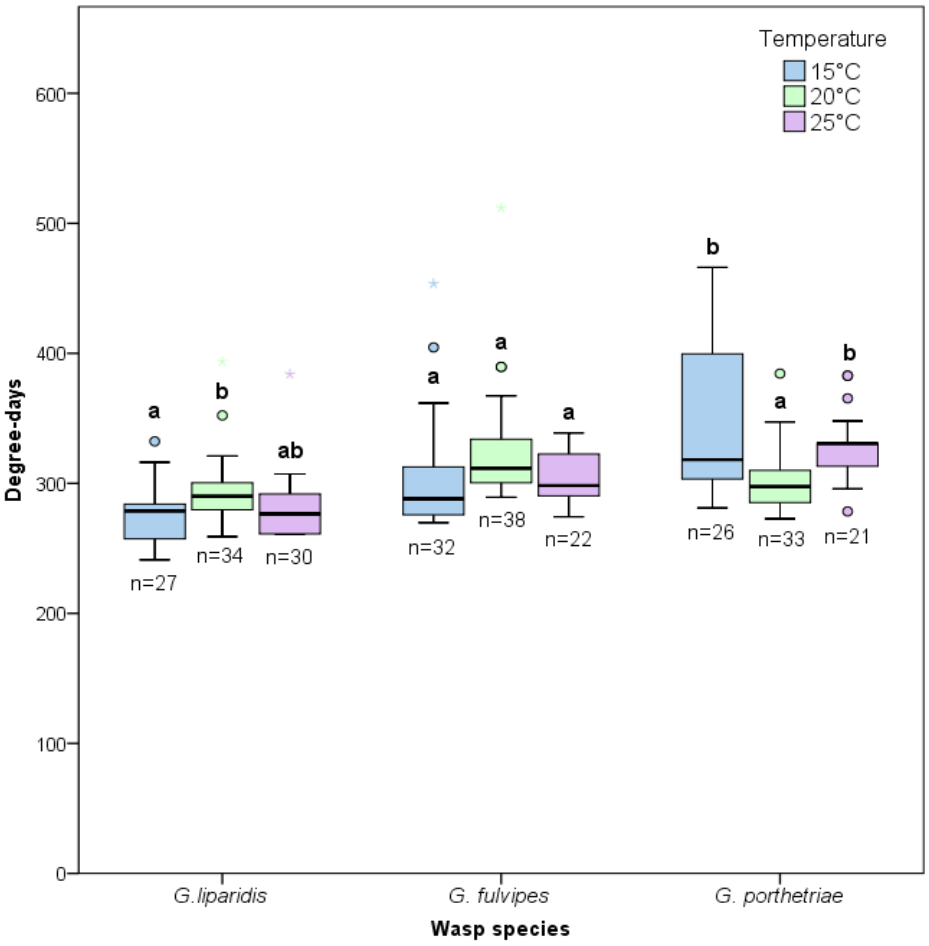

Figure 38: Accumulation of degree-days of *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15ºC, 20ºC and 25ºC during the total development. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75^{th} percentile – 25^{th} percentile). Error bars indicate minimum and maximum values. Significant differences in DD are indicated by different letters above the boxplots (ANOVA, post hoc Scheffé $P<0.05$), n = number of hosts.

The thermal constants for the endoparasitic and total development increased with increasing temperature in *G. liparidis* and *G. fulvipes*. The opposite trend was observed in *G. porthetriae* where the number of degree-days decreased with increasing temperature. On average, the highest value of degree-days was recorded at 20°C in the two gregarious species *G. liparidis* and *G. fulvipes*, while at 15°C it was highest in the solitary species *G. porthetriae* (Tables 11-13).

Table 11: Linear regression equations for endoparasitic development, LDT and degree-days (DD) of *G. liparidis*, *G. fulvipes* and *G. porthetriae*, R^2: coefficient of determination, LDT: lower development threshold, DD: thermal constant (degree-days, means ± SE). Data were obtained from rearing experiments at 15°C, 20°C and 25°C.

Endoparasitic development					
Wasp species	Temperature	Regression equation	R^2	LDT (°C) ±SE	DD (± SE)
G. liparidis	15°C	y = 0.005x - 0.0499	0.92	**9.90** ± 0.0058	189.89 ± 4.81
	20°C				213.54 ± 4.76
	25°C				196.30 ± 3.90
					199.91
G. fulvipes	15°C	y = 0.0044x - 0.0388	0.89	**8.78** ± 0.0062	223.17 ± 6.63
	20°C				238.87 ± 6.12
	25°C				224.48 ± 4.91
					228.84
G. porthetriae	15°C	y = 0.0044x - 0.0306	0.81	**6.89** ± 0.0084	255.15 ± 10.18
	20°C				212.07 ± 4.34
	25°C				238.33 ± 5.95
					235.18

Table 12: Linear regression equations for the pupal stage, LDT and degree days (DD) of *G. liparidis*, *G. fulvipes* and *G. porthetriae*. R^2: coefficient of determination, LDT: lower development threshold, DD: thermal constant (degree-days, means ± SE). Data were obtained from rearing experiments at 15°C, 20°C and 25°C.

Pupal stage					
Wasp species	Temperature	Regression equation	R^2	LDT (°C) ±SE	DD (± SE)
G. liparidis	15°C	y = 0.0121x - 0.119	0.94	**9.22** ± 0.0122	85.62 ± 0.25
	20°C				80.53 ± 1.03
	25°C				84.16 ± 1.55
					83.44
G. fulvipes	15°C	y = 0.0112x - 0.096	0.93	**8.54** ± 0.0115	89.83 ± 1.44
	20°C				89.57 ± 1.27
	25°C				89.78 ± 1.75
					89.73
G. porthetriae	15°C	y = 0.0099x - 0.0784	0.93	**7.94** ± 0.0103	106.71 ± 2.11
	20°C				97.94 ± 1.34
	25°C				103.98 ± 1.59
					102.88

Table 13: Linear regression equations for total development, LDT and degree days (DD) of *G. liparidis*, *G. fulvipes* and *G. porthetriae*. R^2: coefficient of determination, LDT: lower development threshold, DD: thermal constant (degree-days, means ± SE). Data were obtained from rearing experiments at 15°C, 20°C and 25°C.

Total development					
Wasp species	Temperature	Regression equation	R^2	LDT (°C) ±SE	DD (± SE)
G. liparidis	15°C	y = 0.0035x - 0.034	0.96	**9.64** ± 0.0030	274.55 ± 4.31
	20°C				294.35 ± 4.22
	25°C				281.60 ± 4.30
					283.50
G. fulvipes	15°C	y = 0.0032x - 0.0287	0.93	**8.87** ± 0.0035	302.67 ± 7.19
	20°C				323.94 ± 6.49
	25°C				305.00 ± 5.17
					310.54
G. porthetriae	15°C	y = 0.0032x - 0.024	0.91	**7.60** ± 0,0040	344.67 ± 10.47
	20°C				300.98 ± 4.33
	25°C				328.94 ± 5.61
					324.86

3.7 Adult wasps

3.7.1 Longevity

Longevity of adult wasps was investigated at 15°C and 20°C for the two gregarious wasp species *G. liparidis* and *G. fulvipes.* Longevity was significantly higher at 15°C than at 20°C in both wasp species (Figure 39-40) (t-Test, P<0.05).

