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Varying degree of physiological integration among host instars and their endoparasitoid affects stress-induced mortality

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Abstract

In natural populations of insect herbivores, genetic differentiation is likely to occur due to variation in host plant utilization and selection by the local community of organisms with which they interact. In parasitoids, engaging in intimate associations with their host during immature development, local variation may exist in host quality for parasitoid development. We compared the development of a gregarious endoparasitoid, *Cotesia glomerata* L. (Hymenoptera: Braconidae), collected in The Netherlands, in three strains and three caterpillar instars (L1–L3) of its main host, *Pieris brassicae* L. (Lepidoptera: Pieridae). Hosts had been collected in The Netherlands and France, and were reared in the laboratory for one generation. We also used an established Dutch laboratory strain that had not been exposed to parasitoids for at least 24 generations. Parasitoid survival to adulthood was inversely correlated with host instar at parasitism. Adult parasitoid body mass was largest when hosts were parasitized as L1 and smallest when hosts were parasitized as L3, whereas egg-to-adult development time was quickest on L3 hosts and slowest on L1 hosts. Higher survival and faster development of *C. glomerata* on French L2 hosts also showed that there is variation in host-instar-related suitability. Many L2 and most L3 caterpillars that were parasitized exhibited signs of pathogen infection and perished within a few days of parasitism, whereas this never happened when hosts were parasitized as L1 or in non-parasitized control caterpillars. Our results reveal that, irrespective of the host strain, L1 hosts are optimally synchronized with *C. glomerata* development. By contrast, the high precocious mortality of L3 larvae may be due to stress-induced regulation by the parasitoid in order to 'force' its developmental program into synchrony with the developing parasitoid larvae. Our results underscore a potentially important role played by pathogens in mediating herbivore–parasitoid interactions that are host-instar-dependent in their expression.

Introduction

The quality and quantity of resources are well known to affect the performance of consumers up the food chain that exploit them. For example, plants that differ in amounts of nutrients or toxic secondary metabolites can have variable effects upon the performance of herbivores that depend on them for resources (Awmack & Leather, 2002; Schoonhoven et al., 2005). Similarly, some types of prey are nutritionally of higher quality for predators than other types of prey (Rickers et al., 2006; Wilder et al., 2010; Ruehl & Trexler, 2013). Parasitoid wasps (Hymenoptera) are model organisms for studying resource-related constraints on development and fitness. They are

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insects whose larvae develop on or in the bodies of other insects (the host), whereas the adults are free-living (Godfray, 1994). Unlike most herbivores and predators, parasitoids are dependent on a small parcel of resources contained in individual hosts that are often not much larger than the adult parasitoid (Harvey, 2005). Consequently, this group of insects is under extremely strong selection pressure for the optimal utilization and allocation of resources to different and potentially competing fitness functions such as reproduction and survival.

Survival and development of parasitoids is known to depend on various characteristics of the host, such as its size or stage at parasitism and its nutritional status that is often mediated by host diet (Vinson & Iwantsch, 1980b; Mackauer & Sequeira, 1993; Godfray, 1994; Harvey, 2005; Ode, 2006; Gols & Harvey, 2009). Host-related traits that affect host quality, such as growth rate and size, may vary across host populations at the landscape scale (Bukovinszky et al., 2008), although thus far this has been little studied. In the host, there is also, conversely, strong selection to prevent successful parasitism (Kraaijeveld & Godfray, 1997). Hosts therefore have evolved a wide array of mechanisms to avoid parasitism including the expression of behavioral, morphological, and immunological defenses (Gross, 1993; Strand & Pech, 1995). Immunity against parasitism relies to a large extent on the ability of the host to encapsulate the parasitoid egg(s) (Godfray, 1994) and this ability is known to vary in some associations depending on host stage/age at parasitism (Bukovinszky et al., 2009) and among populations of the host species (Kraaijeveld et al., 1998).

One large group of parasitoids, collectively termed 'koinobionts', attacks hosts that continue feeding and growing during parasitoid development (Askew & Shaw, 1986). For koinobionts, the host represents a potentially dynamic resource that may vary considerably in size between oviposition and when it is killed by the developing parasitoid larva(e). Many koinobiont parasitoids are not restricted to attacking a single host stage, but can parasitize a range of host stages, such as different instars of their hosts. These hosts may differ profoundly in several aspects, such as the potency of their immune responses (Strand & Pech, 1995), the amount of resources available to the parasitoid offspring (Sequeira & Mackauer, 1992; Harvey et al., 1994, 2004), and the degree of physiological integration between the host and the parasitoid (Lawrence, 1990).

The prevailing theory in parasitoid–host interactions is that larger hosts are of higher 'quality' than smaller hosts because adult parasitoid size is very often positively correlated with host size at parasitism (Charnov, 1982; King, 1989; Mackauer & Sequeira, 1993; Godfray, 1994). Body size is often suggested as the primary force in selection because it is positively correlated with important demographic parameters that contribute to fitness such as fecundity, longevity, mate-finding, and dispersal capability and is referred to as the 'size-advantage hypothesis' (Mackauer & Sequeira, 1993). However, for koinobionts, the relationship between initial and terminal host size vs. adult parasitoid size is not always well defined. Ultimately what often determines parasitoid size is the size of the host when it is destroyed by the parasitoid larvae (Cloutier et al., 1991). Some koinobiont parasitoids attack tiny, nutritionally inadequate hosts and do not arrest host growth until it has reached a critical size or stage that optimizes the amount of resources available to the parasitoid progeny (Smilowitz & Iwantsch, 1973; Harvey et al., 1994, 2000; Harvey & Strand, 2002). Despite this, most studies with koinobionts have indeed found that host size at oviposition is usually correlated with emerging adult parasitoid size (Mackauer & Sequeira, 1993; Godfray, 1994; Harvey, 2005).

