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Coexistence of competing parasitoids: which is the fugitive and where does it hide?

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Most insects harbour a community of parasitoids that coexist in spite of competition for resources. One potential mechanism for coexistence of competitors is a tradeoff between dispersiveness and local competitive ability. Here we present a study of competition between the specialized parasitoids *Hyposoter horticola* and *Cotesia melitaearum* sharing the Glanville fritillary butterfly, *Melitaea cinxia*. Within one host generation, the parasitoid larvae interact inside the host during each of the three *C. melitaearum* generations. We founds that in the summer when the host is small, the solitary *H. horticola* is the superior competitor, suppressing the gregarious *C. melitaearum* as eggs or small larvae. When multiparasitism occurs in the autumn the two parasitoid species engage in physical combat and *C. melitaearum* is favoured. Finally, a previous study showed that in the third *C. melitaearum* generation the univoltine *H. horticola* grows quickly during its final instar, excluding young *C. melitaearum* simply through limited time and resources. We found that contrary to expectations of the evolution of gregariousness, *C. melitaearum*, which lives in sibling groups, has biting mandibles in the first instar while the solitary *H. horticola* has suctorial mouthparts. Previous studies suggest that the two parasitoids co-exist because *H. Horticola* is dispersive and *C. melitaearum* is a strong local competitor. However, putting together the results of this experiment and out recent understanding of the adult wasp foraging behaviours and large scale population dynamics, we conclude that *H. horticola* is both a superior local competitor and more dispersive than *C. melitaearum. Cotesia melitaearum* has no impact on the population dynamics of *H. horticola*, persisting as a fugitive using a small fraction the larvae left unparasitized by *H. horticola*.

Parasitoids compete directly with other parasitoid species for hosts. Some species may prevail over others, resulting in competitive exclusion, as had been demonstrated in biological control introductions (Luck and Podoler 1985, Schellhorn et al. 2003, Mills 2006), invasions (Henneman and Memmott 2001), and experiments (Bográn et al. 2002). Nevertheless, multiple parasitoid species generally persist together (Hawkins 2000, van Nouhuys and Hanski 2005) through neutral processes or by partitioning the host resource.

Different modes of host resource partitioning occur over a range of spatial and temporal scales. At a landscape scale, competing parasitoids that are equally dispersive may co-exist if the habitat is made up of independent ephemeral patches (Hanski 1981). Competitors may more easily share a host at a landscape scale if one species is mobile and the other is a superior local competitor. Then the dispersive species persists, as a fugitive, in host patches yet to be discovered by the sedentary species (Hutchinson 1951). This is well established theoretically (Holt 1997, Yu and Wilson 2001, Amarasekare 2003) and has been observed to occur among competing plant species (Tilman 1994), and to some extent among animals (Hanski and Ranta 1983, Yu et al. 2004). It has been explored using parasitoids (Lei and Hanski 1998, Amarasekare 2000, Pelosse et al. 2007) but has not been shown definitively to occur.

Where parasitoid species occur together, and use the same host lifestage, individuals experience interspecific competition as adult females or while developing in or on a host. Many species of parasitoids avoid hosts parasitized by conspecifics. However, it is less common for adult females to avoid hosts parasitized by heterospecifics (Turlings et al. 1985, van Alphen and Visser 1990, Lann et al. 2008). Similarly, direct conflict has been observed among conspecific adult parasitoids (Goubault et al. 2005), but not between heterospecific adult females. Instead, with the exception of ovicide (Collier et al. 2007) direct competitive interaction occurs between the heterospecific immature parasitoids in or on the host (Godfray 1994).

When multiple adult parasitoids attempt to parasitize a single host individual, only a single larva of solitary parasitoids or a fraction of the larvae of gregarious parasitoids successfully develop to adulthood. The extra parasitoid individuals die as eggs or larvae, primarily as a result of contest or resource competition (Salt 1961,

Godfray 1994) and physiological suppression (Quicke 1997). The outcome of larval competition between parasitoid species depends on their relative morphologies (primarily if they have fighting mandibles or not), physiological interactions with the host, mobility inside the host, as well as on the relative timing of parasitism and rates of development. For the most part, the resident larval parasitoid kills those that arrive later (Marktl et al. 2002), mobile parasitoids kill less mobile ones (Marris and Casperd 1996, Pexton and Mayhew 2001), and those with weapons such as mandibles (Harvey and Partridge 1987), armoured caudal appendages (Chow and MacKauer 1984), or physiological defence (Fisher 1961) kill those without. However, when species with different life histories compete the outcome is not easily predicted (Bonsall et al. 2002).