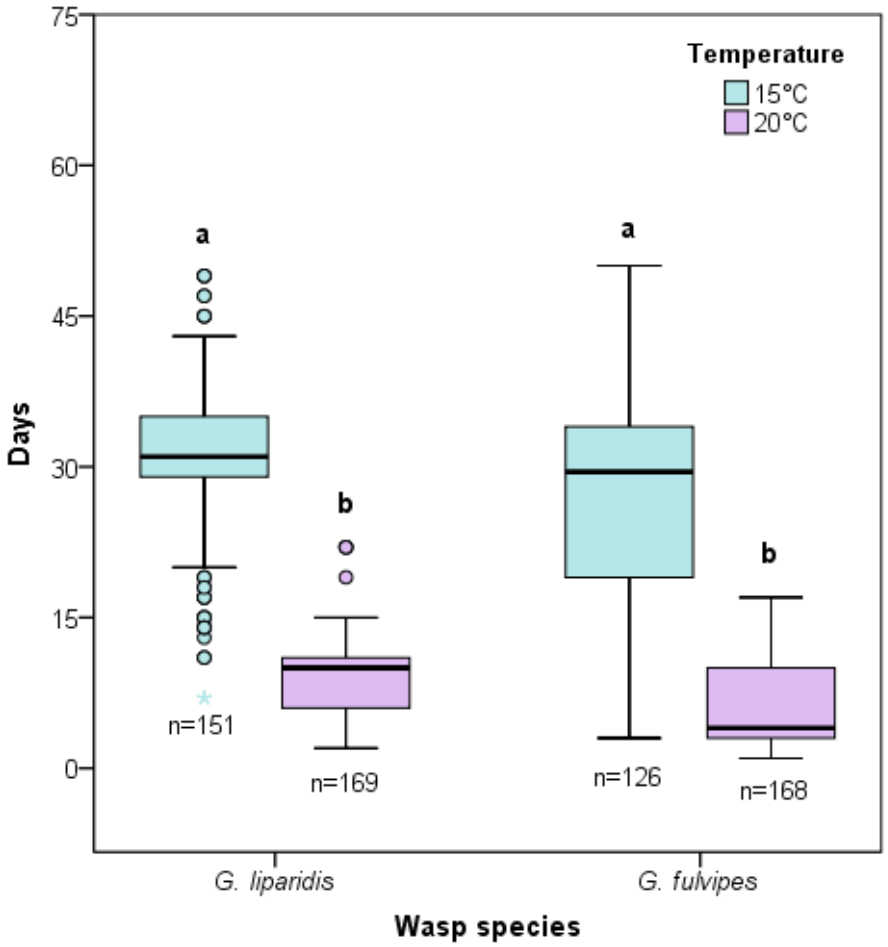

Figure 39: Adult wasp longevity (days) of *G. liparidis* and *G. fulvipes* at 15°C and 20°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in longevity are indicated by different letters above the boxplots (t-Test, P<0.05), n = number wasps.

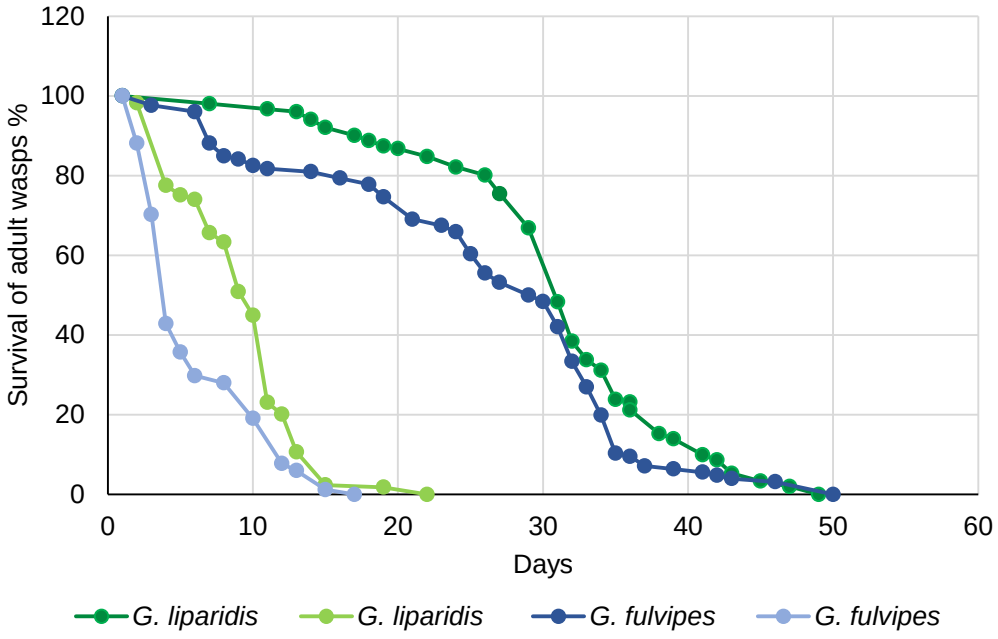

Figure 40: *G. liparidis* and *G. fulvipes* adult wasp survival (%) at 15°C and 20°C, respectively.

Table 14: Wasp longevity (days, mean ± SE) of *G. liparidis* and *G. fulvipes* at 15°C and 20°C. Significant differences between males and females are indicated by different lowercase letters. Significant differences between species are indicated by different uppercase letters (see figures 40 and 41).

T	***G. liparidis***				***G. fulvipes***			
	Male		Female		Male		Female	
	N	mean ± SE	N	mean ± SE	N	mean ± SE	N	mean ± SE
15°C	123	31.5 ± 0.7 aA	28	28.6 ± 2.4 aA	98	27.8 ± 1.1 aB	28	20.6 ± 2.0 bB
20°C	134	9.7 ± 0.4 aA	35	7.7 ± 0.6 bA	128	5.3 ± 0.3 aB	40	8.7 ± 0.7 bA

G. liparidis wasp longevity at 15 ºC was not significantly different for males and females (t-Test, P>0.05). On average, longevity was 31.5 days for males and 28.6 for females. The maximum lifetimes were 47 days for males and 49 days for females, minimum lifetimes 7 and 11 days, respectively. In *G. fulvipes* longevity was significantly shorter than in *G. liparidis*, for both males and females (males 28 days, females 21 days). The maximum lifetime was 50 days for males and 35 for females, the minimum was 3 days for both sexes.

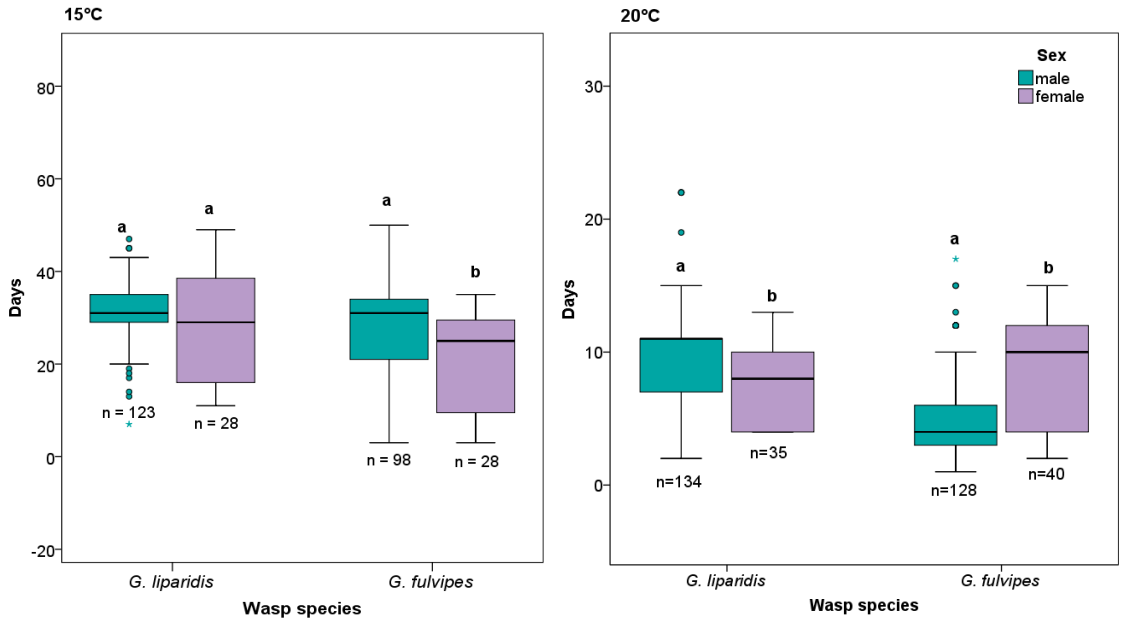

Figure 41: Adult wasp longevity (days), of *G. liparidis* and *G. fulvipes* males and females at 15°C and 20°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences are indicated by different letters above the boxplots (t-Test, P<0.05), n = number of wasps.