In contrast with theory and the results of most studies, a few studies with koinobionts have found that adult parasitoid size is inversely correlated with host size at oviposition over all host instars compared (Harvey et al., 1999; Harvey, 2000). This developmental pattern is difficult to explain, but several factors could be involved. Later instars of many holometabolous insects have much more potent physiological defenses than early instars (Brodeur & Vet, 1995; Rantala & Roff, 2005; Bukovinszky et al., 2009). This may result in a trade-off between overcoming immunosuppression vs. growth and, ultimately, body size. Development of some koinobionts is also much better physiologically integrated with their growing hosts when they lay their eggs in early rather than in late instars. This means that parasitism inflicts less physiological stress when hosts are parasitized as early compared to later instars, and that the parasitoid larvae conform more easily to the developmental program of their hosts (Lawrence, 1990).

Thus far, little attention has been paid to koinobiont parasitoid development when concomitantly reared on various instars and populations of the same host species. The aim of this study was therefore to compare development of the gregarious koinobiont endoparasitoid *Cotesia glomerata* L. (Hymenoptera: Braconidae) in different strains of its preferred host, the large white cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae). Previous work has shown that *C. glomerata* tends to prefer neonate larvae for parasitism over progressively older hosts (Pashalidou et al., 2015), which might be based on the strength of host defenses which increase with host instar (Brodeur & Vet, 1995). Moreover, when *C. glomerata* parasitizes first-instar (L1) host caterpillars, the offspring

develops slower but develops into larger adults than when wasps parasitize second-instar (L2) and third-instar (L3) caterpillars (Harvey, 2000). Here we compared development time and adult biomass of *C. glomerata* in hosts that were parasitized when they were L1–L3 caterpillars. Two wild *P. brassicae* strains were used, one collected in The Netherlands and one in France that had been reared in the laboratory for one generation. In addition, we used our laboratory strain that had been reared for at least 24 generations in the laboratory and had not been exposed to parasitism during this period. We expected that successful parasitoid development would decrease with host instar, but that this relationship would not differ depending on host strain.

Our results suggest that *C. glomerata* adjusts its growth more passively when parasitizing young hosts, whereas the costs associated with increased host regulation when parasitizing older hosts induce stress that leads to precocious host (and parasitoid) mortality. Possible factors responsible for this mortality are discussed.

Materials and methods

Plants and insects

Brussels sprout plants (*Brassica oleracea* L. var. *gemmifera* cv. Cyrus, Brassicaceae) were used as experimental plants and as food plants in the insect cultures. Plants were grown from seeds in potting soil 'no. 4' (Lentse Potgrond, Katwijk, The Netherlands) and were 5–6 weeks old when used in the experiments. Insects in the experiments were reared on excess food. Plants were grown in a greenhouse at 18–26 °C, 50–70% r.h., and L16:D8 photoperiod. Day light was supplemented with SON-T lights (500 μmol photons m^2 s^{-1}; Philips Lighting, Eindhoven, The Netherlands).

Larvae of *P. brassicae* are specialist feeders on brassicaceous plants. The *P. brassicae* culture maintained at Wageningen University (referred to as LAB) originated from cabbage fields growing in the vicinity of the university and had been reared in the laboratory for several years. In addition to using caterpillars from the general culture, we collected larvae from black mustard (*Brassica nigra* L.) plants growing near Wageningen, The Netherlands (referred to as NL) and from cabbage plants in Rennes, France (referred to as FR). These caterpillars were reared for one generation on Brussels sprout plants in the laboratory before they were used in the experiment.

Young *P. brassicae* instars (L1–L3) are the preferred host stages for parasitism by *C. glomerata* (Brodeur et al., 1996). When the parasitized host reaches the final fifth instar, the parasitoid larvae are full-grown and egress from the host and form tight cocoon clusters. *Cotesia glomerata* used in this study were obtained from the general culture that originated from cabbage fields near the university but had been reared in the laboratory for less than 10 generations on *P. brassicae*. When adults emerged from cocoons they were maintained in Bugdorm insect cages (30 × 30 × 30 cm; MegaView Science, Talchung, Taiwan) to allow mating. Wasps were provided with water and 10% sugar water. Females used for parasitism were 3–7 days old. Insects were maintained at 50–70% r.h. and L16:D8 photoperiod in climate rooms (22 ± 1 °C) or greenhouses (22 ± 2 °C).

