

Small-scale spatial resource partitioning in a hyperparasitoid community

Jeffrey A. Harvey · Helen Snaas · Miriama Malcicka · Bertanne Visser · T. Martijn Bezemer

Received: 27 March 2014 / Accepted: 10 July 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Plant-herbivore-natural enemy associations underpin ecological communities, and such interactions may go up to four (or even more) trophic levels. Here, over the course of a growing season, we compared the diversity of secondary hyperparasitoids associated with a common host, *Cotesia glomerata*, a specialized larval endoparasitoid of cabbage butterfly caterpillars that in turn feed on brassicaceous plants. Cocoon clusters of *C. glomerata* were pinned to ~30 *Brassica nigra* plants by pinning them either to branches in the canopy (~1.5 m high) or to the base of the stem near the ground. The cocoons were collected a week later and reared to determine which hyperparasitoid species emerged from them. This was done in four consecutive months (June–September). Cocoons placed in the canopy were primarily attacked by specialized winged hyperparasitoids (*Lysibia nana*, *Acrolyta nens*), whereas cocoons on the ground were attacked by both winged and generalist wingless hyperparasitoids

(*Gelis acarorum*, *G. agilis*), although this changed with season. There was much more temporal variation in the diversity and number of species attacking cocoons in the canopy than on the ground; the abundance of *L. nana* and *A. nens* varied from month to month, whereas *P. semotus* was only prevalent in August. By contrast, *G. acarorum* was abundant in all of the samples placed near the ground. Our results show that hyperparasitoids partition host resources at remarkably small vertical spatial scales. We argue that spatial differences in the distribution of natural enemies can contribute to the diversity patterns observed in the field.

Keywords *Acrolyta nens* · *Brassica nigra* · Competition · *Gelis* sp. · *Lysibia nana* · Multitrophic interactions · Niche · *Pieris brassicae*

Handling Editor: Heikki Hokkanen.

J. A. Harvey (✉) · H. Snaas · M. Malcicka · B. Visser · T. M. Bezemer
Department of Terrestrial Ecology, Netherlands Institute of Ecology, Droevendaalsesteeg 10, 6700 EH Wageningen, The Netherlands
e-mail: j.harvey@nioo.knaw.nl

J. A. Harvey · M. Malcicka
Department of Ecological Sciences, Section Animal Ecology, VU University Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

B. Visser
Ecology of Multitrophic Systems Research Team, Functional Ecology Group, Institut de Recherche sur la Biologie de l'Insecte (IRBI) UMR 7261 CNRS, Université François-Rabelais, Avenue Monge, Parc Grandmont, 37200 Tours, France

Introduction

Ecological communities consist of networks of interacting species occupying different trophic levels within food webs. Some of the best studied trophic interactions in food webs involve plant–insect associations. Insect herbivores feed on plants and are in turn attacked by natural enemies such as predators and parasitic wasps (or parasitoids). Food webs can be more complex, however, when parasitoids are attacked by hyperparasitoids (Harvey et al. 2009, 2011). Ultimately, the length of food chains is determined by a wide range of ecological and physiological factors (Pimm and Lawton 1977; Kondoh and Ninomaya 2009). The strength of interactions among different species within food webs can affect the stability and resilience of communities and ecosystems at larger scales (McCann et al. 1998). The extent and strength of these interactions may,

however, also vary spatially and temporally, due to life-history differentiation of the species involved and the structure of the local habitats in which they occur. Understanding the factors facilitating coexistence among multiple species remains an important question in ecology (Lawton and Hassell 1984; Hawkins 2000).

In some habitats, competition for access to resources may be strong, mainly during the summer months when populations of many multivoltine insects peak. In this situation, selection may favour the evolution of different strategies to reduce interspecific competition. For instance, some species of white (pierid) butterflies prefer different habitat types for oviposition, even though their offspring develop on the same host plant species (Gossard and Jones 1977; Ohsaki 1979). Natural enemies of insect herbivores may also partition their prey or host resources in different ways. Some predators, for instance, adopt a sit-and-wait strategy on the plant and attack any passing herbivore (Inoue and Marsura 1983; Blailey 1985; Schmitz 2008), whereas others are active foragers (Edgar 1969; Inoue and Marsura 1983; Schmitz 2008).

Most predators have very broad diets and will attack many different kinds of prey, thereby reducing competition. However, many herbivores are also attacked by endoparasitoid wasps. Unlike predators, parasitoids are often restricted to attacking certain stages of one or only a few host species (Godfray 1994). Eggs, larvae and pupae of the jack pine sawfly, *Diprion swaneii* (Hymenoptera: Diprionidae) for example, each harbour their own specific parasitoid guilds (Price 1972). However, each guild is represented by several parasitoid species (Price 1972), and these species compete directly for the same resource. Hence, in spite of the fact that parasitoids have evolved different development and host usage strategies, competition among parasitoid species within guilds is still expected to be a major factor in determining the structure and function of multitrophic communities (Force 1974; Woodcock and Vanbergen 2008; Stone et al. 2012; Harvey et al. 2013). As a result of interspecific competition, the temporal or spatial niche of a parasitoid species or the breadth of that niche may change (Lawton and Hassell 1984; Hawkins 2000; Pedersen and Mills 2004). For example, several species of parasitoids that compete for same herbivore hosts differ in their preferences for the height in the foliage at which they search and attack hosts, thus reducing competition among them (Tscharntke 1992; Van Laerhoven and Stephen 2002; Garcia-Medel et al. 2007; Rossi et al. 2006). These niche differences can even occur within a single parasitoid genus. Wieber et al. (1995) showed that four hyperparasitoids in the genus *Gelis* that attack cocoons of the gypsy moth parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae) differed in where they attacked cocoons within a tree. *Gelis apantelis* and *G.*