The parasitoid wasps *Cotesia melitaearum* (Hymenoptera: Braconidae) and *Hyposoter horticola* (Hymenoptera: Ichneumonidae) share the host butterfly, *Melitaea cinxia* (Lepidoptera: Nymphalidae), in the Åland islands in Finland. As an adult *H. horticola* is mobile, moving easily among local host populations at a large spatial scale. There is strong intraspecific competition among adult female *H. horticola*, independent of host density (van Nouhuys and Ehrnsten 2004, van Nouhuys and Kaartinen 2008). *Cotesia melitaearum* on the other hand, is sedentary and constrained to aggregated local host populations (Lei and Camara 1999, van Nouhuys and Hanski 2002, Kankare et al. 2005b). It has a high potential rate of increase because it is multivoltine and gregarious (lays multiple eggs in a host) and it responds numerically to local host population size (van Nouhuys and Tay 2001, van Nouhuys and Lei 2004). The pattern of occurrence and some of the behaviour of these two wasps suggests that they are able to coexist because *H. horticola* is dispersive, and *C. melitaearum* is a superior competitor locally. This was suggested by Lei and Hanski (1998), based on analysis of perceived local and regional distribution in natural populations. Here we revisit the hypothesis after gathering more information.

We present a factorial laboratory experiment and set of dissections to assess the mode and outcome of competition between immature *H. horticola* and *C. melitaearum* inside of the host larvae. The performance of each wasp species with and without competition is compared in terms of rate of successful parasitism and weight of adult progeny. For *C. melitaearum*, which experiences both interspecific and intraspecific competition, we also measured the effect of interspecific competition on brood size. We dissected singly and multiply parasitized host larvae in order to observe the phenology of the parasitoids, the stages of development in which they interact, and evidence of physical combat or encapsulization. In addition, since parasitism can cause hosts to die before parasitoids are fully developed, we analyzed the effects of multiparasitism on premature host mortality.

In order to address the hypothesis that the wasps coexist through a tradeoff between local competitive ability and dispersal, their interaction must be evaluated both while they are within the host (as eggs or larvae), and as adults outside the hosts at local and regional scales. The results of our experiment on larval competition are discussed in combination with previous studies using the same research system. These are: an experimental study of the third annual episode of competition (Fig. 1) (van Nouhuys and Tay 2001), behaviour of the adult wasps as a local scale (Lei and Camara 1999, van Nouhuys and Ehrnsten 2004, van Nouhuys and Kaartinen 2008) and the regional population dynamics of the wasps (van Nouhuys and Hanski 2002).

Material and methods

The host butterfly

The Glanville fritillary butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae) inhabits small meadows as a classical metapopulation the Åland islands, Finland (Nieminen et al. 2004). The butterfly is univoltine. Adults fly in June, and lay eggs in clusters of 100 to 200 on the host plants *Plantago lanceolata* and *Veronica spicata* (Plantaginaceae). After hatching the larvae live gregariously in silken nests on the host plants throughout their development, including a winter diapause, until they disperse to pupate in the leaf litter mid May of the following year (Kuussaari et al. 2004).

The parasitoids

There are two primary parasitoids of *M. cinxia* larvae in the Åland islands. *Cotesia melitaearum* is a taxonomic aggregate of cryptic parasitoid species associated with checkerspot butterflies in the genera *Euphydryas* and *Melitaea* (Kankare and Shaw 2004). The taxon of *C. melitaearum* (agg.) that uses *M. cinxia* has no other host species (Kankare et al. 2005a). Larvae of *C. melitaearum* pass through two to three generations during one host generation (year) (Fig. 1). The summer generation develops in small, 1st to 3rd host instars. Adults of this generation then oviposit into 4th or 5th instar host larvae just before diapause. The autumn generation diapauses as larvae inside of the host larvae, pupates the following spring, and oviposits into large 6th instar hosts (Lei et al. 1997, van Nouhuys and Lei 2004). *Cotesia melitaearum* lay gregarious clutches of eggs in hosts. The brood size of the summer generation is 1 or 2, the autumn generation is 2 to 4, and the spring generation is 15 to 30 (Lei et al. 1997).

The population dynamics of *C. melitaearum* are unstable. The wasp only occupies a small fraction of local *M. cinxia* populations (5–10%), and the rate of parasitism within local populations is generally low. In summer typically less than 2% of the hosts are parasitized. This increases to 4 to 9% in the autumn (overwintering) generation. The natural parasitism rate of the third generation is variable and difficult to measure but can, under the right conditions, reach 90% of host larvae (Lei et al. 1997, van Nouhuys and Tay 2001) (Table 1).

The other parasitoid, *H. horticola* is a large (body length ~12 mm), mobile, solitary endoparasitoid, which has only one generation per host generation. Female *H. horticola* parasitize young host larvae that have not yet hatched from the egg (van Nouhuys and Ehrnsten 2004). The parasitoid larva develops inside of the host larva until the following spring, when it consumes the entire final instar host and pupates within dried caterpillar

