Cascading effects of host size and host plant species on parasitoid resource allocation

T E R E S A   M .   S T O E P L E R ,[1]   J O H N   T .   L I L L[1] and S H A N N O N   M . M U R P H Y[2]   [1]Department of Biological Sciences, The George Washington University, Washington, District of Columbia, U.S.A. and [2]Department of Biological Sciences, University of Denver, Denver, Colorado, U.S.A.

Abstract. 1. The bottom-up factors that determine parasitoid host use are an important area of research in insect ecology. Host size is likely to be a primary cue for foraging parasitoids due to its potential influence on offspring development time, the risk of multiparasitism, and host immunocompetence. Host size is mediated in part by host-plant traits that influence herbivore growth and potentially affect a herbivore's quality as a host for parasitoids.

2. Here, we tested how caterpillar host size and host plant species influence adult fly parasitoid size and whether host size influences wasp parasitoid sex allocation. We measured the hind tibia lengths and determined the sex of wasp and fly parasitoids reared from 11 common host species of polyphagous caterpillars (Limacodidae) that were in turn reared on foliage of seven different host plant species.

3. We also tested how host caterpillar species, host caterpillar size, and host and parasitoid phenology affect how the parasitoid community partitions host resources. We found evidence that parasitoids primarily partition their shared hosts based on size, but not by host species or phenology. One index of specialisation (d') supports our observation that these parasitoids are quite generalised within the Limacodidae. In general, wasps were reared from caterpillars collected in early instars, while flies were reared from caterpillars collected in late instars. Furthermore, for at least one species of solitary wasp, host size influenced sex allocation of offspring by ovipositing females.

4. Host-plant quality indirectly affected the size attained by a tachinid fly parasitoid through its direct effects on the size and performance of the caterpillar host. The host plants that resulted in the highest caterpillar host performance in the absence of enemies also yielded the largest parasitoid flies, which suggests that host plant quality can cascade up to influence the third trophic level.

Key words. Bottom-up, Braconidae, Eulophidae, herbivore, host choice, Limacodidae, stage-specific parasitism, Tachinidae, tritrophic interactions.

Introduction

The factors that determine host use by insect parasitoids and their implications for parasitoid fitness are important areas of research in insect ecology and in biological control applications (Caron *et al*., 2010; Henry *et al*., 2010). Host size, which is a proxy for the developmental stage of the herbivorous host, is likely to be a primary cue for foraging parasitoids due to

its potential influence on offspring development time, the risk of multiparasitism, and host immunocompetence (King, 1987; Bukovinszky *et al*., 2009). A number of studies have found that host size is positively correlated with adult parasitoid size (e.g. Lampson *et al*., 1996; Fidgen *et al*., 2000; Teder & Tammaru, 2002, but see Kouamé & Mackauer, 1991), and evidence for a positive relationship between parasitoid size and fitness in a number of parasitoid groups suggests that parasitoid host choice should be under strong selection (Godfray, 1994; Visser, 1994; Nakamura, 1995; Allen & Hunt, 2001; King & Napoleon, 2006). Larger hosts, or hosts with the potential to grow large, are assumed to be

Correspondence: Teresa M. Stoepler, Department of Biological Sciences, The George Washington University, 2023 G St. NW, Suite 340, Washington, DC 20052, U.S.A. E-mail: teresa.stoepler@gmail.com

superior to small hosts because they represent greater resource quantity for parasitoid development (Charnov, 1982; Charnov & Skinner, 1985). However, for parasitoids that are able to attack a range of host sizes, the use of large hosts may result in increased development time relative to smaller hosts (Harvey *et al*., 2004) or may increase parasitoid mortality risk if excess resources cannot be consumed by larvae and they are unable to escape the host's integument to complete development (Harvey, 1996). In addition, because the first parasitoid to attack a host generally outcompetes later-attacking parasitoids in multiparasitised hosts (Hågver, 1988; Bokonon-Ganta *et al*., 1996), this competitive advantage may outweigh the benefits of attacking larger hosts in some cases, which are also less abundant in nature than smaller, early instar hosts (Price, 1972). Studies comparing community-level responses of natural parasitoid assemblages attacking hosts of different sizes are needed to help clarify the importance of host size for parasitoid host selection.