obscurus (Hymenoptera: Ichneumonidae) preferred to attack cocoons in lower parts of the tree, whereas *G. tenellus* preferentially attacked cocoons located higher in the tree canopy (Wieber et al. 1995).

In addition to spatially segregating their resources, insects may also temporally partition their resources to reduce or even avoid competition. Several butterfly caterpillar species for example prefer to feed during day, whereas moth larvae that feed on the same host plant prefer to feed at night (Schultz 1983). It has also been shown that parasitoid or hyperparasitoid species that attack the same multivoltine host occur at different periods within the same season (e.g. Wieber et al. 1995). Weseloh (1978) for example, studied the complex of hyperparasitoid species that attack cocoons of the gypsy moth parasitoid *C. melanoscela* and showed that hyperparasitoids within the genus *Gelis* generally were active earlier in the season than other hyperparasitoids.

This study compares temporal and spatial patterns of segregation of secondary hyperparasitoids associated with the same primary parasitoid host, *Cotesia glomerata* L. (Hymenoptera: Braconidae) over the course of a spring–summer season in the Netherlands. The importance of hyperparasitoids in shaping multitrophic communities is the subject of ongoing debate (Sullivan and Völkl 1999; Brodeur 2000; Poelman et al. 2012), but hyperparasitoids certainly can influence the success of some biological control programs by reducing primary parasitoid numbers (Weseloh 1978; Sullivan and Völkl 1999; Nofemela 2013). *Cotesia glomerata* is a gregarious endoparasitoid that lays broods of up to 40 eggs in young caterpillars of the large cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) (Harvey 2000; Gu et al. 2003). Larvae of this butterfly in turn lay clutches of up to 150 eggs on wild and cultivated plants in the family Brassicaceae, with the black mustard, *Brassica nigra* (Brassicales: Brassicaceae) being one of its major food plants over much of Eurasia (Feltwell 1982). Both *P. brassicae* and *C. glomerata* have 2 or more generations per year throughout most of their range, and both may be found in the field as late as September or even October (Fei et al. 2014). Pupae and pre-pupae of *C. glomerata* in cocoons are in turn attacked by up to 10 species of hyperparasitoids in the Netherlands (Poelman et al. 2012). Individual cocoon clusters of *C. glomerata* collected from *B. nigra* plants in a garden plot occasionally yielded one species of primary and three species of secondary hyperparasitoids (Harvey et al. 2009), suggesting that competition for access to *C. glomerata* larvae and pupae can be severe, especially in simple landscapes.

Hyperparasitoid diversity was compared from cocoons physically attached to wild *B. nigra* plants growing in close proximity over successive months from June through September of 2011. Cocoons were pinned to two separate parts of the same plants: on upper stems (the ‘canopy’) 1–1.5

metres above the ground and on the stem at ground level. A previous study examining the spatial segregation of hyperparasitoid host use (Wieber et al. 1995) was based on much larger vertical scales than that which we compared here. The main aim was to determine if there are seasonal and spatial differences in the hyperparasitoid communities associated with cocoons of *C. glomerata*. We hypothesized that various species of hyperparasitoids exhibit different adaptations that make them better able to exploit cocoons in different months and on different parts of *B. nigra* plants. We argue that competition probably plays a strong role in shaping the niche and dietary breath of hyperparasitoids associated with *C. glomerata* and other potential hosts.

Methods and materials

Insects

Hosts and parasitoids were maintained at 25 ± 2 °C under a 16:8 h L:D regime. Cultures of *C. glomerata* and *P. brassicae* were obtained from insects reared at Wageningen University (WUR), the Netherlands, that were originally collected from agricultural fields in the vicinity of the University. All *P. brassicae* larvae used in these experiments had been maintained on *Brassica oleracea* var. *Cyrus* (Brussels sprouts) at WUR.

Cotesia glomerata were reared following Harvey (2000). Adult female wasps typically oviposit 10–40 eggs into first (L1) to third (L3) instar larvae of *P. brassicae*. During their development, the parasitoid larvae feed primarily on host haemolymph and fat body. When they are mature, the larvae emerge from the host caterpillar late during its final instar, and they immediately spin cocoons on the host plant adjacent to the host, which perishes within a few days.

Experimental protocol

Larvae of *P. brassicae* were initially parasitized by females of *C. glomerata* in the first instar (L1) in rearing cages (35 × 35 × 35 cm). Cabbage leaves containing >300 L1 larvae were placed into cages containing male and female parasitoids for approximately 30 min. The leaves were pinned to the side of the cage by inserting ordinary pins through the stem and the cage mesh; this allows easier access of the wasp to the caterpillars. After 30 min, the leaves were removed from the parasitoid cages and the larvae were transferred to undamaged cabbage plants in larger rearing cages (1 m × 60 cm × 60 cm). Each cage contained four food plants that were refreshed every 3 days, or earlier if required. When the parasitoid larvae egressed from the host caterpillar, they were allowed to spin cocoons and form individual clusters that contain

~20–40 cocoons. Clusters were collected in large Petri dishes (18 cm diameter) for experiments to be conducted in the field.