Experimental design

We compared the development of *C. glomerata* in three host-age classes (L1–L3) for each of the three *P. brassicae* lines (LAB, NL, and FR). Thus, in total nine parasitism groups were compared. Caterpillars of the various age classes used for parasitism had molted the day prior to the experiments. For parasitism, caterpillars were presented to a single *C. glomerata* female in a small plastic vial (3 × 6 cm). Parasitism was considered successful when the female inserted her ovipositor for at least 10 s in the host caterpillar. As the number of eggs laid declines when the wasp is parasitizing multiple hosts in short sequence, females were used no more than 7×. Fifty caterpillars were parasitized for each age-class by host strain combination, which, after parasitism, were equally divided over two cages (40 × 40 × 55 cm). Caterpillars were provided with Brussels sprout plants as food. The 18 cages (three age classes × three host strains × two replicates) were randomly distributed in a climate room maintained at 25 ± 1 °C, 50–70% r.h., and L16:D8 photoperiod. Parasitized caterpillars were allowed to move and feed freely within a cage until parasitoid larvae egressed from their host and pupated. Cocoon broods were collected and placed in 9.5-cm Petri dishes, one brood per dish. When adult wasps emerged, the day of emergence and their sex were recorded. Petri dishes were checked several times per day for wasp eclosion. Eclosed wasps were killed by freezing. Up to the first 10 wasps of each sex that emerged per brood were weighed to the nearest μg on a CP2P microbalance (Sartorius, Göttingen, Germany) to determine their dry mass. As controls, two groups of 25 non-parasitized neonate caterpillars of each line were placed in cages with Brussels sprout plants as food, and their survival to pupation as well as their pupal (fresh) mass were recorded.

Statistical analysis

To analyze the effect of host stage at parasitism on development and biomass of the parasitoid wasps, we used a linear mixed model as there were both fixed and random sources of variation. The experimental unit was a cage in

which 25 parasitized caterpillars were released and there were two cages of each host instar-line combination. Host stage (three levels) and host strain (three levels) and their interaction term were entered as fixed factors, whereas the variation between the two cages and the variation among the host caterpillars within each cage were modeled as random effects. The response variables in the analyses were development time and adult fresh mass, which were analyzed separately for males and females. As biomass of the adult *C. glomerata* wasps is negatively correlated with the number of developing parasitoid larvae in a host (Harvey, 2000), the number of wasps emerging from a host was entered as a covariate in the statistical model. If any of the main effects was significant, pairwise differences among factor levels were determined with Tukey–Kramer-corrected least significant difference (LSD) tests. The effects of host age at parasitism and host strain on survival to adulthood were analyzed with a generalized linear model with a binomial distribution and logit link function. The response variable in the analysis was the number of hosts out of 25 producing wasps in each cage. Data were analyzed in SAS v.9.3 (SAS Institute, Cary, NC, USA).

Results

To determine whether there was any difference in survival or growth among caterpillars of the three lines, groups of healthy caterpillars were reared to adulthood under the same conditions as the parasitized ones. Survival of non-parasitized caterpillars varied between 94 and 100% for the three lines. Pupal mass of *P. brassicae* did not differ among the three lines ($F_{2,2.9} = 3.21$, $P = 0.18$).

Out of the 450 host caterpillars that were parasitized, only three developed into butterfly pupae. The other caterpillars produced wasps or died. Host stage at parasitism strongly affected survival to adult wasp eclosion ($\chi^2 = 72.6$, d.f. $= 2$, $P<0.001$, Figure 1). When hosts were parasitized as L1, on average 92% of the parasitized caterpillars produced adult *C. glomerata*. The interaction between host stage at parasitism and host strain was also significant ($\chi^2 = 25.5$, d.f. $= 2$, $P<0.001$). With the exception of French hosts parasitized as L2 (in which survival was similar as in L1 hosts), of the hosts parasitized as L2 and L3 on average only 27% produced adult wasps. Parasitized hosts that did not survive until parasitoid egression exhibited signs of pathogenic infection, with precocious death followed by blackening and desiccation. Some of these caterpillars were collected and stored in the freezer for further analysis (see Discussion).

Brood sizes–i.e., the number of cocoons developing from a single host–were highly variable. These varied with instar at parasitism ($F_{2,235} = 7.16$, $P = 0.001$) and also

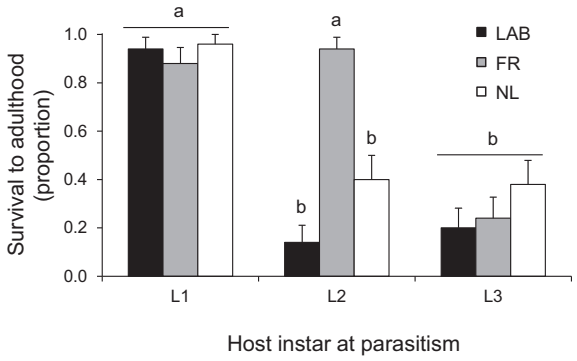

Figure 1 Mean (+ SE) survival of *Cotesia glomerata* to adulthood when they had parasitized different instars (L1–L3) of *Pieris brassicae*. Parasitoid survival is determined as the number of hosts that produced adult wasps out of the 25 hosts that were initially parasitized and is presented as the mean proportion based on two replicate experiments. Hosts originated from the Wageningen laboratory strain (LAB), or were collected from the field near Wageningen (NL) or Rennes, France (FR), and were reared in the laboratory for one generation. Means capped with different letters are significantly different (LSD tests: $P<0.05$).