At 20°C longevity of the sexes differed significantly for both *Glyptapanteles* species. In *G. liparidis* longevity for males was 9.7 days, for females 7.7 days. Maximum lifetime was 22 days (males) and 13 days (females), minimum lifetimes were 2 days (males) and 4 days (females), respectively. Average longevity of *G. fulvipes* was 5.3 days (males) and 8.7 days (females). For males the maximum lifetime was 17 days and for females 15 days, minimum lifetimes were 1 day (males) and 2 days (females) (Figure 41) (t-Test, P<0.05).

At 15°C, the lifetime for *G. liparidis* and *G. fulvipes* males and females were significantly different. At 20°C, the difference in male longevity between two wasp species was significant (t-Test, P>0.05), for females there was no significant difference (Figure 42) (t-Test, P>0.05).

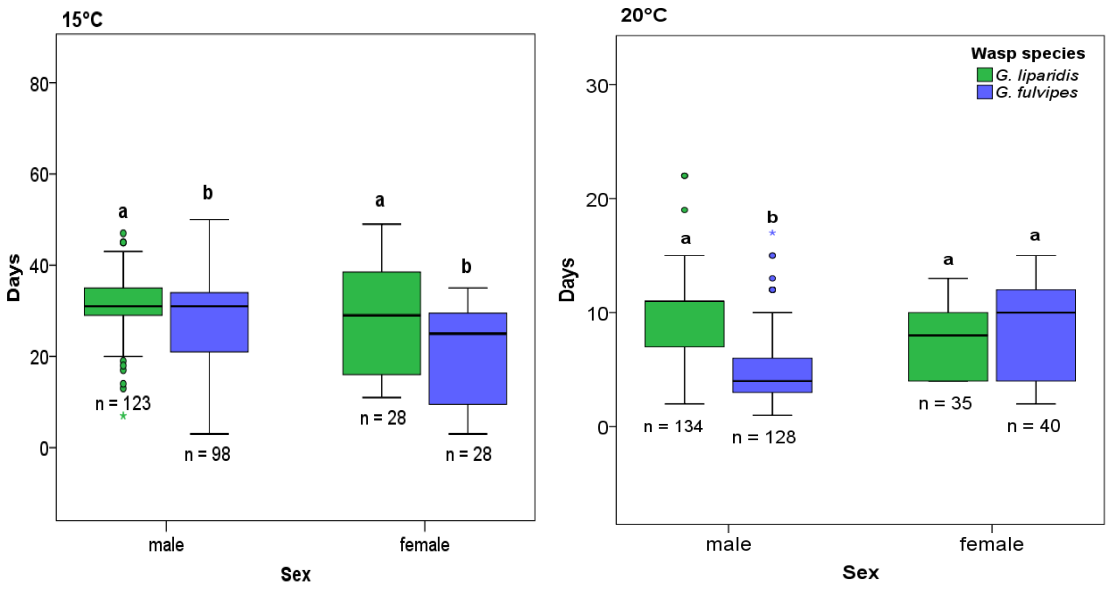

Figure 42: Adult wasp longevity (days) for males and females, of *G. liparidis* and *G. fulvipes* at 15°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences are indicated by different letters above the boxplots (t-Test, P<0.05), n = number of wasps.

3.7.2 Biomass

At every temperature regime, the biomass of *G. porthetriae* male wasps was significantly higher than the biomass of the two other species. The differences in biomass between *G. liparidis* and *G. fulvipes* were not significant (Figure 43).

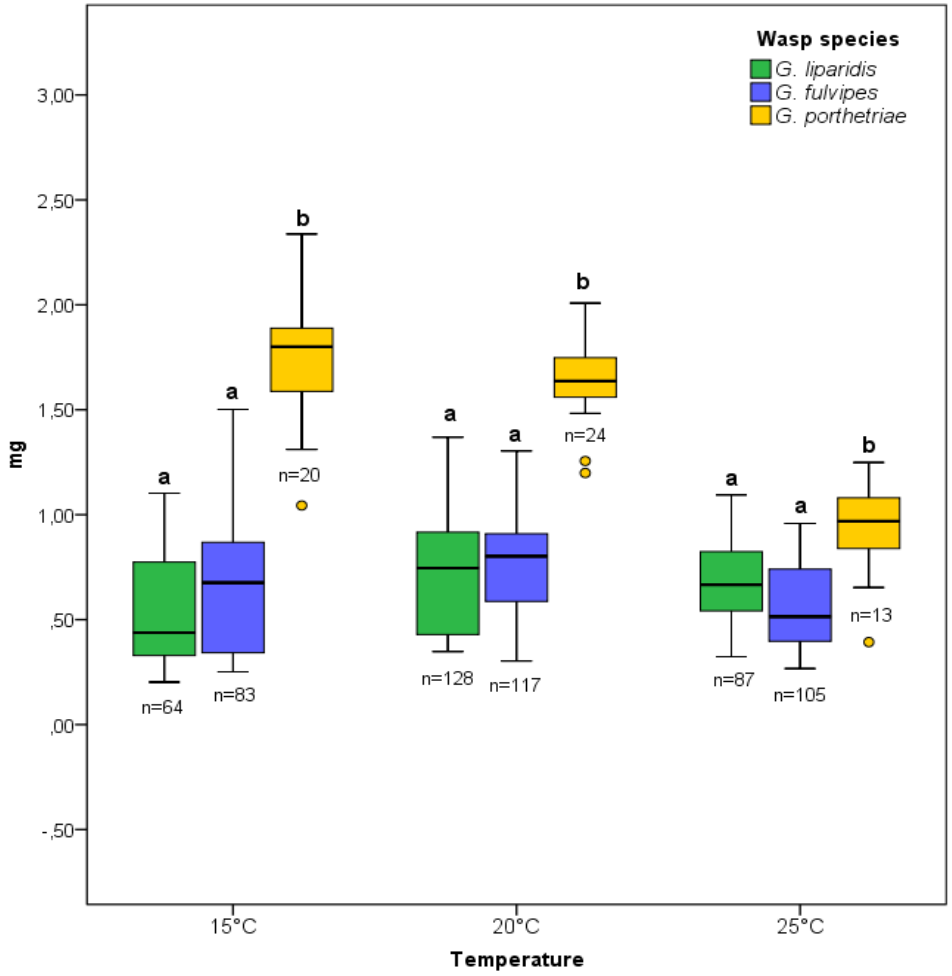

Figure 43: Body mass of male wasps (mg) of *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15°C, 20°C and 25°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in body mass are indicated by different letters above the boxplots (P<0.05), n = number of wasps.

The maximum body mass of *G. porthetriae* males occurred at 15°C and was 2.34 mg, and the minimum biomass was 1.04 mg. At 25°C the highest weight was only 1.25 mg, the minimum body mass was 0.39 mg. For *G. liparidis* and *G. porthetriae* maximum body masses were observed at 20°C with 1.37 mg and 1.30 mg, respectively. The lowest body masses at 20°C were 0.35 mg and 0.30 mg, respectively. The minimum biomass of the two gregarious species occurred at 15°C with 0.20 mg in *G. liparidis* and 0.25 mg in *G. fulvipes.* The highest male body masses at 15°C were 1.10 mg (*G. liparidis*) and 1.50 mg (*G. fulvipes*), respectively (Table 15).

Table 15: Adult wasp biomass (mg, means ± SE) of *G. liparidis*, *G. fulvipes* and *G. porthetriae* males and females at 15°C, 20°C and 25°C.

