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Development of a solitary koinobiont hyperparasitoid in different instars of its primary and secondary hosts

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ABSTRACT

Parasitoid wasps are excellent organisms for studying the allocation of host resources to different fitness functions such as adult body mass and development time. Koinobiont parasitoids attack hosts that continue feeding and growing during parasitism, whereas idiobiont parasitoids attack non-growing host stages or paralyzed hosts. Many adult female koinobionts attack a broad range of host stages and are therefore faced with a different set of dynamic challenges compared with idiobionts, where host resources are largely static. Thus far studies on solitary koinobionts have been almost exclusively based on primary parasitoids, yet it is known that many of these are in turn attacked by both koinobiont and idiobiont hyperparasitoids. Here we compare parasitism and development of a primary koinobiont hyperparasitoid, *Mesochorus gemellus* (Hymenoptera: Ichneumonidae) in larvae of the gregarious primary koinobiont parasitoid, *Cotesia glomerata* (Hymenoptera: Braconidae) developing in the secondary herbivore host, *Pieris brassicae* (Lepidoptera: Pieridae). As far as we know this is the first study to examine development of a solitary primary hyperparasitoid in different stages of its secondary herbivore host. *Pieris brassicae* caterpillars were parasitized as L1 by *C. glomerata* and then these parasitized caterpillars were presented in separate cohorts to *M. gemellus* as L3, L4 or L5 instar *P. brassicae*. Different instars of the secondary hosts were used as proxies for different developmental stages of the primary host, *C. glomerata*. Larvae of *C. glomerata* in L5 *P. brassicae* were significantly longer than those in L3 and L4 caterpillars. Irrespective of secondary host instar, every parasitoid cluster was hyperparasitized by *M. gemellus* but all only produced male progeny. Male development time decreased with host stage attacked, whereas adult male body mass did not, which shows that *M. gemellus* is able to optimally exploit older host larvae in terms of adult size despite their decreasing mass during the pupal stage. Across a range of cocoon masses, hyperparasitoid adult male body mass was approximately 84% as large as primary parasitoids, revealing that *M. gemellus* is almost as efficient at exploiting host resources as secondary (pupal) hyperparasitoids.

1. Introduction

Parasitoid wasps are different from other carnivorous insects in that the larvae develop inside or on the bodies of other arthropods whereas the adults are free-living (Godfray, 1994). In many instances, the body size of the host is only marginally larger than that of the adult parasitoid, revealing that these insects are exceedingly efficient at utilizing limited resources and in allocating them to body and reproductive tissues (Mackauer and Sequeira, 1993; Brodeur and Boivin, 2004; Godfray, 1994; Harvey, 2005; Pennacchio and Strand, 2006). Parasitoids are an extremely diverse group of insects and exhibit profound variation in life histories and development strategies (Quicke, 1997, 2015). Two of the most important of these strategies are 'dichotomous' and based on differences in the way that resources are used during the course of parasitism. Idiobiont parasitoids are species that attack hosts that do not feed or grow, such as eggs or pupae, or hosts which are permanently paralyzed by the female parasitoid before oviposition (Askew and Shaw, 1986). Resources for idiobionts are mostly fixed at oviposition, meaning that the size of the adult parasitoid will be

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largely (although not entirely) based on host size at parasitism. Koinobiont parasitoids, on the other hand, are species that attack hosts that continue to feed, grow and move about during at least the early stages of parasitism (Askew and Shaw, 1986; Mackauer and Sequeira, 1993). These hosts, by virtue of their growth, represent dynamic resources, especially when small, early instar hosts are parasitized and which need to grow considerably to meet the minimal nutritional requirements of the parasitoid(s) developing inside them (Slansky, 1986).

Koinobiosis is adaptive in that it enables parasitoids to attack a wide range of host stages, thus reducing selection pressure for a limited maternal choice of hosts (Cloutier et al., 1991). Some koinobionts of lepidopterous hosts, for example, can parasitize and develop in all host instars (Harvey et al., 2000), whereas others prefer early host instars or even eggs but kill the host towards the end of its larval development (Brown et al., 1993; Soller and Lanzrein, 1996; Harvey et al., 2000). By being able to attack several host (or early) instars instead of one, a female parasitoid can be less choosy when foraging for hosts (Slansky, 1986). There are other advantages of a koinobiont lifestyle as well. For instance, when intra- or interspecific competition for hosts is high, individuals parasitizing smaller hosts earlier may be more successful in intrinsic competition (Slansky, 1986; Strand et al., 1990; De Moraes and Mescher, 2005; Harvey et al., 2013). Furthermore, smaller hosts have less potent metabolic defenses than larger hosts, making the internal host environment less challenging for parasitoid eggs and larvae (Lawrence, 1990; Strand and Pech, 1995).