the interaction between strain and replicate was significant ($F_{2,235} = 5.57$, $P = 0.004$). Brood sizes were larger or equal in hosts parasitized as L2 than in hosts parasitized as L1 or L3 (Figure 2). Adult mass correlated negatively with brood size and this was significant for both females and males [females: $F_{1,216} = 18.2$, $P<0.001$, adult mass (mg) $= 1.489–0.0056 \times$ brood size; males: $F_{1,236.9} = 19.3$, $P<0.001$, adult mass $= 1.187–0.0044 \times$ brood size]. Sex ratios were highly female-biased (median: 63.5% females, interquartile range: 56.0–80.5%). Here all-male broods were excluded ($n = 23$) as unmated females will only produce male offspring. Corrected for brood size, adult mass of both female and male *C. glomerata* wasps was affected by host stage at parasitism (females: $F_{2,6.62} = 18.3$, $P = 0.002$; males: $F_{2,7.83} = 55.5$, $P<0.001$). Overall, wasps were >30% heavier when they had parasitized L1 and L2 hosts than when they had parasitized L3 hosts (Figure 3). Though the patterns were similar for both sexes, only for males was there also an interactive effect of host strain and host stage at parasitism (females: $F_{4,6.32} = 2.8$, $P = 0.12$; males: $F_{4,6.91} = 6.17$, $P = 0.02$). When *C. glomerata* developed on the laboratory and the French *P. brassicae* strain, the mass of male wasps was similar in hosts parasitized as L1 and L2 and lower in hosts parasitized as L3 (Figure 3B). However, in the Dutch *P. brassicae* field line, adult mass of the wasps was lower in hosts that were parasitized as L2 and L3 than when hosts were parasitized as L1.

Both host stage at parasitism and host strain affected development time of female (host stage: $F_{2,7.93} = 13.6$,

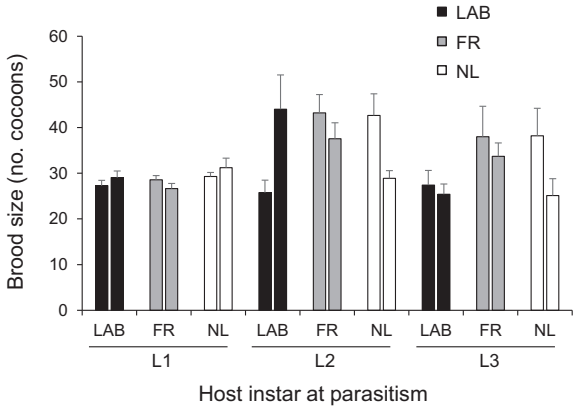

Figure 2 Mean (+ SE) brood sizes of *Cotesia glomerata*, i.e., the number of cocoons developing from a single host, when hosts were parasitized as first (L1), second (L2), or third (L3) instar caterpillars 1 day after molting. Of each developmental stage, 25 parasitized caterpillars were maintained together in a single cage with two cages per developmental stage. Adjacent bars give the mean values for the two replicates. Hosts originated from the Wageningen laboratory strain (LAB), or were collected from the field near Wageningen (NL) or Rennes, France (FR), and were reared in the laboratory for one generation.

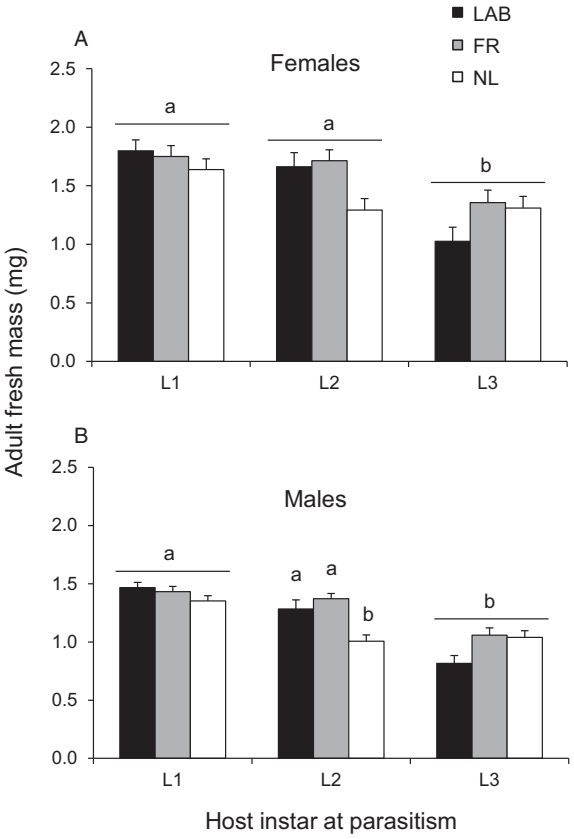

Figure 3 Mean (+ SE) adult fresh mass (mg) of (A) female and (B) male *Cotesia glomerata* when hosts were parasitized as first (L1), second (L2), or third (L3) instar caterpillars 1 day after molting. Of each developmental stage, 25 parasitized caterpillars were maintained together in a single cage with two cages per developmental stage. Hosts originated from the Wageningen laboratory strain (LAB), or were collected from the field near Wageningen (NL) or Rennes, France (FR), and were reared in the laboratory for one generation. Means within a panel capped with different letters are significantly different (LSD tests: $P<0.05$).

$P = 0.003$; host strain: $F_{2,7.95} = 8.31$, $P = 0.01$; Figure 4A) and male *C. glomerata* (host stage: $F_{2,7.27} = 19.4$, $P = 0.001$; host strain: $F_{2,7.28} = 7.42$, $P = 0.02$; Figure 4B). In both sexes, development was slowest (by approximately 1.5 day) when wasps developed in hosts that were parasitized as L1 and wasps developed faster (by approximately 1 day) in hosts collected in France than in those collected in The Netherlands and the Dutch laboratory strain (LSD test: $P<0.05$).