T	*G. liparidis*		*G. fulvipes*		*G. porthetriae*	
	Male	Female	Male	Female	Male	Female
15°C	0.54±0.03 n=64	0.77±0.06 n=33	0.64±0.03 n=83	0.81±0.05 n=48	1.76±0.07 n=20	1.82±0.07 n=6
20°C	0.73±0.02 n=128	1.17±0.03 n=64	0.75±0.02 n=117	1.23±0.02 n=60	1.64±0.04 n=24	1.96±0.04 n=9
25°C	0.67±0.02 n=87	1.05±0.03 n=65	0.58±0.02 n=105	1.02±0.04 n=35	0.93±0.06 n=13	1.25±0.06 n=8

The biomasses of female *G. porthetriae* wasps were significantly higher at every temperature condition in comparison to the two other species. The differences in female wasp body mass between *G. liparidis* and *G. fulvipes* were not significant (Figure 44). The maximum biomass at 20°C was 2.18 mg in *G. porthetriae*, 1.67 mg in *G. liparidis* and 1.46 mg in *G. fulvipes*. The lowest body masses at 20°C were 1.86 mg, 0.49 mg and 0.89 mg for *G. porthetriae*, *G. liparidis* and *G. fulvipes*, respectively. The female minimum weight of *G. porthetriae* at 25°C was 0.99 mg, of *G. liparidis* and *G. fulvipes* the minimum weights were 0.35 and 0.34 mg at 15°C, respectively.

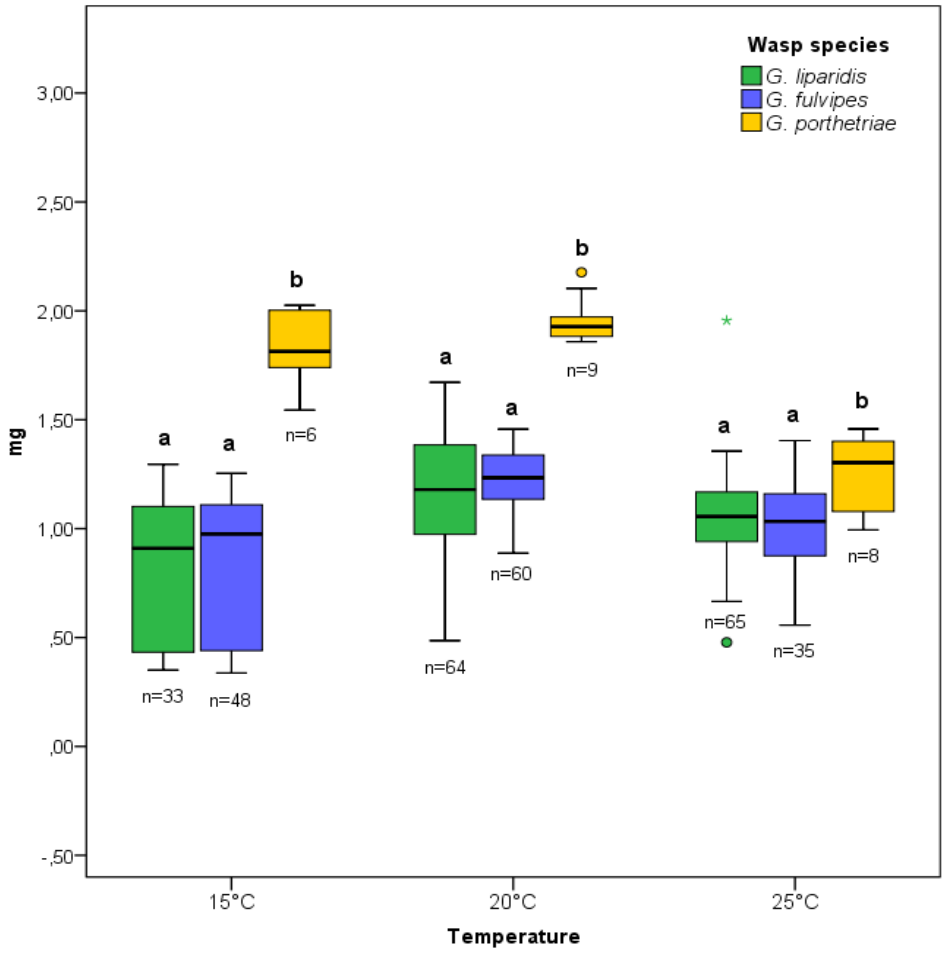

Figure 44: Body mass of female wasps (mg) of *G. liparidis*, *G. fulvipes* and *G. porthetriae* at 15°C, 20°C and 25°C. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in body mass are indicated by different letters above the boxplots (P<0.05), n = number of wasps.

At all temperatures (15°C, 20°C, 25°C) and wasp species females were significantly bigger than males, except for *G. porthetriae* at 15°C (see Figures 45-47).

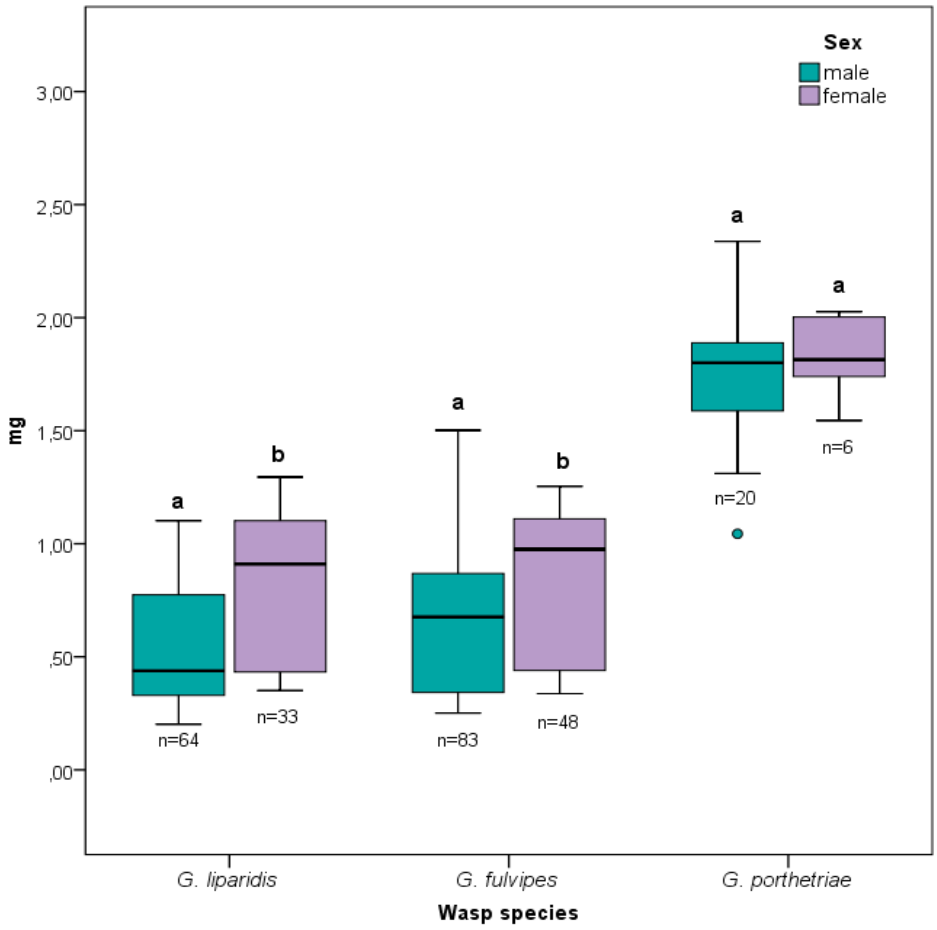

Figure 45: Adult wasp biomass (mg) at 15°C of *G. liparidis*, *G. fulvipes* and *G. porthetriae*. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in biomass are indicated by different letters above the boxplots (t-Test, P<0.05), n = number of wasps.