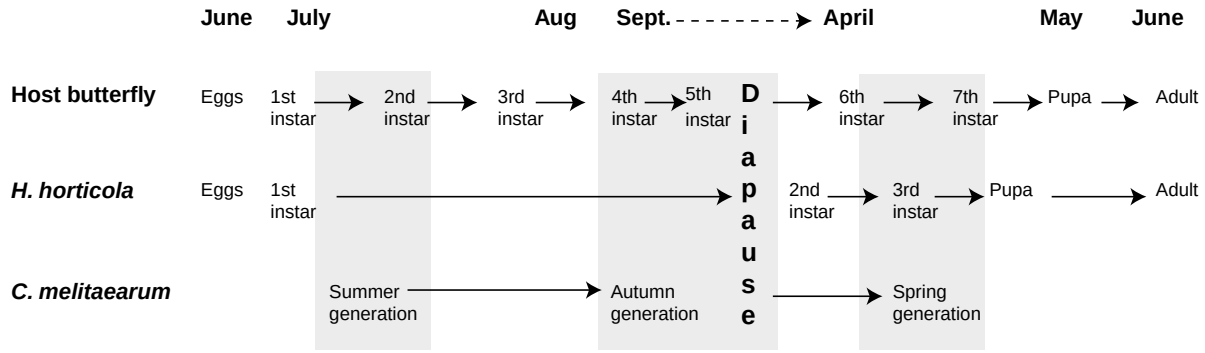

Figure 1. The annual phenologies of the host butterfly, *Melitaea cinxia* (univoltine), and the parasitoids *Hyposoter horticola* (univoltine) and *Cotesia melitaearum* (tri-voltine). The three shaded boxes indicate the summer, autumn and spring episodes of interspecific competition among larval parasitoids.

integument. Adult wasps emerge three to four weeks later (Lei et al. 1997). Unlike *C. melitaearum, H. horticola* has extremely stable population dynamics, occupying virtually all local host populations, and consistently parasitizes about 1/3 of the host larvae in each (van Nouhuys and Hanski 2002, van Nouhuys and Ehrnsten 2004).

The competition experiment

The *M. cinxia* larvae used in the experiment were offspring of butterflies derived from a mixture of populations in the Åland islands. Host larvae parasitized by *H. horticola* were obtained by placing 20 host plants, each with one egg clusters on it, in natural populations during the parasitoid flight season. After parasitism the first instar larvae were brought back into the laboratory. Predators ate six of the clusters, leaving approximately 2000 larvae. The rate of parasitism is reliably about 1/3 of the larvae in each host cluster (van Nouhuys and Ehrnsten 2004), and parasitism of each cluster was confirmed by dissection of several individuals. While the host larvae were still in their first instar we mixed them together and then placed 30 in each of 22 plastic cups. The larvae were reared in cups of 30 because they live gregariously, especially before diapause. The cups were then allotted randomly into three treatments groups (Table 2): eight replicates of *H. horticola* alone (H), seven replicates to be parasitized by summer generation *C. melitaearum* females (H+C1), and seven replicates to be parasitized by autumn generation *C. melitaearum* females (H+C2). At the same time we separated cups of 30 first instar laboratory reared host larvae (unparasitized by *H. horticola*) into two treatments, four replicates to be parasitized by summer generation *C. melitaearum* alone (C1), and four replicates to be parasitized by autumn generation *C. melitaearum* alone (C2) (Table 2). We did not include a treatment of entirely unparasitized larvae.

For the summer generation *C. melitaearum* treatments (H+C1 and C1) the host larvae were parasitized in their second instar, and the autumn generation *C. melitaearum* treatments (H+C2 and C2) were parasitized in their fourth instar. For both treatments we offered individual host larvae to individual laboratory reared, mated *C. melitaearum* females. For the summer generation a total of 28 different *C. melitaearum* females parasitized the host larvae during six days (25–30 July). For the autumn generation 15 *C. melitaearum* females parasitized the host larvae over five days (14–18 August). For each larva, oviposition insertion and behaviour associated with oviposition was observed and we saw no indication that ovipositing *C. melitaearum* females distinguished between previously unparasitised hosts and hosts already parasitized by *H. horticola*. In a previous study dissection confirmed that when ovipositor insertion was observed, eggs had been deposited in both unparasitised larvae and those parasitized by *H. horticola* (van Nouhuys and Tay 2001). Each wasp was observed to parasitize one to three larvae from a cup, and then moved to a different cup. After each wasp had been used for several cups (1 to 3) in a day it was fed and left for the next day. To avoid confounding variation in rate of parasitism among wasps with treatments, we

Table 1. Effect of competition for *H. horticola* and *C. melitaearum,* and rate of parasitism by *C. melitaearum* in natural populations during the three annual *C. melitaearum* generations.

	Summer–C1	Autumn–C2	Spring–C3[1]
H. horticola mortality due to *C. m.* (all hosts parasitized by *C. m.*)	0	50%	0[1]
C. melitaearum mortality due to *H. h.* (mortality in group)*	100% (33%)	50% (17%)**	100% (33%)[1]
C. m. average brood size[2]	1.2	2.5	17.4
C. m. fraction of larvae parasitized in occupied population[2, 3]	0.02	0.07	0.37 (0 to 0.9)
C. m. fraction of populations occupied[4]	0.02 to 0.2 (mean 0.07) over 14 years		

[1]van Nouhuys and Tay 2001; [2]Lei et al. 1997; [3]van Nouhuys and Lei 2004; [4]van Nouhyus unpubl.
*only 1/3 of larvae are parasitized by *H. horticola,* so there is a 66% chance a *C. melitaearum* will encounter and unparasitized host.
**the effect of *H. horticola* on the autumn generation of *C. melitaearum* was not statistically significant in this study. However an estimate of mortality is included here because some *H. horticola* survived, which must have been at the expense of *C. melitaearum*. This inconsistency was probably due to high variability of survival among replicates.