Despite behavioral plasticity that enables many koinobionts to develop in vastly different host sizes or stages, many studies have shown that parasitoid development may differ profoundly depending on the instar in which they were initially laid as eggs by their mother. Most evidence suggests that wasps developing from later host instars are larger, and develop faster, than conspecifics developing in smaller or younger hosts (reviewed by Godfray, 1994 and Harvey, 2005). This is because more resources are immediately available for exploitation by the parasitoid larvae; younger hosts may be too small to support rapid parasitoid development and the progeny thus need to arrest or slow their development until the host reaches a minimum size where the amount of resources is sufficient. However, the situation is far from straightforward. Some koinobionts developing in early host instars delay their development but exhibit little variation in adult size at eclosion, whereas others show the opposite developmental pattern. Harvey and Strand (2002) found that many koinobionts developing in early instars differentially trade-off size and development time depending upon extrinsic threats in nature such as predation. Species attacking early instars of concealed-feeding hosts favored extended development time in order to allow the host to grow to a large size where adult parasitoid size was also larger; by contrast, species attacking exposed-feeding hosts developed rapidly in small hosts but killed them at an earlier stage and were themselves smaller adults as a result. Some parasitoids also exhibited intermediate patterns with both extended development time and reduced size when developing in early instar hosts, revealing a strategy of ontogenetic compromise.

The above studies have been done almost exclusively with primary parasitoids (in the third trophic level) that attack insect herbivores feeding in turn on plants. However, many primary parasitoids are, in turn, attacked by hyperparasitoids (Sullivan, 1987; Sullivan and Völkl, 1999). Hyperparasitoids fall into two groups: primary hyperparasitoids that lay their eggs into the bodies of primary parasitoid larvae while they are inside of the herbivore host, and secondary hyperparasitoids that lay their eggs onto or inside the pre-pupae and/or pupae of their primary parasitoid hosts once they have left the herbivore host. The latter most often lay their eggs inside of the cocoon that the parasitoid has constructed after egression (Harvey, 2008). Whereas most secondary hyperparasitoids are idiobionts, the vast majority of primary hyperparasitoids are koinobionts that do not kill their host until it has successfully emerged from the body of the herbivore host. This is because parasitoids, like other insects, cannot pupate in a wet environment, and must therefore seek a dry site to construct a cocoon (Chapman, 2013). Koinobiont endoparasitoids have evolved two strategies of dealing with this problem. Some species consume all (or most) of the host before pupation (=tissue-feeders), or else they 'escape' the host by chewing a hole in its cuticle and emerging through this to pupate (=hemolymph-feeders; Harvey, 2005). The adaptive significance of the latter strategy has been recently discussed by Harvey and Malcicka (in press). Primary hyperparasitoids are primarily tissue feeders and thus consume most host tissues inside of the cocoon.

This study examines development of a primary solitary koinobiont endo-hyperparasitoid, *Mesochorus gemellus* Holmgren (Hymenoptera: Ichneumonidae) in larvae of the gregarious primary koinobiont endoparasitoid, *Cotesia glomerata* L. (Hymenoptera: Braconidae) in turn developing in caterpillars of its host, the large cabbage white butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae). Caterpillars of *P. brassicae* were parasitized by *C. glomerata* as L1 and then presented to *M. gemellus* females as L3, L4 or L5 when larvae of *C. glomerata* are also at different stages of development. *C. glomerata* only emerges from *P. brassicae* caterpillars and related hosts late during their final instar (Gu et al., 2003). A previous study (Harvey et al., 2012) compared development of a gregarious primary hyperparasitoid, *Baryscapus galactopus* Ratzeburg (Hymenoptera: Eulophidae) in L3-L5 instars of *P. brassicae* parasitized as L2 by *C. glomerata*. They found that development time of the hyperparasitoid was much longer in L3 hosts than in later instars, but that body mass was lower in hosts (hyper)parasitized as L5. One of the problems in interpreting the results was that *B. galactopus* typically produced broods of 3–5 per larva of *C. glomerata*, making it difficult to separate resource-related constraints imposed by host age/size and gregariousness in *B. galactopus*. When resources are limited, the parasitoid larvae may engage in scramble or even contest competition that complicate fitness-related effects of host quality. This is not a problem here, however, because *M. gemellus* is solitary. Our main hypotheses are that (1) development time will be longer in L3 than in L4 or L5 hosts because *M. gemellus* cannot complete its development until the primary parasitoid egresses from its caterpillar host and constructs a cocoon, and (b) adult hyperparasitoid mass will be lowest when developing in L5 *P. brassicae*, because *C. glomerata* development will not be arrested until several days after cocoon construction and pupation, during which time differentiation of tissues into body parts such as antennae, legs, and the head capsule will be well underway. Our results are discussed in relation to host-size/parasitoid fitness studies and models with primary koinobiont endoparasitoids.

2. Methods and materials

2.1. Insects

Hosts and parasitoids were maintained at 22 ± 1 °C under a 16:8 h L:D regime. Cultures of *P. brassicae* and *C. glomerata* were obtained from insects reared at Wageningen University (WUR), The Netherlands. These had been originally collected from agricultural fields in the vicinity of the University. Adult female *C. glomerata* habitually lay 10–40 eggs into first or second (L1-L2) instars of *P. brassicae*. During their development the parasitoid larvae feed primarily on host hemolymph and fat body. Fully grown parasitoid larvae emerge from the host caterpillar late during its final instar, and they immediately spin cocoons on the host plant adjacent to