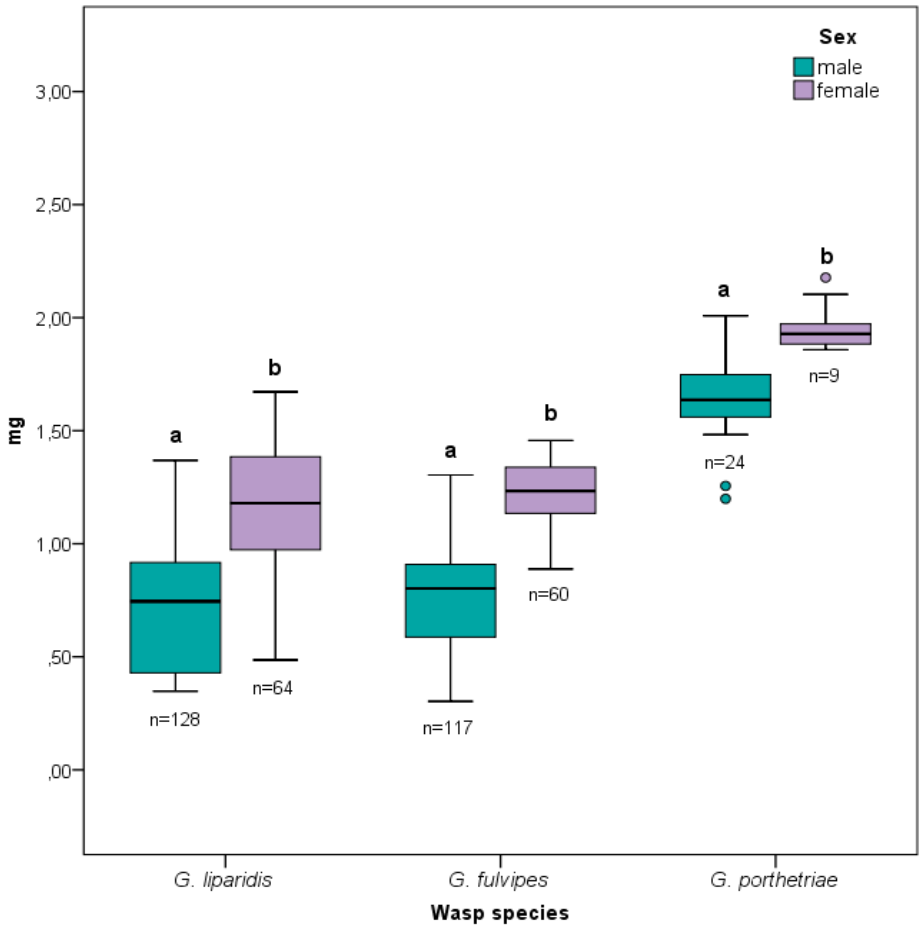

Figure 46: Adult wasp biomass (mg) at 20°C of *G. liparidis*, *G. fulvipes* and *G. porthetriae*. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in biomass are indicated by different letters above the boxplots (t-Test, P<0.05), n = number of wasps.

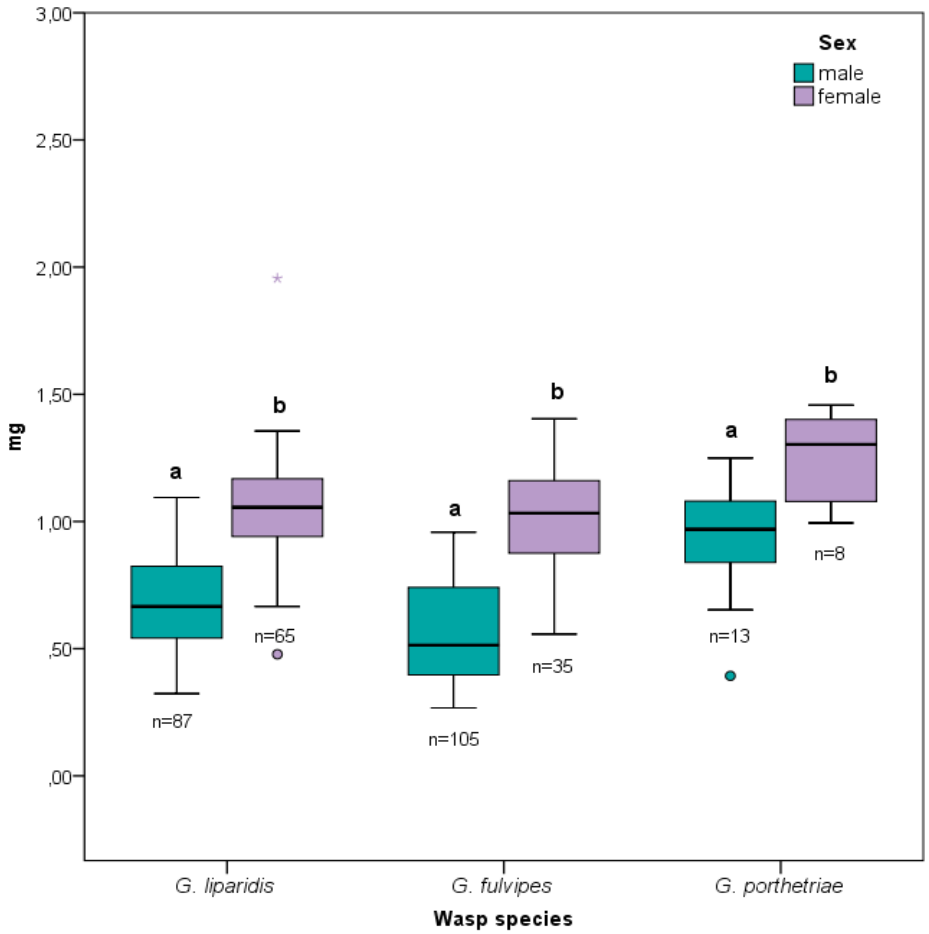

Figure 47: Adult wasp biomass (mg) at 25°C of *G. liparidis*, *G. fulvipes* and *G. porthetriae*. The horizontal line inside the box represents the median, the length of the box corresponds to the interquartile range (75th percentile – 25th percentile). Error bars indicate minimum and maximum values. Significant differences in biomass are indicated by different letters above the boxplots (t-Test, P<0.05), n = number of wasps.

3.7.3 Offspring sex ratio

All wasp species produced more males than females, regardless of the temperature. The parasitoid offspring sex ratio (male:female) was 6:1 for *G. liparidis*, 5:1 for *G. fulvipes* and 2.5:1 for *G. porthetriae*. Tables 16-17 show absolute and relative numbers of male and female offspring per host for the three wasp species at all temperature regimes.

Table 16: Male and female offspring (mean ± SE) of *G. liparidis* and *G. fulvipes* at 15°C, 20°C and 25°C, per host larva, T= temperature, N= number of host larvae *L. dispar.*

T	***G. liparidis***			***G. fulvipes***		
	Male	Female	N	Male	Female	N
15°C	10.88 ± 4.26	1.83 ± 1.37	24	9.52 ± 3.17	3.57 ± 1.53	21
20°C	15.70 ± 3.98	2.89 ± 1.20	27	13.45 ± 3.03	3.81 ± 1.83	31
25°C	24.17 ± 3.64	5.52 ± 1.45	29	14.32 ± 2.32	2.50 ± 0.86	22

Table 17: Percentage of male and female offspring of *G. liparidis*, *G. fulvipes* and *G. porthetriae* emerging from *L. dispar* host larva, T= temperature, N= number of host larvae.