Table 2. Experimental treatments, sample sizes and mortality.

Treatment	Replicates[1]	Dissected live	Dissected dead	Mortality %
H	8	38	81	44.3
H+C1	7	76	81	65.9
H+C2	7	20	98	55.4
C1	4	20	59	75.6
C2	4	20	70	77.8

Treatments (H) parasitized by *H. horticola*, (H+C1) parasitized by *H. horticola* and summer generation *C. melitaearum*, (H+C2) parasitized by *H. horticola* and autumn generation *C. melitaearum*, (C1) parasitized by summer generation *C. melitaearum* only, (C2) =parasitized by autumn generation *C. melitaearum* only.
[1]30 larvae in each replicate treatment.

presented larvae from the different treatments alternately, and randomized the replicate cups within the treatments each cycle. In this way multiple wasps parasitized the larvae in each cup, and each wasp parasitized larvae in cups from both treatments. After parasitism the larvae were kept under ambient laboratory conditions and fed freshly gathered *P. lanceolata* daily.

The larvae spent winter diapause in plastic vials in an outdoor root cellar. After diapause they continued to develop in the laboratory and ultimately pupated into butterflies, yielded *H. horticola* or *C. melitaearum* pupae, or died. The remains of host larvae that had produced gregarious *C. melitaearum* cocoons were dissected and the number and state (intact, wounded or encapsulated) of dead parasitoid larvae was recorded. If the host larva developed into *H. horticola* only the integument remained so it could not be dissected. Host larvae that died before anything pupated from them were dissected to record the presence or absence of either parasitoid. Parasitoid pupae were kept in plastic tubes until adult wasps emerged. Within four hours of adult emergence they were frozen and then dried for 14 days in silica gel, and weighed using a microbalance. The sex of each *C. melitaearum* adult was also recorded.

Statistical analysis

Using a series of logistic regression and ANOVA models we analyzed the association of competition with rate of successful parasitism (survival to pupation), brood size of *C. melitaearum*, adult wasp weight, and premature host mortality.

Rate of successful parasitism

The effect of interspecific competition on survival to pupation was analyzed using logistic regression (SAS PROC GENMOD, SAS Institute Inc. 1999). The two parasitoid species were analyzed separately. For each host larva that did not die prematurely, the presence/absence of a parasitoid cocoon (or cocoons) was analyzed as a function of treatment and cup (replicate), with cup nested in treatment. We analyzed the differences between the treatments further by contrasting the outcome of singly parasitized treatments with double parasitized competition treatments. One cup from control treatment C1 and one from C2 had to be excluded from the analysis because contamination by one *H. horticola* parasitized larvae in each occurred early in the experiment.

Brood size of C. melitaearum larvae

One way in which interspecific competition can influence a gregarious parasitoid is through its effect on the number of siblings successfully developing in a host. We used Poisson regression (SAS PROC GENMOD) to analyze the association between the numbers of *C. melitaearum* pupating per host and the treatment (C1, C2, H+C1 and H+C2). Simultaneous contrasts were conducted in order to distinguish among treatments.

Weight and sex of adult wasps

Competition for resources within the host can cause wasps to be small; for a parasitoid small size is generally associated with low fitness, both in terms of fecundity and longevity (Brodeur and Boivin 2004). We analyzed the effect of interspecific competition on weight of *H. horticola* using ANOVA (SAS PROC GLM) with adult dry weight as the response variable, and treatment and cup nested in treatment as explanatory variables.

Competition can also influence the sex of parasitoid offspring, either because the mother controls the sex of eggs based on the host condition (Godfray 1994), or perhaps by differential mortality between male and female parasitoid larvae. Since *C. melitaearum* is gregarious, it experiences both intraspecific and interspecific competition, both of which may affect weight and sex. Furthermore, weight and sex in the genus *Cotesia* are related to each other because females are larger than males. Therefore, we analyzed the effect of interspecific competition on weight of Cotesia using ANOVA (SAS PROC GLM), with weight as the response variable, and treatment, cup nested in treatment, brood size (for intraspecific competition) and sex as explanatory variables. We also analyzed the association of sex with competition using logistic regression (JMP ver. 7, 2007) with sex as the dependent variable and treatment, cup, and brood size as factors, and weight as an offset covariate. We did not analyze the sex of *H. horticola*.