Discussion

The results of this study show that there was an almost linear, inverse correlation between host instar at parasitism in *P. brassicae* and quality of the host for the development of its major parasitoid, *C. glomerata*. Parasitoid survival and body mass were by far the highest in hosts stung as L1 and lowest in hosts stung as L3, irrespective of host population, with L2 hosts of intermediate quality. Thus, despite the fact that L3 *P. brassicae* caterpillar hosts are considerably larger than L1 caterpillars, this size advantage clearly did not benefit *C. glomerata* parasitoids in terms of adult mass and survival. Among parasitoids that did survive on L3 hosts, development time of *C. glomerata* was shorter compared to wasps developing from hosts that were parasitized as L1. Given that extended development time incurs potential costs on parasitoid survival and fitness (e.g., the slow-growth-high-mortality-hypothesis; Benrey & Denno, 1997), this reveals at least a partial trade-off among fitness correlates in parasitoids developing in the different instars.

Although *C. glomerata* is known to attack as many as four host species in the field across its broad range (Feltwell, 1982), in The Netherlands, it is considered a fairly specialized parasitoid of *P. brassicae* caterpillars (Brodeur et al., 1998; Geervliet et al., 2000; Harvey, 2000). For a koinobiont parasitoid like *C. glomerata*, the host is a dynamic resource which differs considerably in size during the course of parasitism (Harvey et al., 1994). Irrespective of instar at parasitism, the host is not killed until very late in the final (fifth) instar. Our results show that L1 *P. brassicae* caterpillars are clearly the most suitable host stage for

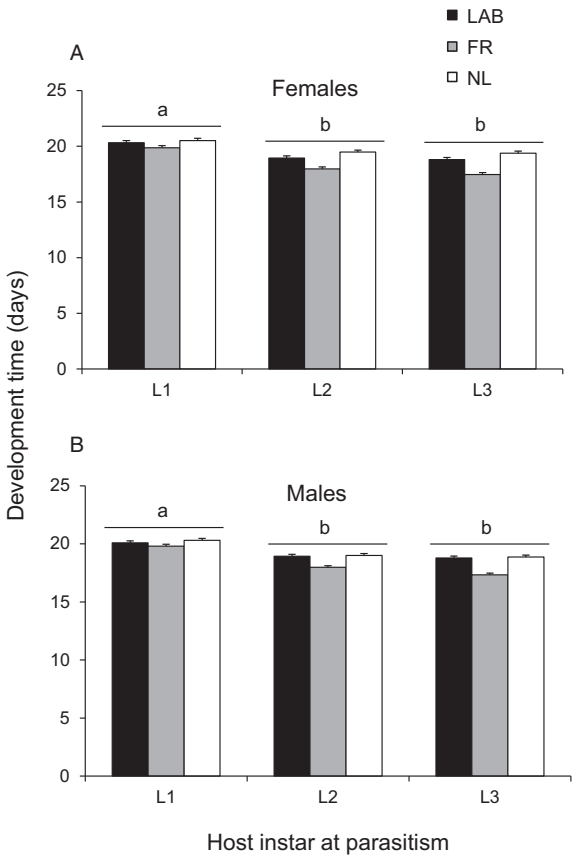

Figure 4 Mean (+ SE) egg-to-adult developmental time (days) of (A) female and (B) male *Cotesia glomerata* when hosts were parasitized as first (L1), second (L2), or third (L3) instar caterpillars 1 day after molting. Of each developmental stage, 25 parasitized caterpillars were maintained together in a single cage with two cages per developmental stage. Hosts originated from the Wageningen laboratory strain (LAB), or were collected from the field near Wageningen (NL) or Rennes, France (FR), and were reared in the laboratory for one generation. Means within a panel capped with different letters are significantly different (LSD tests: $P<0.05$).

the development of *C. glomerata* in terms of adult mass and especially survival. Harvey (2000) also reported an inverse relationship between host instar at parasitism, adult parasitoid mass, and development time in *C. glomerata* attacking larvae of *P. brassicae*, but survival was not measured. Previous work has also shown that *C. glomerata* is more successful in parasitizing hosts on mustard or cabbage plants in patches with L1 *P. brassicae* larvae, than on plants in patches with L2 or L3 *P. brassicae* larvae (Brodeur et al., 1996; Pashalidou et al., 2015). The more effective location of the 'optimal' L1 host stage is mediated by plant volatiles induced by the host and contact cues from the host itself (Mattiacci & Dicke, 1995; Pashalidou et al., 2015). However, the effects of instar-specific differences in host quality or behavioral/immunological defenses on the observed higher parasitism rates of L1 larvae vs. L2–L3 larvae in the field have not been investigated.