Brassica nigra plants are abundant in the field between mid-June and early-September. In 2011, many plants were growing adjacent to the Netherlands Institute of Ecology (NIOO) buildings in Wageningen, the Netherlands. The plants had apparently germinated at different times during the spring meaning that flowering stages of different individual plants persisted over the first 2–3 months. No other wild or cultivated brassicaceous plants were growing anywhere close to the site of the experiment at any time when it was carried out. In each monthly replicate, 30–36 plants were selected and marked with small tags. Non-flowering plants were used in the first month replicate (=June), whereas in monthly replicates 2 and 3 (July and August), the plants were flowering. In the fourth month replicate (=September), the plants were no longer flowering, but were instead producing seeds. The plants selected for the experiment were at least 1.8 m tall and growing in small groups at various locations around the institute over an area of ~2–3 ha. On marked plants, individual single cocoon clusters (<24 h old) were pinned to an upper branch of a single mustard plant, as well as to the base of the stem, using mounting pins. The pins perforated the cocoon silk (but not any of the pupae) and the plant stem at vertical angles to reduce the risk of displacement. Clusters attached to the base of the stem were often in the vicinity of dense vegetation at ground level. Plants selected were at least several metres apart. After 1 week (7 days), irrespective of weather conditions during the experimental period, the cocoons were collected and each cluster was placed into a small Petri dish (12 cm dia.) that was marked with plant number and location. The cocoons were maintained in climate rooms under the same conditions for the cultures and were any hyperparasitoids that emerged from them were identified to species. The experiment was initiated in the last week of June, 2011, and was repeated at 4–5 week intervals in July, August and September. In each monthly replicate, different plants were used, although they were all growing in the same habitat (Table 1).

It should be noted that it was not possible to count the cocoons at the beginning of the experiment because they were tightly clustered. Typical brood sizes in *C. glomerata* are 20–40 per host, and we attempted to ensure continuity by selecting broods of approximately the same size for attachments to the plants.

Data analyses

All data were analysed in SPSS version 19. The main and interactive effects of location (canopy/bottom) and period (June–September) on the number of cocoons per cluster

Table 1 Numbers and species of secondary hyperparasitoids that emerged from cocoons of *Cotesia glomerata* that had been placed in the canopy and bottom of *Brassica nigra* plants in weekly periods over 4 consecutive months between June and September, 2011

Species	June-canopy	June-bottom	July-canopy	July-bottom	August-canopy	August-bottom	September-canopy	September-bottom
<i>Lysibia nana</i>	22	34	0	0	14	7	0	0
<i>Acrolyta nens</i>	33	82	0	6	2	0	105	0
<i>Bathytrix aenea</i>	0	0	0	5	7	0	12	3
<i>Gelis areator</i>	0	0	0	0	0	0	5	0
<i>Gelis acarorum</i>	0	49	0	103	0	52	0	19
<i>Gelis agilis</i>	0	13	1	26	3	1	0	5
<i>Pteromalus semotus</i>	0	0	0	9	68	0	0	0

that were hyperparasitized, and on the number of hyperparasitoid species that emerged from a clutch were analysed using generalized linear models with Poisson distribution and log-linear link function. Subsequently, for each hyperparasitoid species separately, the effects of location and period on the proportion of clutches from which the species emerged (prevalence) were analysed using binary logistic regression. The hyperparasitoid species *Gelis acarorum* was only found in clusters that were located at the bottom of the plant (see results). Data for this species were therefore not analysed with binary logistic regression but using a chi-square test for the effects of location and for the effects of sampling period separately.

Results

Over the four different sampling periods, a total of seven species of hyperparasitoids emerged from the cocoon clusters: *Lysibia nana* (Hymenoptera: Ichneumonidae), *Acrolyta nens* (Hymenoptera: Ichneumonidae), *G. acarorum* (Hymenoptera: Ichneumonidae), *G. agilis* (Hymenoptera: Ichneumonidae), *G. areator* (Hymenoptera: Ichneumonidae), *Pteromalus semotus* (Hymenoptera: Pteromalidae) and *Bathytrix aenea* (Hymenoptera: Ichneumonidae) (Fig. 1). The most abundant species collected from the ground samples was *G. acarorum*, whereas the most abundant species collected from the canopy samples was *A. nens*. In the June month replicate, two cocoon clusters in the canopy and 18 on the ground produced hyperparasitoids; this ratio in the following months was: 1:15 (July), 15:13 (August) and 19:9 (September), indicating that cocoons clusters in the canopy became increasingly susceptible to hyperparasitoids with time, whereas the opposite pattern to some extent occurred in cocoons placed on the ground.