Host size also has the potential to influence parasitoid sex allocation, a component of parasitoid fitness (Charnov *et al*., 1981; Jones, 1982; Tillman & Cate, 1993). In parasitic wasps, sex determination is haplodiploid: fertilised eggs become daughters (diploid) and unfertilised eggs become sons (haploid; reviewed in Heimpel & de Boer, 2008). Therefore, offspring sex is under the direct control of the ovipositing female. Sex allocation theory predicts that a greater proportion of female eggs should be placed in large hosts while male eggs should be preferentially placed in smaller hosts (Charnov, 1982). This theory assumes that host size is directly related to parasitoid size and fitness, and that the fitness consequences of developing within a small host are greater for females than for males (Charnov, 1982).

Host plant nutritional quality (Sarfraz *et al*., 2009) and host plant chemistry (Ode, 2006; Gols *et al*., 2008) also have the potential to affect parasitoid development and performance through their effects on the host. Host plant traits can alter an herbivore's quality as a host for parasitoids by affecting herbivore growth rate and development time (Awmack & Leather, 2002) and plant chemistry can directly affect parasitoid survival and growth (reviewed in Ode, 2006). Because many herbivorous hosts actively sequester plant secondary compounds in their hemolymph or in other tissues (reviewed in Opitz & Müller, 2009), these compounds can be an important environmental factor for the development of parasitoid larvae. However, there is evidence to support both positive (Gentry & Dyer, 2002) and negative (Campbell & Duffey, 1979; Barbosa *et al*., 1986) effects of these sequestered compounds on parasitoid performance. Some parasitoids may also be able to secondarily sequester allelochemicals from their hosts conferring protection against attack by hyperparasitoids (Campbell & Duffey, 1979; Bowers, 2003; Reudler Talsma, 2007), adding another layer of complexity to the process of parasitoid host selection.

The duration of host–parasitoid association, or the length of time from parasitoid oviposition to host death, is of prime importance to parasitoid oviposition decisions and for determining the outcome of competition with other parasitoids. The duration of host association may be influenced by host

size or developmental stage at the time of attack, the existence and timing of host and/or parasitoid diapause, and parasitoid development time (Godfray, 1994). Parasitoid development time is likely based on a combination of factors related to the life history of the parasitoid as well as the quality of the host (Harvey *et al*., 2004), as determined by both its nutritional quality and perhaps the presence/abundance of other parasitoids feeding on the same host (Godfray, 1994; Harvey, 2005). For parasitoids that arrest host feeding and growth, or those that allow their hosts to continue to grow but have a relatively short duration of host association, the host resources at the time of adult oviposition closely approximate those available for offspring development (Askew & Shaw, 1986). However, for parasitoids with longer periods of host association, which attack hosts that will subsequently continue to feed and grow significantly larger, the size of the host at the time of attack may not be a reliable indicator of its final size at the time of parasitoid emergence (Mackauer & Sequeira, 1993). Therefore, parasitoids that are associated with their hosts for longer periods of time should be under selection to make use of other cues related to their host's growth potential (Harvey & Vet, 1997; Li & Mills, 2004).

Parasitoids with prolonged host association are contained within the host's body as the host continues to feed and grow on the host plant. For polyphagous or oligophagous herbivores, host plant association can have strong repercussions for both herbivore growth rate and body mass (Barbosa & Greenblatt, 1979; Parry & Goyer, 2004; Singer *et al*., 2004; Rajapakse & Walter, 2007). Some generalist herbivore species are polyphagous at the population level, but individual larvae are functionally monophagous because they are confined to the host plant that neonates first encounter through female oviposition of via larval dispersal (e.g. ballooning) (Zalucki *et al*., 2002). Thus, larvae that are confined to a single host plant individual for the course of their development would be expected to vary in performance on different host plant species in response to the relative quality of that host plant. With regard to parasitoids with prolonged periods of host association that use these types of generalist herbivores as hosts, we might expect them to choose hosts based on environmental and host plant cues that would allow them to assess future herbivore performance (i.e. to select those hosts and hosts plants that confer the greatest growth potential).

Although host choice is relatively well studied in the parasitic Hymenoptera, fewer studies have examined the factors that affect host choice in tachinid flies, many of which remain within their hosts for prolonged periods as the host continues to grow and develop after attack (Stireman *et al*., 2006). In theory, this life history feature allows tachinids to attack a wider range of host species and/or developmental stages (Harvey *et al*., 1994). However, few previous studies have directly compared the host stages attacked by parasitoids with variable life-history strategies or have investigated the consequences of attacking different host stages on parasitoid fitness components, particularly in natural systems (see also Kato, 1994; Lill, 1999; Lee & Pemberton, 2007).