Premature host mortality

Parasitism is a stress that can cause premature host mortality (Bernstein et al. 2002, Beckage and Gelman 2004, Brodeur and Boivin 2004). Each of the 369 larvae that died during the experiment was dissected and the species, numbers and stages of immature parasitoids found inside was recorded. To understand what factors affected the premature mortality of the hosts overall, we used logistic regression (SAS PROC GENMOD) to analyze

the association of cup, treatment and parasitoid species with premature death of the hosts (died before anything pupated from it/survived to yield a butterfly or parasitoid pupae). Each species was then analyzed separately to find out if the two parasitoid species affected the host mortality differently. In one analysis only the treatments with *H. horticola* were included (H, H+C1 and H+C2), and in the second analysis only the treatments with *C. melitaearum* were included (C1, C2, H+C1 and H+C2). For both analyses we included rearing cup (replicate), treatment, parasitism (presence or absence of each parasitoid species) and the interaction of parasitism and treatment as factors.

Dissections

In addition to the outcome of competition, we were interested in the phenology of the parasitoid larvae, and evidence of the nature of their interactions. Therefore, we dissected parasitized host larvae during the experiment. Larvae from each of the single species parasitism treatments (H, C1 and C2) were dissected just before diapause. Larvae from the H+C1 treatment were dissected seven to ten days after the parasitism by *C. melitaearum*, and larvae from the H+C2 treatment were dissected eleven days after parasitism, which was when the larvae were ready for diapause (Table 2). The numbers of dissected larvae varied among treatments because there was a high rate of mortality in some treatments and we wanted to maintain sufficient group sizes in each replicate treatment. The host larvae were dissected in insect saline under a dissecting microscope. From each dissected host larva we recorded the species, numbers and stages of immature parasitoids found, and whether each parasitoid larva was alive or dead. We dissected additional parasitized larvae (not part of the experimental treatments described above) in order to clarify the development rates and identify basic morphological features of each instar of both parasitoids.

Results

The outcome of competition

The rate of successful parasitism by *H. horticola* was not affected by the summer generation of *C. melitaearum* (H = H+C1; $\chi^2_{1\ DF} = 0.91$, $p = 0.34$), but was negatively effected by the autumn generation of *C. melitaearum* ((H > H+C2; $\chi^2_{1\ DF} = 6.58$, $p = 0.01$) (Fig. 2a). Correspondingly, there was low success of summer *C. melitaearum* using *H. horticola* infected hosts (C1 > H+C1; $\chi^2_{1\ DF} = 8.041$, $p = 0.005$), but no effect of *H. horticola* on the autumn generation of *C. melitaearum* (C2 = H+C2; $\chi^2_{1\ DF} = 0.03$, $p = 0.86$) (Fig. 2b). Within each generation, there was no significant difference between brood sizes of *C. melitaearum* pupating from the singly parasitized treatments and the doubly parasitized treatments (C1 vs H+C1 and C2 vs H+C2), indicating that competing with *H. horticola* did not have an incremental effect on the number of *C. melitaearum* in a brood.

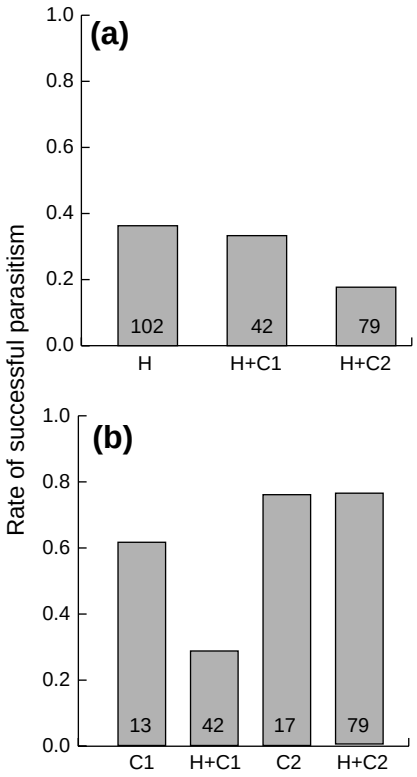

Figure 2. The fraction of host larvae that yielded *Hyposoter horticola* (a) and *Cotesia melitaearum* (b) in each treatment. The numbers in the bars indicate the number of larvae in the sample (those that survived to produce parasitoid pupae or become butterfly pupae). The treatments are *H. horticola* (H), summer (C1) and autumn (C2) *C. melitaearum*, *H. horticola* and summer *C. melitaearum* (H+C1), *H. horticola* and autumn *C. melitaearum* (H+C2).

The effect of competition on weight and sex of the adult wasps

The dry weight of *H. horticola* was relatively uniform (4.2–4.4 mg), and indistinguishable between treatments ($F_{2\ DF} = 1.07$, $p = 0.35$). However, the sample size was small due to mishandling of the *H. horticola* pupae (34 of the 65 pupae died) and there was a slight trend for *H. horticola* from the H+C2 treatment to weigh less than the control (H) treatment. The sex of *H. horticola* was not analyzed.