The number of cocoons from which hyperparasitoids emerged differed significantly between the two locations

on the plant (Wald $\chi^2 = 148.33$; $P < 0.0001$) and between sampling periods (Wald $\chi^2 = 15.24$; $P < 0.0001$; Fig. 2). During the first two sampling periods, the number of cocoons that were hyperparasitized was significantly higher for clusters that were placed at the bottom of the plant than for clutches in the canopy. However, in August, there was not a statistically significant difference between the locations, while in September, this pattern was reversed, presumably because of a large number of captured *G. acarorum* (Fig. 2), resulting in a highly significant interaction between location and sampling period (Wald $\chi^2 = 124.93$; $P < 0.0001$).

From the majority of cocoon clusters (80 %) placed in the canopy or on the ground, only one species emerged and the maximum number of species that emerged from a cluster was three. Most canopy-placed and ground-placed clusters also yielded different species of hyperparasitoids. The number of hyperparasitoid species that emerged per cluster of cocoons differed significantly between the two locations on the plant (Wald $\chi^2 = 23.23$; $P < 0.0001$) and between the sampling periods (Wald $\chi^2 = 18.71$; $P < 0.0001$). The number of species that emerged from clusters placed in the canopy increased in August and September only while the number of species that emerged from clusters placed at the bottom was more constant over time, resulting in a significant interaction between location and sampling period (Wald $\chi^2 = 19.24$; $P < 0.0001$; Fig. 3).

The proportion of cocoon clusters producing each of six hyperparasitoid species (densities of *G. areator* were too low to be analysed) from canopy- and ground-placed cocoon clusters is shown in Fig. 4). There was considerable seasonal and spatial variation in the abundance of the emerging hyperparasitoids. However, *G. acarorum* clearly dominates in ground-placed cocoons, whereas in the canopy, there is more variation in species composition. Analyses of the effects of location and sampling period for each hyperparasitoid species separately showed that the



Fig. 1 Six species of secondary hyperparasitoids emerging from clusters of cocoons of *Cotesia glomerata* attached to a branch in the canopy or at the base of a stem of *Brassica nigra* plants. **a** *Gelis acarorum*; **b** *G. agilis*; **c** *G. areator*; **d** *Acrolyta nens*; **e** *Lysibia nana*; **f** *Pteromalus semotus*

prevalence of *A. nens* was significantly affected by location (Wald = 17.85; $P < 0.0001$) and by sampling period (Wald 5.90; $P = 0.015$). However, the effects of location differed strongly between sampling periods. During the first sampling period, in early- summer, *A. nens* was found primarily in clutches at the bottom of the plant, while during the last two sampling periods, in mid- to late-summer, this pattern was reversed, resulting in a highly significant interaction between period and location (Wald = 20.86; $P < 0.0001$).

During each of the four sampling periods, *G. acarorum* was only found in clutches located at the bottom of the plant, resulting in a significant effect of location ($\chi_1 = 8.19$; $P = 0.004$), and this effect did not differ between sampling periods ($\chi_1 = 7.48$; $P = 0.06$). A similar pattern was observed for the related species *Gelis agilis*, but this was not significant. The prevalence of *L. nana*, *P. semotus* and *Bathyrix aenea* was also not significantly affected by location or sampling period. The species *G.*

areator only emerged from two clutches, and data for this species were not statistically analysed.

Discussion

The results of this investigation reveal clear differences in hyperparasitoid species composition when cocoons of *C. glomerata* were pinned to upper branches or lower stems of *B. nigra* plants. Moreover, hyperparasitoid abundance and species composition changed from late-spring to summer during the growing season of *B. nigra* plants occurring within the same habitat. Hyperparasitoid species richness was generally higher in cocoons placed in the canopy than on the ground. Hyperparasitoids developing in canopy-placed cocoons were generally winged and capable of flight, whereas the vast majority of ground-placed cocoons were attacked by the wingless hyperparasitoids *G. acarorum* and *G. agilis* (with the former species predominating).

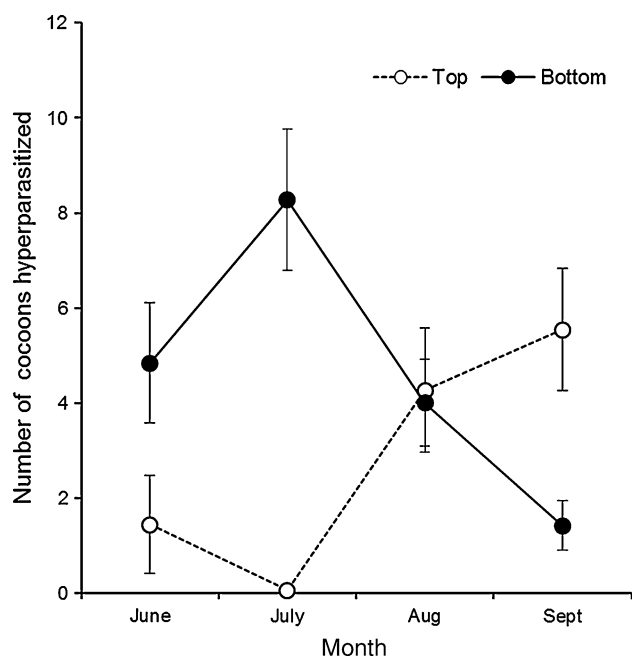


Fig. 2 Mean total number of *Cotesia glomerata* cocoon clusters hyperparasitized that were attached to branches in the canopy (open circles) or at the base of the stem (dark circles) over a 4-month period in the growing season of 2011. Numbers represent mean from clusters collected and returned to the laboratory. Vertical line bars represent standard error of the mean