T	***G. liparidis***			***G. fulvipes***			***G. porthetriae***		
	male	female	N	male	female	N	male	female	N
15°C	86	14	24	73	27	21	77	23	26
20°C	85	15	27	78	22	31	73	27	33
25°C	81	19	29	85	15	22	62	38	21

4. Discussion

4.1 Rate of parasitization

Successful parasitism of endoparasitic wasps indicates the parasitoids' potential and ability to overcome the immune responses of their host larvae. Schopf & Steinberger (1996) studied the rate of parasitization by *G. liparidis* using various instars of *L. dispar* as hosts and concluded that the wasp prefers early instars for oviposition because the parasitoids emerged successfully from 77% to 90% of the host larvae parasitized in premolt to the 2nd instar to the middle of the 3rd instar. In my experiment host larvae were parasitized on the first day of the 3rd (*G. liparidis* and *G. porthetriae*) or 2nd instar (*G. porthetriae*), according to the wasp's preferred host size in the field. 'Successful parasitization' was used for each host larva from which parasitoids emerged or in case of premature host death parasitoid larvae were found in the host hemocoel. The term 'non successful parasitization' referred to hosts that pupated or when no parasitoid egg/larva were found upon dissection. Parasitization success was higher for *G. liparidis* and *G. fulvipes* than for *G. porthetriae*, regardless of the ambient temperature. Successful wasp emergence was highest at 20°C, regardless of the wasp species. If the rate of successful parasitization represents the ability of the parasitoid to overcome the host immune responses this temperature can be considered as the most suitable for the endoparasitic development of the wasps. Ipso facto, the highest number of larvae considered 'not successfully parasitized', were found in *G. porthetriae* followed by *G. fulvipes* and *G. liparidis*. The reason for unsuccessful parasitization includes parasitoid death because of the host immune responses (i.e. encapsulation and melanization of the parasitoid egg/larva) or no parasitoid eggs were injected into the host at oviposition (i.e. pseudoparasitization).

4.2 Host mortality

For koinobiont endoparasitoids, which develop in a living, feeding and molting host, successful development in and emergence from the host is a great challenge: they live in intimate physical contact with their host and must avoid damaging important organs that would be lethal for both, host and parasitoid (Godfray 1994). To overcome the host immune responses during endoparasitic development, females of several braconid subfamilies introduce not only eggs and venom into the host hemocoel, but also a

symbiotic virus (termed polydnavirus). These maternal factors plus the parasitoid larvae manipulate the host immune defenses, nutrition and physiology. Prior to parasitoid emergence, the host is developmentally arrested and never pupates (Schafellner et al. 2004, 2007). Sometimes even suitable hosts do not survive these manipulations and the parasitoid also dies. However, premature death of host and parasitoid could also be the result of a concurrent pathogen infection. Upon dissection, I never found any hint of bacterial, fungal or viral infections of the deceased larvae, but low mortality occurred with every wasp species at all temperature variants. Since mortality was lowest at 20°C and higher at the two marginal temperatures, it appears that temperature can exacerbate or mitigate the reaction of the host towards the manipulation by the parasitic wasps, rendering parasitoid survival and development more or less favorable.

Development disorders of the parasitized host, such as molting problems, pupal malformations or growth retardation, were induced by all wasp species. Even in cases when no parasitoids emerged from the host and the host remained alive, the maternal factors that were introduced during parasitization had detrimental effects on host development and metamorphosis.

4.3 Parasitoid loads

Gregarious wasps that are able to lay many eggs per host are able to adjust the number of eggs during oviposition to the host's size and quality (Strand 2002, Schafellner et al. 2007), injecting more eggs in larger hosts that provide a greater quantity of resources than smaller hosts. Schopf & Steinberger (1996) offered three instars of *L. dispar* larvae to *G. liparidis* females and found a higher number of offspring from larger hosts. Häckermann et al. (2007) observed the opposite from *Hyssopus pallidus* (Hymenoptera: Eulophidae), a gregarious ectoparasitoid of larval instars of *Cydia* species (Lepidoptera, Tortricidae). Here, clutch size produced per host weight unit was significantly higher in smaller *Cydia molesta* than in larger *Cydia pomonella*. The authors conclude that parasitoid females are able to assess the nutritional quality of an encountered host and adjust clutch size accordingly. Thus, host size is not the only parameter to explain the nutritional quality of a given host.

The amount of eggs injected by a gregarious female at oviposition depends on many factors such as the number of eggs the female can lay at a given moment, the storage

capacity of the ovarioles, and – in case of synovigenic wasps – the number of eggs that have reached maturity, which in turn depends on the female's fitness, its nutritional status and age (Jervis & Kidd 1996). In my experiment the two gregarious wasp species parasitized hosts of equal sizes and the same developmental stage and the average number of eggs per host was higher in hosts parasitized by *G. liparidis*, however, differences in clutch size were not significant.

4.4 Parasitoid development

Temperature has substantial effects on insect distribution, colonization, survival, abundance, behavior, fitness and life history traits (Campbell et al. 1974, Spanoudis & Andreadis 2012). The three parasitoid species used in my experiments developed successfully under all test temperatures with decreasing development time as the rearing temperatures increased. Endoparasitic and total development of the solitary *G. porthetriae* was significantly shorter at the lower temperature regimes than development of the two gregarious species, *G. liparidis* and *G. fulvipes*. The solitary parasitoid develops as the only 'resident' in the host hemocoel, i.e. the larva possesses food resources in excess and does not have to compete with conspecifics for food and space; thus, the larva completed development fast. It is assumed that larvae of many koinobionts avoid growing too big in young hosts, because if the host is exhausted before the parasitoid has completed development, both will perish (Harvey et al. 1994, Eliopoulos & Stathas 2003).

The impact of temperature on the development of larval endoparasitoids is superimposed by effects from a continuously changing composition of the host hemolymph to which the parasitoid larvae are exposed, thus, temperature effects on the immature wasp stages are difficult to interpret because of the strong dependence of the wasp larvae on the host's physiological reaction (Eliopoulos & Stathas 2003).

The development strategy of parasitoids is also influenced by the mortality risk of the host during endoparasitic development (Harvey 2005). Parasitoids in environments with high risk of host mortality (e.g. free feeding hosts exposed to high predation rates) were obviously selected for higher growth rates and shorter development times in comparison to parasitoids developing in hosts from low-risk environments (e.g. concealed hosts). Similarly, parasitoids in expanding populations are under selection pressure for faster

development and shorter generation times (Seyahooei et al. 2011). As Malina & Praslička (2008) point out the effective parasitoid should develop very quickly.

A curious phenomenon was observed in my experiments when the wasps emerged from their hosts. A significant number of the emerging parasitoids did not spin a cocoon, but instead the larvae remained stuck to the bottom of the Petri dish and perished without being able to pupate. The reason of this phenomenon is not known. I can only speculate that the humidity inside the glass chambers was too high so that many parasitoids were 'glued' to the glass and could not start spinning.

4.5 Lower development thresholds

In the experiment, the *Glyptapanteles* species developed in the same host species under the same temperature and moisture conditions. The lower developmental thresholds were similar for the three species, but the solitary *G. porthetriae* had clearly the lowest threshold for endoparasitic, pupal and total immature development (i.e. from oviposition until wasp eclosion from cocoons). The only difference in rearing conditions was the host size at parasitization; the solitary species developed in second to third-instar gypsy moth larvae, while the gregarious species developed in third to fourth-instar hosts. According to Campbell et al. (1974) the threshold values of parasitoids are higher than those of their hosts so that the build-up of the parasitoid population will be delayed until ambient temperatures increase and the hosts are well developed in spring. All *Glyptapanteles* species in temperate climates that use gypsy moth larvae as hosts depend on alternate hosts for overwintering. Thus, the timing of wasp emergence from winter diapause determines the coincidence with gypsy moth hatch and larval development in the field. For obvious reasons the parasitoid's season cannot start earlier than that of the host.

4.6 Thermal constants (degree-days)

The thermal constant is commonly used in phenology models to predict the time of appearance of particular development stages of agricultural or forest pests in the field. Knowledge about degree-days is crucial to apply well-timed control measures. Nahrung et al. (2004) developed a degree-day model to predict the timing of appearance and larval duration of the eucalypt leaf beetle, C*hrysophtharta agricola* (Coleoptera: Chrysomelidae)

in order to apply *Bacillus thuringiensis* (Bt) when it is most lethal, in early instars. Phenological degree day models are also used as warning systems for the occurrence of natural enemies of the pest species. For the European earwig, *Forficula auricularia* (Dermaptera: Forficulidae), an important natural enemy in pipfruit orchards, a day degree model was calculated to avoid negative effects of crucial orchard management on earwig mortality, such a spray application and soil tillage (Moerkens et al. 2011). Thus, using data from my experiments such as the lower development thresholds and the thermal sums, it is possible to develop a preliminary temperature-driven phenology model for three *Glyptapanteles* species that can predict the appearance of the wasps from *L. dispar* larvae in the forest and help to avoid negative impacts on the parasitoid population due to badly timed applications of pesticides.