All three *P. brassicae* host strains exhibited an inverse relationship between host stage at parasitism and successful emergence of adult wasps, but this effect was not equally strong in each host strain. For instance, in the French *P. brassicae* strain, survival of the parasitoids was similar when they had been parasitized as L1 or L2, and only declined when hosts were parasitized as L3, whereas in the Dutch laboratory and field-collected strain parasitoid survival already declined when hosts were parasitized as L2. In addition, wasps developed faster in French *P. brassicae* larvae than on the two Dutch host strains, irrespective of host instar that was parasitized. This suggests that there is variation in host quality for *C. glomerata* development among the various strains of *P. brassicae* used in this study. Subtle genetic differences among the *P. brassicae* strains resulting from differences in selection pressure exerted by French *C. glomerata* and/or other parasites may explain why the French *P. brassicae* were slightly better hosts for the Dutch *C. glomerata* strain in this study or why precocious death in this strain only occurred when hosts were parasitized as L3. It would be interesting to also study variation in *C. glomerata* populations in their ability to parasitize hosts originating from the same and different locations as where the parasitoid originated. Interestingly, the results for the Dutch laboratory strain were similar as those for the field-collected strain, despite being reared in the laboratory for many generations under 'crowded' conditions. This result suggests that relaxation of selection by *C. glomerata* did not noticeably change the response of *P. brassicae* to *C. glomerata* parasitism.

Successful parasitism depends on extrinsic factors (e.g., the ability to find hosts) and intrinsic factors such as host quality (Vinson & Iwantsch, 1980b; Vinson, 1998). Host quality is determined, for example, by the ability of the parasitoid larvae to conform to or to adjust development of the host to its own nutritional and physiological requirements (Vinson & Iwantsch, 1980a). Females of *C. glomerata* may lay up to 60 eggs in a host during a single oviposition event. Parasitized hosts may grow larger compared to healthy caterpillars depending on the number of wasp larvae developing in a single host (Smallegange et al., 2008), but this primarily occurs when the host is superparasitized (Harvey, 2000; Gu et al., 2003). When parasitizing L1 *P. brassicae* hosts, *C. glomerata* conforms its own development to that of the host by delaying egression from the host until the host is large enough to fulfill the nutritional requirements for parasitoid larval development. Hosts parasitized as L2 or L3 appear to grow at a rate that does

not require growth adjustment of the parasitoid larvae. Thus, *C. glomerata* may adjust or conform its development depending on egg clutch size and host stage at parasitism. Adjustment of host development can be achieved through a combination of regulatory input from the larvae (e.g., selective tissue feeding, secretions) and the adult female (e.g., venoms and/or polydnaviruses injected into the host at oviposition; Beckage & Gelman, 2004).

Immunological defenses based on the ability of the host to encapsulate parasitoid eggs is a well-studied factor that may prevent successful parasitism (Vinson, 1990). In many endoparasitoids, encapsulation ability has been shown to increase with host instar (Brodeur & Vet, 1995; Bukovinszky et al., 2009). However, in this study, only three of the parasitized caterpillars (irrespective of host instar at parasitism) developed into healthy pupae. The caterpillars that did not produce parasitoids died prematurely when they were L4 or early L5 and showed signs similar to those resulting from pathogenic infection; the caterpillars turned black and died, followed by desiccation of the carcasses. Preliminary molecular diagnostics ruled out infection with baculoviruses that are known to commonly infect Lepidoptera (Harrison et al., 2012). Identification of pathogenic bacteria has not been pursued as the caterpillars were already dead when collected for analysis, thus allowing secondary infection from gut bacteria or those present in the environment. Precocious death of the host, preceded by melanization of the host, may also have resulted from the host being hypersensitive to venom or other secretions that were injected by the parasitoid mother or her offspring.

An area that has not been explored in general is the health of the host during parasitism. Infection and susceptibility to pathogens is often enhanced under conditions of stress such as nutritional stress when developing on poor-quality diet (Cory & Hoover, 2006). Natural populations of Lepidoptera also support a diversity of virus infections that are covert, i.e., clear disease symptoms are absent (Williams et al., 2017). The conversion from covert to overt infection can be triggered by high host density, the food plant of the host, marked changes in abiotic conditions, as well as parasitism (Stoltz & Makkay, 2003; Myers & Cory, 2016; Williams et al., 2017). Given that parasitism represents a profound stress on the host during its development, this stress may be differentially expressed in different stages of hosts depending on the degree of physiological integration between the host and parasitoid during parasitism.

Host immunity to parasitism itself can also be compromised under conditions of environmental stress, as has been reported for aphids that were less resistant to parasitism at high temperatures (Bensadia et al., 2006). Parasitism has huge impact on the physiology of the host. Immune responses directed at the parasitoid and adjustment of the host's physiology to the requirements of the developing parasitoid larvae may increase host vulnerability to other threats such as pathogen infection. Though opposite results have been reported as well. For example, *Trichoplusia ni* (Hübner) caterpillars were less susceptible to nucleopolyhedrovirus when parasitized by *Hyposoter exiguae* (Viereck) (Beegle & Oatman, 1974), which may be caused by antimicrobial properties of peptides present in the venom injected by the parasitoid mother at oviposition (Moreau, 2013). Given the high degree of host specificity of these viruses, parasitism-induced changes in the physiology of the host may also render it less susceptible to infection by these viruses when parasitized (Beegle & Oatman, 1974). In the present study, all cages with insects were maintained in the same room and were in close proximity of each other. If pathogen infection was indeed causing precocious death of the caterpillars, it suggests that the pathogen was present in the room, but only invaded the weaker host caterpillars. Alternatively, infection concerned a latent pathogen that only became infectious upon parasitism.