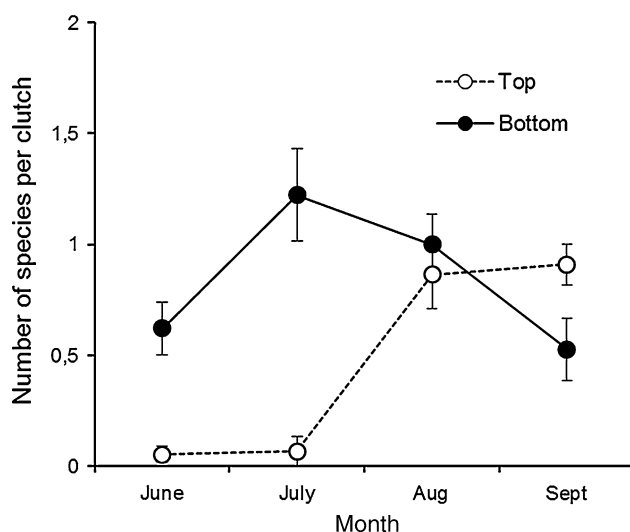


Fig. 3 Number of hyperparasitoid species emerging from *Cotesia glomerata* cocoon clusters attached to branches in the canopy (open circles) or at the base of the stem (dark circles) over a 4-month period in the growing season of 2011. Vertical line bars represent standard error of the mean

Remarkably, cocoons on the ground were also less frequently attacked by three of the winged (and more specialized on the cocoons of *Cotesia* spp.) hyperparasitoids, showing that these species forage over a wide area of the host plant.

Our results demonstrate that the same resources (parasitoid cocoons) can be partitioned at extremely small spatial scales, as well as over longer temporal scales in the same habitat. The distance between cocoons placed in the canopy and on the stem at ground level was usually only about 1.5 metres or even less. Laszlo and Tothmeresz (2013) compared resource use patterns in 4 species of parasitoids of a gall-inducing wasp and found that co-existence among the parasitoids was facilitated by a combination of life-history traits and seasonal phenology at different spatial scales in the landscape. Romero and Harwood (2010) also found that the diet of linyphiid spiders in agroecosystems varied considerably during day and night periods, with detrital (ground) prey dominating the spider diet at night and canopy prey dominating the spider diet at day.

In primary parasitoids, competition can be reduced, at least marginally, between parasitoids attacking the same host species through the evolution of host-stage specialization and thus the formation of different parasitoid guilds (Hawkins 1994). Thus, eggs, larvae and pupae all may harbour species of parasitoids that will not parasitize, or else that cannot develop, in other stages of the host (Godfray 1994). However, host-stage differentiation is not always clear cut. Some parasitoid females obligatorily oviposit into host eggs, but their progeny develop in the larval stages (so-called egg-larval parasitoids). Moreover, some parasitoids hatch in early-larval instars of their hosts, but may pupate in late larval instars or even host pupae (Godfray 1994). Many herbivores are also attacked by several to many parasitoids within the same guild (Price 1972; Hawkins 1994). Under these conditions, competition for access to and control of host resources may be intense (Harvey et al. 2013). Parasitoids have evolved a number of intricate ways of excluding interspecific competitors and thus to monopolize host resources. These include morphological, physiological and behavioural traits and strategies (Force 1974; Harvey et al. 2013). However, as we have shown here, competition can be diffused by habitat-related parameters in conjunction with the biology of the hyperparasitoids.

Cocoons of *C. glomerata* are attacked by up to 10 species of primary and secondary hyperparasitoids in the field (Poelman 2008; this study). Four of the species recovered here (e.g. *L. nana*, *A. nens*, *B. aenea*, *P. semotus*) are fairly specialized hyperparasitoids of *Cotesia* sp. cocoons, whereas the others are generalists. A previous field study (Harvey et al. 2009) found that one species of primary (*Baryscapus galaccanopyus*) and two species of secondary (*L. nana* and *A. nens*) hyperparasitoids occasionally emerged from single broods of *C. glomerata* cocoons collected from *B. nigra* plants. Primary hyperparasitoids attack the developing larvae of primary parasitoids inside of the herbivore host,