4.7 Adult wasps

4.7.1 Longevity

Longevity is a practical and commonly used indicator of parasitoid fitness. Adult wasp lifetime is a major factor that impacts the field efficacy of parasitoids used in biological control programs (Wade & Wratten 2007). My experiments clearly revealed longer lifetimes at low temperatures (15°C) compared to increased temperatures (20°C). This result mirrors the effect of temperature on metabolic processes. Low temperatures reduce the metabolic rates and thus, the wasps live longer than at high temperatures (Spanoudis & Andreadis 2012). Two hypotheses provide explanations for short adult lifespans. First, metabolic rates determine the rates of food consumption. Accordingly, fast consumption of an essential resource shortens the lifespan (Seyahooei et al. 2011). Second, ageing of an organism is accompanied with an increasing production of reactive oxygen species (Herman 1957). As a result of respiration, free radicals are generated in the mitochondria that destroy biomolecules and so contribute to ageing.

4.7.2 Biomass

Body size of the adult wasp at emergence from the cocoon reflects directly the resources that were acquired and stored during the immature development inside the host. It is one of the key traits that is often correlated with life-history parameters such as mating efficiency, dispersal capability, and most importantly, reproductive success. A larger body

enables the parasitoid to store more energy and produce more eggs, resulting in longer life span and higher reproductive success (Liu & Ueno 2012). Harvey (2005) describes three developmental patterns of koinobiont parasitoids concerning the relationships between host age, egg-to-adult development time and adult body size. First, adult wasp size increases and development time decreases with host size or stage at parasitism. Second, adult wasp size increases with host size, but development time is unaffected by this parameter. Third, adult wasp size is mostly unaffected by variations of the size of the host at parasitization, but development time is much longer in small than in large hosts. Since I used hosts of similar size/age for the rearing experiments, I cannot attribute the individual wasp species to one of the proposed types. However, the body mass of the solitary *G. porthetriae* wasps was significantly higher than that of the two gregarious species, *G. liparidis* and *G. fulvipes*, regardless of the rearing temperatures. Obviously, the results reflect the importance of competition among conspecifics for limited host resources that comes into effect in the gregarious species but not in the solitary one.

Except for *G. porthetriae* at 15°C, the body mass of females was always significantly higher than that of males, indicating that female wasps are able to convert host biomass more efficiently into adult biomass than males. There was a clear temperature effect on adult wasp body mass; for all species, both sexes attained the highest body mass when the parasitized hosts were kept at 20°C. This temperature seems the most suitable for resource allocation during parasitoid development.

4.7.3 Offspring sex ratio

The haplo-diploid sex determination system of most parasitoid wasps provides females a means of controlling the offspring sex ratio, because they can adjust the proportion of fertilized eggs at oviposition (Jervis & Kidd 1996). For many species, the male offspring ratio increases with (1) maternal age at oviposition or the number of days after insemination, (2) the age of the male parent or the number of copulations, (3) extreme temperatures, (4) decreasing host size, age, or quality, (5) female wasp density and (6) the number of progeny per host. According to the offspring sex allocation theory, females lay more fertilized eggs into superior quality hosts, while unfertilized eggs are injected into hosts of lower quality (Godfray 1994). Under laboratory conditions wasp sex ratios are often strongly skewed towards males, indicating that the rearing conditions are not

favorable for yielding higher female offspring. According to Nussbaumer & Schopf (2000) this is the main reason why the sex ratio in the laboratory differs from that observed in the field. In my experiments offspring sex ratios (males:females) in the two gregarious species were 6:1 (*G. liparidis*) and 5:1 (*G. fulvipes*), respectively. In contrast, the sex ratio of the solitary *G. porthetriae* was 2.5:1 which seems to be more related to natural conditions.

5. Summary

The three *Glyptapanteles* species developed successfully in and egressed from their gypsy moth hosts at 15°C, 20°C and 25°C, with the middle temperature regime being the most favorable one. At 20°C, the highest number of parasitoids egressed from the host, the highest number of parasitoids pupated and the lowest number of hosts/parasitoids died.

Endoparasitic development decreased with increasing temperature and was faster for *G. porthetriae* than for *G. liparidis* and *G. fulvipes* at 15°C and 20°C, but there was no difference at 25°C. The calculated lower development thresholds for endoparasitic development, pupal stage and total immature development were lowest for *G. porthetriae* followed by *G. fulvipes* and *G. liparidis*. This indicates that the solitary species *G. porthetriae* is able to start developing at lower temperatures in the field, reflecting the biology of the wasp. *G. porthetriae* wasps appear earlier in spring and parasitize younger instars than the two gregarious wasps.

Adult wasps of the gregarious wasp species lived three times longer at 15°C than at 20°C. A longer life span increases the chances of the wasps to find suitable hosts for oviposition.

At all temperature regimes, adult body masses of males and females were not significantly different between *G. liparidis* and *G. fulvipes*, but they were significantly higher for *G. porthetriae*.

The offspring sex ratios were significantly male-biased, more in the gregarious wasp species, less in the solitary species.

6. References

Abbasipour H., 1996. Biology of Grass Feeding Noctuidae (Lepidoptera) and their parasitoids in North East England. Doctoral thesis at the University of Newcastle, United Kingdom.

Alalouni U., Schädler M., Brandl R., 2013. Natural enemies and environmental factors affecting the population dynamics of the gypsy moth. Journal of Applied Entomology 137: 721-738.

Beck S.D., 1983. Insect thermoperiodism. Annual Review of Entomology 29: 91-108.

Bell R.A., Owens C.D., Shapiro M., Tardif, J.M. 1981. Development of mass-rearing technology. *In*: C.C. Doane & M.L. Mc Manus (eds.), The gypsy moth: Research toward integrated pest management. U.S.D.A., Technical Bulletin 1584: 599-633.

Campbell A., B.D. Frazer N. Gilbert A.P. Gutierrez, M. Mackauer, 1974. Temperature requirements of some aphids and their parasities. Journal of Applied Ecology 11: 431-438.

Capinera J. L., (Editor) 2008. Encyclopedia of entomology. Springer. 4346 pp.

Chen S., Fleischer S. J., Saunders M. C., Thomas M. B., 2015. The influence of diurnal temperature variation on degree day accumulation and insect life history. PLoS (ONE) 10(3).

Cho Y., Kwon O., Nam S., 2007. Effect of host age on life cycle and morphological characteristics of *Glyptapanteles liparidis* (Hymenoptera: Braconidae), a parasitoid of *Acronicta rumicis* (Lepidoptera: Noctuidae). Entomological Research 37: 103-107.

Chown S. L., Nicholson S. W., 2004. Insect physiological ecology. Mechanism and patterns. Oxford University Press, New York. 243 p.

Clausen C. P., 1940. Entomophagous insects. McGraw-Hill Book Company, Inc. 27 pp.

Eliopoulos P.A., Stathas G.J., 2003. Temperature-dependent development of the koinobiont endoparasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae): Effect of host instar. Environmental Entomology 32(5): 1049-1055.

Elkinton J. S., Liebhold A. M., 1990. Population dynamic of the gypsy moth in North America. Annual Review of Entomology 35: 571-96.

Fromm F. 2014. Untersuchungen zum Eignung von Raupen des Goldafters *Euproctis chrysorrhoea* (Lepidoptera: Erebiade) als Überwinterungswirt für die Brackwespe *Glyptapanteles liparidis* (Hymenoptera: Braconidae). Master thesis. BOKU, Wien. 67 pp.