A total of 221 *C. Melitaearum* were weighed (40 females, 178 males and three individuals of uncertain sex). The dry weights of the wasps varied between 0.148 mg and 0.586 mg. Analysis of variance showed no significant difference in the wasp's weights among treatments ($F_{3\ DF} = 0.68$, $p = 0.27$). As expected, female *C. melitaearum* were heavier than males ($F_{1\ DF} = 44.81$, $p = 0.0001$; Fig. 3). Additionally, adult *C. melitaearum* weight decreased with increasing brood size ($F_{1\ DF} = 4.57$, $p = 0.033$; Fig. 3), indicating that *C. melitaearum* suffer from intraspecific competition. Though there were many more male than female *C. melitaearum*, sex was unrelated to treatment.

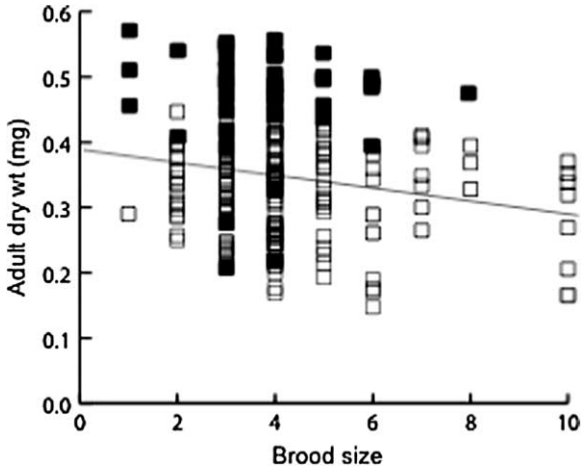

Figure 3. The dry weight of adult *C. melitaearum* as a function of brood size. filled symbols are female and open symbols are male. Weight decreases with increasing brood size. The linear regression line is $y = 0.40 - 01x$, significant at $p = 0.03$.

Premature mortality of the host larvae

About 40% the host larvae died during development, before any parasitoids had pupated. This premature mortality differed among treatments (Table 3). Mortality of host larvae was significantly lower in the *H. horticola* only treatment (H) than in either *C. melitaearum* treatment (C1 and C2), which is not surprising because 2/3 of the larvae in the H treatment were unparasitized. The mortality in treatment H was also lower than in the competition treatment H+C1, but not significantly lower than in the competition treatment H+C2. The mortality among larvae in the C1 treatment was not different than for those in the H+C1 treatment but, surprisingly, the mortality in C2 was higher than in treatment H+C2 (Table 3).

Development of *H. horticola* and *C. melitaearum* inside of host larvae

Cotesia melitaearum eggs were visible in dissected hosts after two days as transparent elongate forms of about 0.25 mm. They grew to about 0.5 mm before hatching.

Table 3. The logistic regression analysis of the premature death of host larvae.

Source	DF	χ^2	$Pr > \chi^2$
Cup(treatment)	21	65.57	<0.0001
Treatment	4	38.12	<0.0001
Parasitism	3	10.11	0.0177
Contrast:			
H vs H+C1	1	9.95	0.0016 (H <H+C1)
H vs H+C2	1	0.33	0.5637
C1 vs H+C1	1	2.41	0.1208
C2 vs H+C2	1	18.18	<0.0001 (C2 >H+C2)
Parasitized vs unparasitized	1	9.21	0.0024
Parasitized by *C.* vs unparasitized	1	6.88	0.0087
Parasitized by *H.* vs unparasitized	1	3.94	0.0470
Doubly parasitized vs unparasitized	1	6.23	0.0125

Newly hatched *C. melitaearum* were about 0.5 mm long and visibly segmented. The head capsule was lightly sclerotized, and the mandibles were visible and mobile. During the first instar a caudal vesicle developed. The second instar larvae were opaque, with a visible yellow gut. The remains of the first larval skin remained as a collar around the caudal vesicle. In the third larval instar the remains of the second larval skin was visible as a second ring around the caudal vesicle. The cast skin from the third instar remained in the host, near where the fourth instar/prepupal parasitoid leaves the host. The fourth instar had cuticular setae and two pigmented eye spots.

Hyposoter horticola eggs could not be observed because the parasitized larvae were brought into the lab after hatching. Dissection of the ovaries of female *H. horticola* reviled 0.2 mm long elongate white eggs. The first instar *H. horticola* was transparent and had a distinctive caudal appendage. There are no visible mandibles and the mouth was suctorial. The *H. horticola* remained in first instar until the host went into diapause in autumn. It grew to about 0.75 mm (+the 0.1 mm caudal appendage). The second instar *H. horticola* did not have a caudal appendage, and was transparent, with a visible yellow gut. The third instar was not easily distinguishable from the second (with our microscope), but was more opaque and grew to be 5 mm long. The *H. horticola* presumably moults to the last instar or prepupa once it has built a cocoon inside the host integument. It is about 6 mm long, white, and had visible pigmented eyes and mouthparts. Photographs of each of these stages of both parasitoid species are in Punju (2002).

Discussion

The outcome of competition between *C. melitaearum* and *H. horticola* depended on when it occurred during development of *H. horticola*. In the summer (C1 and H+C1) *H. horticola* was the superior competitor, excluding all cohabiting *C. melitaearum*. In the autumn (C2 and H+C2) *C. melitaearum* was superior but not completely exclusive. Though competition decreased parasitoid survival, it did not measurably effect parasitoid weight, or the brood size or sex of *C. melitaearum*. Parasitism, especially by *C. melitaearum*, caused significant premature mortality of host larvae.