Here, we focus on the community of fly and wasp parasitoids that specialise on one group of polyphagous herbivores

(Limacodidae, slug caterpillars) to examine how parasitoids partition host resources and respond to variation in host quality. The objectives of this study were to answer three main questions. (i) How does a community of parasitoids sharing a common set of caterpillar hosts partition these resources? (ii) Does host size influence adult parasitoid size and/or sex allocation and do flies and wasps respond to host size in the same manner? (iii) For a common host caterpillar species, how does host plant quality affect adult parasitoid size? (i.e. does host plant quality 'cascade up' to higher trophic levels?). We tested whether parasitoids partitioned host caterpillars based on caterpillar host species, host size, and host and parasitoid phenology. We hypothesised that the community of oligophagous parasitoids that share limacodid caterpillars as hosts partitions these resources based on size; we therefore predicted that wasps would attack early instars (small hosts) and flies would attack late instars (large hosts). We hypothesised that wasps, which are haplodiploid and can choose the sex of their offspring, would choose larger hosts for their daughters than for their sons. Finally, we hypothesised that indirect effects of host plant species would be most likely to be manifested in tachinid fly parasitoids with extended host association periods. Specifically, we predicted that the host plants on which caterpillars performed best would also be the ones on which the flies performed best when reared from caterpillars feeding on these host plants.

Materials and methods

Study system

Caterpillars in the family Limacodidae feed externally on mature foliage June–October in the eastern U.S.A. Many species are brightly coloured (aposematic) and have both physical and chemical defences, including stinging spines, while others are behaviourally and morphologically cryptic (Murphy *et al.*, 2010). Taken together, these traits suggest that natural enemies have played an important role in their evolution. Indeed, limacodid caterpillars suffer high mortality by parasitoids [30–85% from both parasitic flies (Tachinidae) and wasps (mainly Eulophidae, Braconidae and Ichneumonidae; T. M. Stoepler, unpublished)]. Limacodids are among the most polyphagous caterpillars known and individual species can feed on dozens of plant species (Wagner, 2005). However, each individual caterpillar is largely restricted to feeding on the individual host plant chosen by its mother (Wagner, 2005), which makes this an excellent system for studying both natural enemy and host plant effects on larvae in the field.

The entire community of parasitoids that use limacodid caterpillars as hosts in North America has recently been circumscribed (Gates *et al.*, in press). All of the primary parasitoids of limacodid caterpillars in our study system are technically classified as koinobionts (although there are both ecto- and endoparasitoids), despite significant variation in life-history characteristics associated with each. For this study, we focus on the numerically dominant species at our field site: *Triraphis discoideus* Cresson (Rogadinae: Braconidae:

Hymenoptera), *Platyplectrus americana* Girault (Eulophinae: Eulophidae: Hymenoptera), *Uramya pristis* Walker (Dexiinae: Tachinidae: Diptera), and *Austrophorocera* sp. Townsend (Exoristinae: Tachinidae: Diptera) (hereafter all species are referred to by genus). All four of these parasitoid species are oligophagous, specialising on larvae in the family Limacodidae. *Platyplectrus* is a gregarious larval ectoparasitoid that pupates beneath the host's remains (Gates *et al.*, in press). *Triraphis* is a solitary larval endoparasitoid that emerges from the mummified remains of its host (Kula *et al.*, 2009). *Uramya* is a solitary endoparasitoid and like many tachinids, *Uramya* females are ovolarviparous; the eggs they lay contain first-instar larvae that hatch immediately (Gates *et al.*, in press). *Uramya* emerge from late-instar larvae and typically overwinter as pupae, emerging as adults the following summer (J. T. Lill, pers. obs.). Finally, *Austrophorocera* is a larval–prepupal endoparasitoid that overwinters inside the host prepupa and emerges just prior to host pupation (T. M. Stoepler, pers. obs., see below), probably after the host breaks diapause, with adults typically emerging after overwintering (Murphy *et al.*, 2009). The taxonomic status of *Austrophorocera* is currently being investigated. Adult parasitoid body length ranges, from smallest to largest, are as follows: *Platyplectrus*, 1.5–3 mm; *Triraphis*, 2–6 mm; *Uramya*, 7–12 mm; and *Austrophorocera*, 8.5–13 mm.