To summarize, previous studies have shown that L1 *P. brassicae* hosts are the better host instar for *C. glomerata* with regard to both the ability to successfully attack hosts (Pashalidou et al., 2015) and to overcome host immune responses (Brodeur & Vet, 1995; Bukovinszky et al., 2009). Our data confirm that L1 hosts are optimally synchronized with *C. glomerata* development, but also that there is variation among host strains in the degree that host physiology is conformed to the requirements of parasitoid development. Reduced parasitism success in L2 and L3 host coincided with precocious death of the caterpillar host. Whether this was caused by a pathogen or resulted from hypersensitivity to parasitoid secretions or something else, is not yet clear. Nevertheless, host–parasitoid–pathogen interactions have received little attention and most of these studies have investigated the effect of baculovirus and microsporidium infections in Lepidoptera (Brooks, 1993). Simultaneous infection with a pathogen and parasitism can have severe fitness consequences for the parasitoid often caused by premature death of the host (Vinson & Iwantsch, 1980b; Brooks, 1993; Stoianova et al., 2012). Considering that both parasitoids and baculoviruses are used as biological control agents of pest insects, the combined use may reduce the efficiency of biological control. Given the omnipresence of pathogens in the environment, studies on multitrophic interactions involving plants, herbivorous insects, and their natural enemies should also consider insect pathogens (Cory & Hoover, 2006) as they may have strong impact on host–parasitoid dynamics (Hochberg & Lawton, 1990).

References

Askew RR & Shaw MR (1986) Parasitoid communities: their size, structure, and development. Insect Parasitoids (eds. by J Waage & D Greathead), pp. 225–264. Academic Press, London, UK.

Awmack CS & Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology 47: 817–844.

Beckage NE & Gelman DB (2004) Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Annual Review of Entomology 49: 299–330.

Beegle CC & Oatman ER (1974) Differential susceptibility of parasitized and nonparasitized larvae of *Trichoplusia ni* to a nuclear polyhedrosis virus. Journal of Invertebrate Pathology 24: 188–195.

Benrey B & Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78: 987–999.

Bensadia F, Boudreault S, Guay J-F, Michaud D & Cloutier C (2006) Aphid clonal resistance to a parasitoid fails under heat stress. Journal of Insect Physiology 52: 146–157.

Brodeur J & Vet LEM (1995) Relationships between parasitoid host-range and host-defense: a comparative study of egg encapsulation in two related parasitoid species. Physiological Entomology 20: 7–12.

Brodeur J, Geervliet JBF & Vet LEM (1996) The role of host species, age and defensive behaviour on ovipositional decisions in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species). Entomologia Experimentalis et Applicata 81: 125–132.

Brodeur J, Geervliet JBF & Vet LEM (1998) Effects of *Pieris* host species on life history parameters in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species). Entomologia Experimentalis et Applicata 86: 145–152.

Brooks W (1993) Host-parasitoid-pathogen interactions. Parasites and Pathogens of Insects, Vol. 2 (ed. by NE Beckage, SN Thompson & BA Federici), pp. 231–272. Academic Press, San Diego, CA, USA.

Bukovinszky T, van Veen FJF, Jongema Y & Dicke M (2008) Direct and indirect effects of resource quality on food web structure. Science 319: 804–807.

Bukovinszky T, Poelman EH, Gols R, Prekatsakis G, Vet LEM et al. (2009) Consequences of constitutive and induced variation in plant nutritional quality for immune defence of a herbivore against parasitism. Oecologia 160: 299–308.

Charnov EL (1982) The Theory of Sex Allocation. Princeton University Press, Princeton, NJ, USA.

Cloutier C, Levesque CA, Eaves DM & Mackauer M (1991) Maternal adjustment of sex-ratio in response to host size in the aphid parasitoid *Ephedrus californicus*. Canadian Journal of Zoology 69: 1489–1495.

Cory JS & Hoover K (2006) Plant-mediated effects in insect-pathogen interactions. Trends in Ecology & Evolution 21: 278–286.

Feltwell J (1982) Large White Butterfly – The Biology, Biochemistry and Physiology of *Pieris brassicae* (Linnaeus). Dr W Junk Publishers, The Hague, The Netherlands.

Geervliet JBF, Verdel MSW, Snellen H, Schaub J, Dicke M & Vet LEM (2000) Coexistence and niche segregation by field populations of the parasitoids *Cotesia glomerata* and *C. rubecula* in the Netherlands: predicting field performance from laboratory data. Oecologia 124: 55–63.

Godfray HCJ (1994) Parasitoids - Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ, USA.

Gols R & Harvey JA (2009) Plant-mediated effects in the Brassicaceae on the performance and behaviour of parasitoids. Phytochemistry Reviews 8: 187–206.

Gross P (1993) Insect behavioral and morphological defenses against parasitoids. Annual Review of Entomology 38: 251–273.

Gu HN, Wang Q & Dorn S (2003) Superparasitism in *Cotesia glomerata*: response of hosts and consequences for parasitoids. Ecological Entomology 28: 422–431.

Harrison R, Hoover K & Kaya HK (2012) Baculoviruses and other occluded insect viruses. Insect Pathology 2nd edn (ed. by FE Vega & HK Kaya), pp. 73–131. Academic Press, London, UK.

Harvey JA (2000) Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implications for host quality and parasitoid fitness. Ecological Entomology 25: 267–278.

Harvey JA (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.

Harvey JA & Strand MR (2002) The developmental strategies of endoparasitoid wasps vary with host feeding ecology. Ecology 83: 2439–2451.

Harvey JA, Harvey IF & Thompson DJ (1994) Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. Ecology 75: 1420–1428.