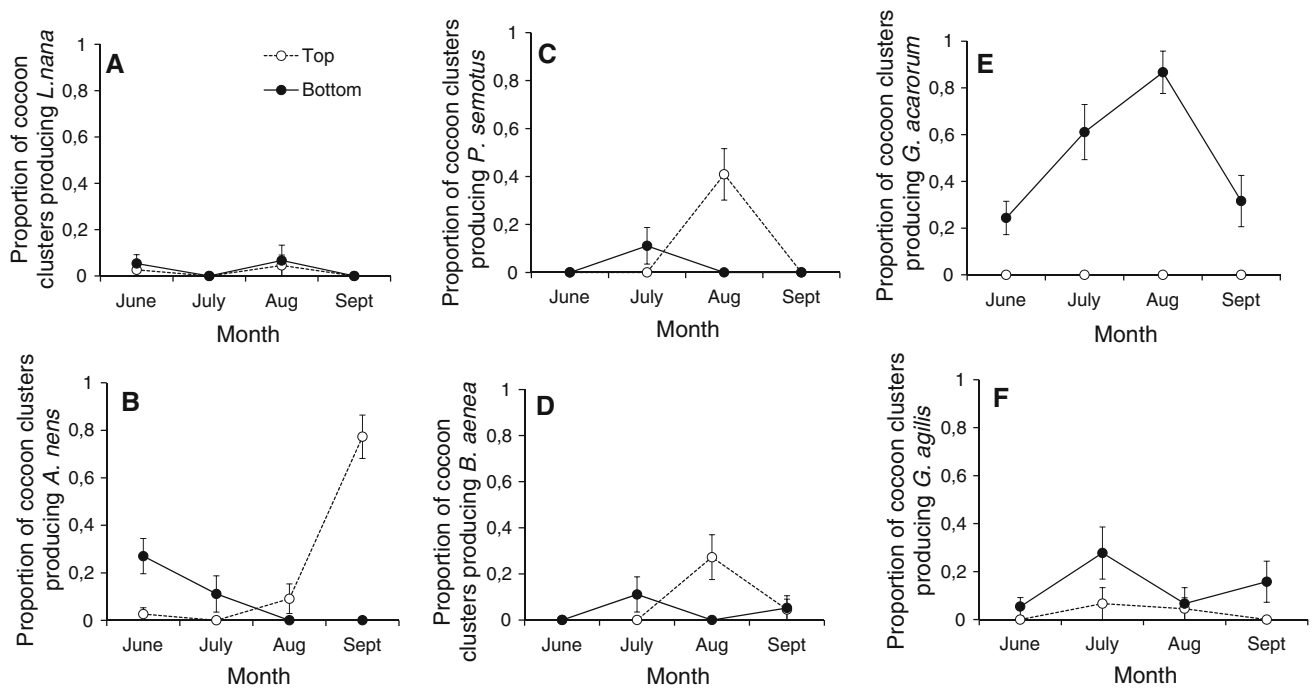


Fig. 4 Proportion of cocoon clusters of *Cotesia glomerata* attached to branches in the canopy (open circles) or at the base of the stem (dark circles) producing 6 hyperparasitoid species over a 4-month

period in the growing season of 2011. Vertical line bars represent standard error of the mean

whereas secondary hyperparasitoids attack the pre-pupae and pupae of primary parasitoids once they have terminated their relationship with the herbivore host. In the case of *B. galaccanopyus*, the female habitually attacks mature *C. glomerata* larvae inside the caterpillar just prior to egression from the host (Harvey et al. 2012). Parasitized pre-pupae of *C. glomerata* then emerge from the dying host and construct cocoons from which adult *B. galaccanopyus* will eventually emerge. The extent to which competition is played out between *B. galaccanopyus* and secondary hyperparasitoids in the field will be the subject of future studies. Other studies in tree systems have shown that cocoons of *C. melanoscela*, a parasitoid of the Gypsy moth, can be attacked by up to 20 species of hyperparasitoids (Weseloh 1978; Wieber et al. 1995, 2001). It is important to note, however, that several hyperparasitoid species, including *Gelis* spp., must host feed prior to oviposition in order to obtain proteins necessary for oogenesis (Jervis and Kidd 1986). *Gelis* spp. are destructive host-feeders and thus kill the host during this process. Host-feeding and oviposition therefore occur on different individual hosts. In some associations, host-feeding-induced mortality far exceeds mortality caused by parasitism (Jervis and Kidd 1986), adding another layer of complexity to the competition problem.

From our data, it appears that some of the hyperparasitoids are at least bi-voltine and have well defined first and second generations, whereas in other species, different

generations were difficult to separate or else the insects were univoltine. The most commonly reported winged hyperparasitoids of *C. glomerata* are *L. nana* and *A. nens* (Poelman 2008), and *A. nens* peaked in abundance in June and August/September but was much less common in the July samples. *P. semotus* was mostly recovered from the late-summer canopy samples, whereas both wingless *Gelis* species (and *G. acarorum* in particular) emerged in large numbers in ground-placed cocoons throughout most of the sampling period but (for *G. acarorum*) especially in August. One important factor in being able to separate different generations is longevity. Winged hyperparasitoids tend to have shorter lifespans than wingless species (Harvey 2008; Harvey et al. 2009) meaning that both *Gelis* species may survive for extended periods, thus blurring the distinction between different generations. The wingless *Gelis* species also inevitably forage over much smaller spatial scales than the winged hyperparasitoids (Harvey 2008; Harvey et al. 2009; Visser et al. 2014). Ground habitats are often densely vegetated, meaning that both wingless *Gelis* species must navigate habitats that are potentially complex structurally. Because of this, they are far less likely to encounter suitable hosts than their winged counterparts, perhaps accounting for their remarkably low reproductive potential (Harvey 2008; Visser et al. in preparation). At the same time, many *Gelis* species are known to have very broad host diets and are able to attack

such diverse hosts as moth pupae and spider egg sacs in addition to parasitoid cocoons (Bezant 1956; Russell 1987; Cobb and Cobb 2004). By contrast, *L. nana* and *A. nens* are specialized on cocoons of parasitoids in the genus *Cotesia* (Schwarz and Shaw 1998).