Fuester R. W., Drea J. J., Gruber F., Hoyer H., Mercadier G., 1983. Larval parasites and other natural enemies of *Lymantria dispar* (Lepidoptera: Lymantriidae) in Burgenland, Austria and Würzburg, Germany. Environmental Entomology 12: 724-737.

Godfray H. C. J., 1994. Parasitoids: Behavioral and evolutionary ecology. Princeton NJ, New Jersey. 473 pp.

Gould J. R., Elkinton J. S., 1990. Temperature-dependent growth of *Cotesia melanoscela* (Hymenoptera: Braconidae), a parasitoid of the Gypsy Moth (Lepidoptera: Lymantriidae). Environmental Entomology 8: 859-865.

Harvey J. A., 2005. Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. Entomologia Experimentalis et Applicata 117: 1-13.

Häckermann J., Rott A. S., Dorn S., 2007. How do different host species influence the performance of a gregarious parasitoid: Host size is not equal to the host quality. Journal of Animal Ecology 76: 376-383.

Hemerik L., Egbert H., 2016. Predicting the potential establishment of two insect species using the simulation environment INSIM. Entomologia Experimentalis et Applicata 12433: 222-229.

Hoch G., Schopf A., Maddox J.V., 1999. Interactions between an entomopathogenic microsporidium and the endoparasitoid *Glyptapanteles liparidis* within their host, the gypsy moth larva. Journal of Invertebrate Pathology 75: 59-68.

Hoch G., Zubrik M., Novotny J., Schopf A., 2001. The natural enemy complex of the gypsy moth *Lymantria dispar* (Lep., Lymantriidae), in different phases of its population

dynamics in eastern Austria and Slovakia – a comparative study. Journal of Applied Entomology 125, 217-227.

Jervis M., Kidd N., 1996. Insect natural enemies. Chapman & Hall. 491 pp.

Kalbacher G., 2008. Untersuchungen zum Parasitoidenkomplex des Schwammspinners *L. dispar* (Lep: Lymantriidae) in seiner Progradations- und Kulminationsphase. Master thesis. BOKU, Wien, 69 pp.

LaSalle J., 1993. Parasitic Hymenoptera, Biological Control and Diversity. CAB International. 197-215 pp.

Leonard D. E., 1981. Bioecology of the Gypsy Moth. In C.C. Doane & M.L. Mc Manus (eds.), The gypsy moth: Research toward Integrated Pest Management. U.S.D.A., Technical Bulletin 1584: 599-633.

Liu H., Ueno T., 2012. The importance of food and host on the fecundity and longevity of a host-feeding parasitoid wasp. Journal of the Faculty of Agriculture Kyushu University 57: 121-125.

Malina R., Praslička J., 2008. Effect of temperature on the developmental rate, longevity and parasitism of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). Plant Protection Science 44: 19-24.

Marktl R.C., Stauffer C., Schopf A., 2002. Intraspecific competition between the braconid endoparasitoids *Glyptapanteles porthetriae* and *G. liparidis* in *Lymantria dispar* larvae. Entomologia Experimentalis et Applicata 105: 97-109.

Marschnig M., 2013. Development of the braconid wasp *Glyptapanteles liparidis* (Hymenoptera: Braconidae) in larvae of the brown-tail moth *Euproctis chrysorrhoea* (Lepidoptera; Noctuidae, Lymantriinae). Master thesis. BOKU, Wien. 55 pp.

Massachusetts, State forester, 1910. Parasites of the gypsy and brown-tail moths introduced into Massachussets. Where they come from. What they are doing. A general survey of the work.

Matthews R.W., 1974. Biology of Braconidae. Annual Review of Entomology 19: 15-32.

McManus M., Csóka G., 2007. History and Impact of Gypsy Moth in North America and Comparison to Recent Outbreaks in Europe. Acta Silvatica & Lignaria Hungarica, 3: 47-64.

Milanović S., Lazerević J., Popović Z., Miletić Z., Kostić M., Radulović Z., Karadžić D., Vuleta A., 2014. Preference and performance of the larvae of *Lymantria dispar* (Lepidoptera: Lymantriidae) on three species of European oaks. European Journal of Entomology 111: 371-378.

Moerkens R., Gobin B., Peusens G., Helsen H., Hilton R., Dib H., Suckling D.M., Herwig L., 2011. Optimizing biocontrol using phenological day degree models: the European earwig in pipfruit orchards. Agricultural and Forest Entomology 13: 301-312.

Nahrung H.F., Allen G.R., Patel V.S., 2004. Day-degree development and phenology modelling of the immature stages of *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae), a pest of eucalypt plantations. Australian Journal of Entomology 43: 177-183.

Nierhaus-Wunderwald D., Wermelinger B., 2001. Der Schwammspinner (*Lymantria dispar* L.) Merkblatt für die Praxis. WSL Birmensdorf. 8 S.

Nussbaumer C., Schopf A., 2000. Development of the solitary larval endoparasitoid *Glyptapanteles porthetriae* (Hymenoptera: Braconidae) in its host *Lymantria dispar* (Lepidoptera: Braconidae). European Journal of Entomology 97: 355-361.

Quicke D. L. J., 1997. Parasitic wasps. Chapman & Hall. 470 pp.

Schafellner C., Marktl R.C., Nussbaumer C., Schopf A., 2004. Parasitism-induced effects of *Glyptapanteles liparidis* (Hym: Braconidae) on the juvenile hormone titer of its host, *Lymantria dispar*: the role of the parasitoid larvae. Journal of Insect Physiology 50: 1181-1189.

Schafellner C., Marktl R.C., Schopf A., 2007. Inhibition of juvenile hormone esterase activity in *Lymantria dispar* (Lepidoptera, Lymantriidae) larvae parasitized by *Glyptapanteles liparidis* (Hymenoptera, Braconidae). Journal of Insect Physiology 53: 858-868.

Schläger S., 2010. Effects of parasitization by *Glyptapanteles liparidis* on morphology and reaction of haemocytes of *Lymantria dispar*. Master thesis. BOKU, Wien. 65 pp.

Schopf A., 1991. The effect of the host age of *Lymantria dispar*-larvae (Lepidoptera: Lymantriidae) on the development of *Glyptapanteles liparidis* (Hymenoptera: Braconidae). Entomophaga 36: 593-604.

Schopf A., Steinberger P., 1996. The influence of the endoparasitic wasp *Glyptapanteles liparidis* (Hymenoptera: Braconidae) on the growth, food consumption, and food utilization of its host larvae *Lymantria dispar* (Lepidoptera: Lymantriidae). European Journal of Entomology 93: 555-568.

Schopf A. 2007. Parasitoide – halb Parasit, halb Räuber. Wie kleine Schlupfwespen große Schwammspinner-Raupen gefügig machen. Biologie in unserer Zeit 37: 290-298.

Seyahooei M.A., Van Alphen J.J.M., Kraaijeveld K., 2011. Metabolic rate affects adult life span independently of developmental rate in parasitoid wasps. Biological Journal of the Linnean Society 103: 45-56.

Shaw M.R., 2006. Habitat considerations for parasitic wasps (Hymenoptera). Journal of Insect Conservation 10: 117-127.

Shaw M.R., Hochberg M.E., 2001. The neglect of parasitic Hymenoptera in insect conservation strategies: The British fauna as a prime example. Journal of Insect Conservation 5: 253-263.

Spanoudis C. G., Andreadis S.S., 2011. Temperature-dependent survival, development and adult longevity of the koinobiont endoparasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae) parasitizing *Plodia interpunctella* (Lepidoptera: Pyralidae). Journal of Pest Science 85: 75-80.

Strand M.R., 2002. The interactions between larval stage parasitoids and their hosts. The behavioural ecology of parasites. CAB International. 358 pp.

Wade M.R., Wratten S.D., 2007. Excised or intact inflorescences? Methodological effects of parasitoid wasp longevity. Biological Control 40: 347-354.

Wu T.H., Shiao, S.F., Okuyama T., 2015. Development of insects under fluctuating temperature: a review and case study. Journal of Applied Entomology 139: 592-599.