Harvey JA, Jervis MA, Gols R, Jiang NQ & Vet LEM (1999) Development of the parasitoid, *Cotesia rubecula* (Hymenoptera: Braconidae) in *Pieris rapae* and *Pieris brassicae* (Lepidoptera: Pieridae): evidence for host regulation. Journal of Insect Physiology 45: 173–182.

Harvey JA, Kadash K & Strand MR (2000) Differences in larval feeding behavior correlate with altered developmental strategies in two parasitic wasps: implications for the size-fitness hypothesis. Oikos 88: 621–629.

Harvey JA, Bezemer TM, Elzinga JA & Strand MR (2004) Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. Ecological Entomology 29: 35–43.

Hochberg ME & Lawton JH (1990) Competition between kingdoms. Trends in Ecology & Evolution 5: 367–371.

King BH (1989) Host-size-dependent sex-ratios among parasitoid wasps: does host growth matter. Oecologia 78: 420–426.

Kraaijeveld AR & Godfray HCJ (1997) Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Nature 389: 278–280.

Kraaijeveld AR, van Alphen JJM & Godfray HCJ (1998) The coevolution of host resistance and parasitoid virulence. Parasitology 116: S29–S45.

Lawrence PO (1990) The biochemical and physiological effects of insect hosts on the development and ecology of their insect parasites - an overview. Archives of Insect Biochemistry and Physiology 13: 217–228.

Mackauer M & Sequeira R (1993) Patterns of development in insect parasites. Parasites and Pathogens of Insects, Vol. 1 (ed. by NE Beckage, SN Thompson & BA Federici), pp. 1–20. Academic Press, New York, NY, USA.

Mattiacci L & Dicke M (1995) The parasitoid *Cotesia glomerata* (Hymenoptera, Braconidae) discriminates between first and fifth larval instars of its host *Pieris brassicae*, on the basis of contact cues from frass, silk, and herbivore-damaged leaf tissue. Journal of Insect Behavior 8: 485–498.

Moreau SJM (2013) 'It stings a bit but it cleans well': venoms of Hymenoptera and their antimicrobial potential. Journal of Insect Physiology 59: 186–204.

Myers JH & Cory JS (2016) Ecology and evolution of pathogens in natural populations of Lepidoptera. Evolutionary Applications 9: 231–247.

Ode PJ (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. Annual Review of Entomology 51: 163–185.

Pashalidou FG, Gols R, Berkhout BW, Weldegergis BT, van Loon JJA et al. (2015) To be in time: egg deposition enhances plant-mediated detection of young caterpillars by parasitoids. Oecologia 177: 477–486.

Rantala MJ & Roff DA (2005) An analysis of trade-offs in immune function, body size and development time in the Mediterranean field cricket, *Gryllus bimaculatus*. Functional Ecology 19: 323–330.

Rickers S, Langel R & Scheu S (2006) Dietary routing of nutrients from prey to offspring in a generalist predator: effects of prey quality. Functional Ecology 20: 124–131.

Ruehl CB & Trexler JC (2013) A suite of prey traits determine predator and nutrient enrichment effects in a tri-trophic food chain. Ecosphere 4: 1–21.

Schoonhoven LM, van Loon JJA & Dicke M (2005) Insect-Plant Biology, 2nd edn. Oxford University Press, Oxford, UK.

Sequeira R & Mackauer M (1992) Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrthosiphon pisum*. Evolutionary Ecology 6: 34–44.

Smallegange RC, van Loon JJA, Blatt SE, Harvey JA & Dicke M (2008) Parasitoid load affects plant fitness in a tri-trophic system. Entomologia Experimentalis et Applicata 128: 172–183.

Smilowitz Z & Iwantsch GF (1973) Relationships between the parasitoid *Hyposoter exiguae* and the cabbage looper, *Trichoplusia ni*: effects of host age on developmental rate of the parasitoid. Environmental Entomology 2: 759–764.

Stoianova E, Williams T, Cisneros J, Muñoz D, Murillo R et al. (2012) Interactions between an ectoparasitoid and a nucleopolyhedrovirus when simultaneously attacking *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Applied Entomology 136: 596–604.

Stoltz D & Makkay A (2003) Overt viral diseases induced from apparent latency following parasitization by the ichneumonid wasp, *Hyposoter exiguae*. Journal of Insect Physiology 49: 483–489.

Strand MR & Pech LL (1995) Immunological basis for compatibility in parasitoid host relationships. Annual Review of Entomology 40: 31–56.

Vinson SB (1990) How parasitoids deal with the immune system of their host: an overview. Archives of Insect Biochemistry and Physiology 13: 3–27.

Vinson SB (1998) The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biological Control 11: 79–96.

Vinson SB & Iwantsch GF (1980a) Host regulation by insect parasitoids. Quarterly Review of Biology 55: 143–165.

Vinson SB & Iwantsch GF (1980b) Host suitability for insect parasitoids. Annual Review of Entomology 25: 397–419.

Wilder SM, Mayntz D, Toft S, Rypstra AL, Pilati A & Vanni MJ (2010) Intraspecific variation in prey quality: a comparison of nutrient presence in prey and nutrient extraction by predators. Oikos 119: 350–358.

Williams T, Virto C, Murillo R & Caballero P (2017) Covert infection of insects by baculoviruses. Frontiers in Microbiology 8: 1337.