Rate of successful parasitism by each species

Hyposoter horticola parasitizes the host as a tiny larva still in the eggshell (van Nouhuys and Ehrnsten 2004), which is before *C. melitaearum* can parasitize it. Given that, the outcome of competition primarily depends on when in the lifecycle of *H. horticola* the *C. melitaearum* is introduced (Table 1). Initially (treatment C1), *H. horticola* did not suffer from competition, and the rate of successful parasitism by *C. melitaearum* was reduced from 61 to 27% of the host larvae. Since only 36.3% of the host larvae were parasitized by *H. horticola*, probably all of the *C. melitaearum* laid in second instar larvae parasitized by

H. horticola died. In contrast, during the autumn *C. melitaearum* generation (C2), only half the *H. horticola* in larvae parasitized by *C. melitaearum* survived, and *C. melitaearum* were equally successful with *H. horticola* as alone. Van Nouhuys and Tay (2001) showed that during the final (spring) *C. melitaearum* generation *C. melitaearum* fail to develop in larvae parasitized by *H. horticola*. Thus, in two out of the three opportunities for competition each host generation, *H. horticola* is the superior competitor.

The causes of parasitoid mortality at each of these three stages differ. Early on (treatment H + C1) *H. horticola* is a small first instar larva and the cause of mortality of the *C. melitaearum* is unclear. However, when we dissected larvae we found a few cases of both parasitoids alive, and a few where *H. horticola* was alive and the *C. melitaearum* were dead. These dead *C. melitaearum* larvae did not show any melanized scars or other marks of physical combat, suggesting that the death had occurred because of some physiological mechanism. For instance, host immune response may be heightened by the present of *H. horticola*, or *C. melitaearum* may become susceptible to host defence under interspecific competition (Brodeur and Boivin 2004, Roberts et al. 2004, Dorn and Beckage 2007). This physiological suppression could also have occurred when *C. melitaearum* was in the egg stage as well, which we would not have detected by dissection.

In competition between the autumn generation of *C. melitaearum* and *H. horticola* (treatment H + C2), dead *H. horticola* larvae with melanized marks were found in the dissections of intact hosts and also in the remains of hosts from which *C. melitaearum* had pupated. Though gregariously developing *Cotesia* parasitoids are considered noncombative because they are not siblicidal (Mayhew and van Alphen 1999), we found that *C. melitaearum* do indeed have mobile sclerotized mandibles that are especially visible in the very young first instar larvae. These mandibles are no longer present in the second instar (Punju 2002). Biting with such sickle-shaped mandibles is the most widely reported means of physical combat in parasitoids (Quicke 1997). We observed that throughout their development *H. horticola*, like the congener *H. didymator* (Bahena et al. 1999), have suctorial moth parts, which are unsuited to biting.

Aggressive behaviour is generally though to be lost as a result of evolution of gregariousness (Godfray 1987, Ode and Rosenheim 1998, Mayhew and van Alphen 1999, Pexton and Mayhew 2004). Some gregarious species have not lost their larval aggressiveness, but instead their mobility is reduced and they do not actively seek competitors (Boivin and Baaren 2000, Pexton et al. 2009). These larvae can perhaps still defend themselves against other species that actively seek competitors (Laing and Corrigan 1987, Shi et al 2004). This might be the case in the autumn competition. *Cotesia melitaearum* larvae do appear less mobile than *H. horticola* larvae when observed live (Punju unpubl.). Additionally, the dead *H. horticola* larvae found in the hosts were in their first instar. First instar *H. horticola* have a caudal appendage, which is likely used for swimming inside of the host larva (Marris and Casperd 1996, Bahena et al. 1999). Finally, the natural difference in the brood sizes between the first (brood size of 1 to 2) and second generation (brood size of 4 to 10) of *C. melitaearum* may also be important. To win the competition in the second generation a *H. horticola* larva would have to kill each of the *C. melitaearum* larvae in the host.

Finally, after diapause, when the third generation *C. melitaearum* parasitize the large host larvae in the spring, *H. horticola* always wins (van Nouhuys and Tay 2001). This parasitism occurs when the *H. horticola* is in its 3rd (final) instar and growing rapidly. Soon after parasitism by *C. melitaearum* the entire host is consumed, and the *H. horticola* pupates. There is not sufficient time or resources for development of the large brood (10 to 30 individuals) of *C. melitaearum*.

Premature host mortality

Parasitism can increase the risk of premature mortality of hosts by depleting limited resources or by rendering the host vulnerable to infection (Brodeur and Boivin 2004). When a parasitized host dies the parasitoids developing inside it also die. Forty percent of the *M. cinxia* died before parasitoid or butterfly pupation. Overall 60–70% of the parasitoids died with their hosts. The mortality was highest for host larvae parasitized by *C. melitaearum* (C1 and C2). Interestingly, in the treatments H + C1 and H + C2, where all the hosts were attacked by *C. melitaearum* in the same way as in the treatments C1 and C2, the premature mortality was lower, suggesting that the presence of *H. horticola* reduced premature mortality, probably by killing *C. melitaearum*.