In summary, this study demonstrates small-scale niche differentiation in a secondary hyperparasitoid community sharing cocoons of the same primary parasitoid. Although cocoons of *C. glomerata* were partitioned to some extent on the basis of placement (canopy vs. ground) and in different seasons, there was still evidence for some level of extrinsic (and perhaps intrinsic) competition among the different hyperparasitoids. It is important to note that *C. glomerata* generally emerges from the host caterpillar and constructs cocoons on the food plant, meaning that they may be largely inaccessible to wingless *Gelis* species foraging primarily on or very close to the ground. On the other hand, *P. brassicae* caterpillars feed on some tall cruciferous species (e.g. *B. nigra*) that grow in riverine habitats among stands of dense vegetation, as well as smaller species (e.g. *Sinapis arvensis*, *Sisymbrium officinale*) whose canopy is much lower. *C. glomerata* developing in caterpillars on different food plant species may be forced to pupate in locations where they are more or less susceptible to different species of hyperparasitoids. Future experiments will be performed to determine if hyperparasitoid communities differ in response to the identity of the food plant on which the herbivore host developed.

Acknowledgments The authors wish to thank Rieta Gols for reading an early draft of the manuscript and Roel Wagenaar for constantly supplying cocoons of *Cotesia glomerata*. Ashes of Ares and Wolfen provided much needed musical support during long experimental days. Photographs of all hyperparasitoids were taken by Tibor Bukovinszky except for *Pteromalus semotus*, which was taken by Alex Gumovsky. Mark Shaw and Martin Schwarz identified the hyperparasitoids.

References

- Bezant ET (1956) *Gelis micrurus* Forster (Hymenoptera: Ichneumonidae) parasitising a lycosid spider egg sac. Entomol Month Mag 92:106
- Blailey PCE (1985) 'A prey in the hand', multi-prey capture behaviour in a sit-and-wait predator, *Ranatra dispar* (Heteroptera: Nepidae), the water stick insect. J Ethol 3:105–112
- Brodeur J (2000) Host specificity and trophic relationships of hyperparasitoids. In: Hochberg ME, Ives AR (eds) Parasitoid Population biology. Princeton University Press, Princeton, New Jersey, pp 163–183
- Cobb LM, Cobb VA (2004) Occurrence of parasitoid wasps, *Baeus* sp and *Gelis* sp., in the egg sacs of the wolf spiders *Pardosa moesta* and *Pardosa sternalis* (Araneae, Lycosidae) in southeastern Idaho. Can Field Nat 118:122–123
- Edgar WD (1969) Prey and predators of the Wolf spider *Lycosa lugubris*. J Zool 159:405–411
- Fei M, Gols R, Harvey JA (2014) Seasonal phenology of interactions involving short-lived annual plants, a multivoltine herbivore and its endoparasitoid wasp. J Anim Ecol 83:234–244
- Feltwell J (1982) Large white butterfly: the biology, biochemistry and physiology of *Pieris brassicae* (Linnaeus). Dr. W. Junk Publishers, The Hague
- Force DC (1974) Ecology of insect host-parasitoid communities. Science 184:624–632
- Garcia-Medel D, Sivinski J, Diaz-Fleischer F, Ramirez-Romano R, Aluja M (2007) Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as a single- or as multiple species cohorts in field cages: influence of fruit location and host density. Biol Cont 43:12–22
- Godfray H CJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ, USA
- Gossard TW, Jones RE (1977) The effects of age and weather on egg laying behaviour in *Pieris rapae* L. J Appl Ecol 14:65–71
- Gu H, Wang Q, Dorm S (2003) Superparasitism in *Cotesia glomerata*: response of hosts and consequences for parasitoids. Ecol Entomol 28:422–431
- 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. Ecol Entomol 25:267–278
- Harvey JA (2008) Comparing and contrasting development and reproductive strategies in the pupal hyperparasitoids *Lysibia nana* and *Gelis agilis* (Hymenoptera: Ichneumonidae). Evol Ecol 22:153–166
- Harvey JA, Wagenaar R, Bezemer TM (2009) Life-history traits in closely related secondary parasitoids sharing the same primary parasitoid host: evolutionary opportunities and constraints. Entomol Exp Appl 132:155–164
- Harvey JA, Pashalidou F, Wagenaar R, Bezemer TM (2011) Intrinsic competition between two secondary hyperparasitoids results in temporal trophic switch. Oikos 120:226–233
- Harvey JA, Gols R, Vet LEM, Kruidhof HM (2012) Development of a hyperparasitoid wasp in different stages of its primary parasitoid and secondary herbivore hosts. J Insect Physiol 58:1463–1468
- Harvey JA, Poelman EH, Tanaka T (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. Ann Rev Entomol 58:333–351
- Hawkins BA (1994) Pattern and process in host-parasitoid interactions. Cambridge University Press, Cambridge, UK
- Hawkins BA (2000) Species coexistence in parasitoid communities: does competition matter? In: Hochberg ME, Ives AR (eds) Parasitoid population biology. Princeton University Press, Princeton, New Jersey, pp 198–213
- Inoue T, Marsura T (1983) Foraging strategy of a mantid, *Paratenodera angustipennis* S.: mechanisms of switching tactics between ambush and active search. Oecologia 56:264–271
- Jervis MA, Kidd NAC (1986) Host-feeding strategies in hymenopteran parasitoids. Biol Rev 61:395–434
- Kondoh M, Ninomaya K (2009) Food chain length and adaptive foraging. Proc Royal Soc B 276:3113–3121
- Laszlo Z, Tothmeresz B (2013) Landscape and local effects on multiparasitoid coexistence. Insect Cons Div 6:354–364
- Lawton JH, Hassell MP (1984) Interspecific competition in insects. In: Huffaker CB, Rab R (eds) Ecological Entomology. Wiley, New York, pp 451–495
- McCann K, Hastings A, Huxel GR (1998) Weak trophic interactions and the balance of nature. Nature 395:794–798
- Nofemela RS (2013) The effect of obligate hyperparasitoids on biological control: differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. Biol Cont 65:218–224
- Ohsaki N (1979) Comparative population studies of three *Pieris* butterflies, *P. rapae*, *P. melete* and *P. napi*, living in the same area. Res Pop Ecol 20:278–296

- Pedersen BS, Mills NJ (2004) Single vs. multiple introduction in biological control: the roles of parasitoid efficiency, antagonism and niche overlap. *J Appl Ecol* 41:973–984
- Pimm SL, Lawton JH (1977) On the number of trophic levels. *Nature* 268:329–331
- Poelman EH, Bruinsma M, Zhu F, Weldegergis BT, Boursault AE, Jongema Y, van Loon JJA, Vet LEM, Harvey JA, Dicke M (2012) Hyperparasitoids use herbivore-induced plant volatiles to locate their parasitoid host. *PLoS Biol* 10:e1001435
- Price PW (1972) Parasitoids utilizing the same host: adaptive nature of differences in size and form. *Ecology* 53:190–195
- Romero SA, Harwood JD (2010) Diel and seasonal patterns of prey availability for epigeal predators: evidence for food limitation is a linyphiid spider community. *Biol Cont* 52:84–90
- Rossi AM, Murray M, Hughes K, Kotowski M, Moon DC, Stiling P (2006) Non-random distribution among a guild of parasitoids: implications for community structure and host survival. *Ecol Entomol* 31:557–563
- Russell DA (1987) Parasitism of the oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae): the New Zealand position in a world perspective. *New Zeal Entomol* 10:13–26
- Schmitz O (2008) Effects of predator hunting mode on grassland ecosystem function. *Science* 319:952–954
- Schultz JC (1983) Habitat selection and foraging tactics in heterogeneous trees. In: Denno RF, McClure MS (eds) *Variable plants and herbivores in natural and managed systems*. Academic Press, New York, pp 61–86
- Schwarz M, Shaw MR (1998) Western Palaearctic Cryptinae (Hymenoptera: Ichneumonidae) in the National Museums of Scotland, with nomenclatural changes, taxonomic notes, rearing records and special references to the British checklist. Part 1. Tribe Cryptini. *Entomol Gaz* 49:101–127
- Stone GN, Lohse K, Nicholls JA, Fuentes-Utrilla P, Sinclair F, Schoenrogge K, Csoka G, Melika G, Nieves-Aldrey JL, Pujade-Villar J, Tavakoli M, Askew RR, Hickerson MJ (2012) Reconstructing community assembly in time and space reveals enemy escape in a western Palearctic insect community. *Curr Biol* 22:532–537
- Sullivan DJ, Völkl W (1999) Hyperparasitism: multitrophic ecology and behavior. *Ann Rev Entomol* 44:291–315
- Tscharntke T (1992) Coexistence, tritrophic interactions and density dependence in a species parasitoid community. *J Anim Ecol* 61:59–67
- Van Laerhoven SL, Stephen FM (2002) Height distribution of adult parasitoids of the spine beetle complex. *Environ Entomol* 31:982–987
- Visser B, Le Lann C, Snaas H, Hardy ICW, Harvey JA (2014) Consequences of resource competition for sex allocation and discriminative behaviors in a hyperparasitoid wasp. *Behav Ecol Sociobiol* 68:105–113
- Weseloh RM (1978) Seasonal and spatial mortality patterns of *Apanteles melanoscelus* due to predators and Gypsy moth hyperparasitoids. *Environ Entomol* 7:662–665
- Wieber AM, Cook SP, Webb RE, Tatman KM, Reardon RC (1995) Niche partitioning by four *Gelis* spp. (Hymenoptera: Ichneumonidae) hyperparasitoids of the primary Gypsy moth parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). *Ann Entomol Soc Am* 88:427–433
- Wieber AM, Webb RE, Reardon RC, Tatman KM (2001) Characterization of the hyperparasitoid complex of *Cotesia melanoscela* (Hymenoptera: Braconidae) at three Maryland locations. *Entomol News* 112:241–254
- Woodcock BA, Vanbergen AJ (2008) Parasitism of the beech leafminer weevil in a woodland: patch size, edge effects and parasitoid species identity. *Insect Cons Div* 1:180–188