Interspecific and intraspecific competition in *C. melitaearum*

We found no effect of developing in a multiparasitized host on the weight of adult wasps of either species, suggesting that they did not suffer direct competition for resources. Perhaps this is because for the most part the second species died before the winner became resource limited. The gregarious *C. melitaearum* experience both interspecific and intraspecific competition; the outcome of one could affect the outcome of the other. We know that there was intraspecific competition among *C. melitaearum* brood mates because adult weight decreased with brood size, as is common in gregarious parasitoid species (Godfray 1987, Mayhew 1998), and has been shown in another study of *C. melitaearum* (van Nouhuys and Laine 2008). However, brood size was unrelated to the outcome of interspecific competition. This is probably again because mortality due to interspecific competition occurred before intraspecific competition for resources inside the host.

The outcome of interspecific competition in natural populations

For both parasitoids, the consequences of larval competition, whatever the outcome, depends on its importance relative to other factors that explain abundance. *Hyposoter horticola* occupies virtually all host populations, finds

essentially all of the host egg clusters and then lays eggs in about a third of the eggs in each cluster (van Nouhuys and Ehrnsten 2004). In a host population occupied by both wasps, each host larva that is parasitized by *H. horticola* has a roughly 20% chance of being doubly parasitized sometime between oviposition and pupation (estimated from Table 1: 1% chance in the summer + 3% chance in the autumn + 19% chance in the spring). However, because *H. horticola* wins in contest with the first and third generation of *C. melitaearum*, and only half the *H. horticola* die in the second generation competition, there is just a 1.5% chance of being killed by *C. melitaearum*. Moving up to a larger spatial scale, *C. melitaearum* occupy an average of 6% of local host populations in Åland (Table 1) so over the entire area competition with *C. melitaearum* reduces parasitism by *H. horticola* by roughly 0.1% $((0.015 \times 0.06) \times 100 = 0.09)$.

For *C. melitaearum* it is a different story. One third of the host larvae that *C. melitaearum* encounter, regardless of where they are in Åland are parasitized by *H. horticola*. Most of these encounters result in failure for *C. melitaearum*. The effect of mortality due to *H. horticola* on *C. melitaearum* population size is complex because of the difference in brood size and rate of parasitism between generations (Table 1). Furthermore, one can argue that population size during the summer generation is most important because less than 2% of larvae in occupied patches are successfully parasitized, only 1 or 2 eggs are laid in a host, and there is a real risk of local extinction (van Nouhuys and Hanski 2000). On the other hand, there is great potential for increase in population size in the spring when rate of parasitism and brood size is high (Table 1), which is known to affect *C. melitaearum* metapopulation size (van Nouhuys and Lei 2004).

Hyposoter horticola, with approximately 1.5% loss to *C. melitaearum* in patches where the two co-occur, is the superior competitor locally. This result is different than presented by Lei and Hanski (1998), who observed that *H. horticola* pupated from 33% of the larvae in gregarious larval groups in the absence of *C. melitaearum*, but only 18% of larvae in groups that also contained larvae parasitized by *C. melitaearum*. This may be an artefact of the behaviour of the parasitized caterpillars, and method of recording the numbers parasitized. Lei and Hanski (1998) also report that *H. horticola* were more frequently found in smaller and more peripheral gregarious host nests than *C. melitaearum*. The explanation of this pattern was that *H. horticola* find nests after *C. melitaearum*, and choose those that are not occupied. However, *H. horticola* is now known to come first, and inhabit virtually all larval nests, with no pattern in relation to larval group size and location within a patch (van Nouhuys and Hanski 2002, van Nouhuys and Ehrnsten 2004).

Because *H. horticola* is the more dispersive of the two wasps and the superior local competitor, we cannot explain their co-existence as a tradeoff between mobility and local competitive ability. Instead, *C. melitaearum* has little impact on *H. horticola* and persists because *H. horticola* consistently leaves about two thirds of each larval group unparasitized. The refuge for *C. melitaearum* is not spatial or temporal, but due to the foraging behaviour of ovipositing female *H. horticola*.

If *H. horticola* were absent *C. melitaearum* would be 1/3 more successful. This could have a negative impact on the host population (Lei and Hanski 1997). However, it may not because of the strong aggregation of secondary parasitoids in response to high *C. melitaearum* density (van Nouhuys and Hanski 2000, van Nouhuys and Tay 2001). If *C. melitaearum* were absent, ca 0.1% more *H. horticola* would survive to pupation. This, or any other moderate amount of mortality would not have a significant effect on the *H. horticola* population size or its impact on the host because there is an excess of foraging *H. horticola* females, and virtually all host egg clusters are found by the wasp, many by multiple individuals (van Nouhuys and Ehrnsten 2004).

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