To verify the stage at which *Austrophorocera* kills its hosts, four host cocoons that were attacked by *Austrophorocera* as larvae in summer 2010 were carefully opened on 8 April 2011. Cocoons represented two hosts used in the current study, *Euclea delphinii* ($n = 3$) and *Lithacodes fasciola* ($n = 1$). If *Austrophorocera* killed its host prior to the spring following host parasitism, these cocoons would each contain an *Austrophorocera* pupa. Alternatively, if *Austrophorocera* had not yet killed its host by this time, these cocoons would each contain a host prepupa; this would indicate that *Austrophorocera* parasitoids have periods of host association of *at least* 6 months even if they were parasitised at the end of the summer in 2010. All four cocoons contained living host prepupae, indicating the latter scenario holds for this species. Cocoons were monitored daily for *Austrophorocera* larval and pupal development. On 21 April, two of the three *E. delphinii* prepupal hosts that survived cocoon dissection were killed when a single *Austrophorocera* larva emerged from each prepupa. These tachinid larvae pupated within 1–3 days. The other host, *L. fasciola*, remained a living prepupa for at least 4 weeks following cocoon dissection. These observations confirm that *Austrophorocera* has an extended period of host association prior to killing its host (a minimum of 6 months) and does not complete larval development until the spring following host attack.

The four species of parasitoids exhibit significant variation in life-history strategies. Both tachinid flies have prolonged host association periods and emerge from either late instars in the case of *Uramya* or prepupae in the case of *Austrophorocera*. It appears that *Austrophorocera* is highly attuned to its host's phenology and typically emerges following overwintering, however, one of its host species (*Euclea*) has a partial second generation causing some *Austrophorocera* to emerge in the

same year as they attack their hosts. Yet, for both non-overwintering and overwintering *Austrophorocera*, the host association period is quite prolonged. The mean number of days from host collection to host death ±1 SE for non-overwintering *Austrophorocera* is 50 ± 2; for overwintering *Austrophorocera*, the host association period is a minimum of 180 days but the exact duration is unknown because host death occurs inside the host cocoon. Similarly, for *Uramya*, which emerges the same year as it attacks the host, the duration of host association is prolonged (mean number of days from host collection to host death ±1 SE: 55 ± 17). In contrast, both wasps typically emerge shortly after attack and have much shorter periods of host association (mean number of days from host collection to host death ±1 SE: *Triraphis*: 13 ± 1, *Platyplectrus*: 19 ± 1).

Rearing

Parasitoids were reared from 11 species of host larvae in the family Limacodidae (Lepidoptera): *Acharia stimulea* Clemens, *Adoneta spinuloides* Herrich-Schäffer, *Euclea delphinii* Boisduval, *Isa textula* Herrich-Schäffer, *Isochaetes beutenmuelleri* Edwards, *Lithacodes fasciola* Herrich-Schäffer, *Natada nasoni* Grote, *Packardia geminata* Packard, *Parasa chloris* Moore, *Phobetron pithecium* J. E. Smith, and *Prolimacodes badia* Hübner (hereafter all species are referred to by genus) over 7 years (2004–2010) as part of our lab's ongoing studies on the ecology of the Limacodidae. Host larvae were a mix of wild-caught larvae collected in the greater Washington DC area at Little Bennett Regional Park, MD, Rock Creek Park, Washington, DC, Plummers Island, MD, and Patuxent National Wildlife Refuge, MD and larvae that were reared from lab colonies and then experimentally exposed to parasitism at these sites (Table 1). The date that each larva was collected from the field

was recorded. All experimentally exposed larvae were fed in the lab with excised foliage of the same host plant species on which they were placed in the field, both prior and subsequent to exposure to parasitism. After collection, wild-caught larvae were also reared in the lab on excised foliage from the same host plant from which they were collected. In total, larvae were reared on leaves of seven different host plants: *Carya glabra* P. Mill (pignut hickory, Juglandaceae), *Fagus grandifolia* Ehrh. (American beech, Fagaceae), *Nyssa sylvatica* Marsh (black gum, Cornaceae), *Prunus serotina* Ehrh. (black cherry, Rosaceae), *Quercus alba* L. (white oak, Fagaceae), *Quercus prinus* L. (chestnut oak, Fagaceae), and *Quercus rubra* L. (northern red oak, Fagaceae) (hereafter all plant species are referred to by their common names). All host larvae were brought back to the lab, their body lengths measured with calipers to the nearest 0.1 mm, and were reared individually in clear plastic deli containers with moistened filter paper discs and fresh leaves provided as needed. The body length of each larva at the time of collection (longest body axis) was used as a proxy for body mass and developmental stage. Instar cannot be reliably determined for limacodids, which can have a variable number of instars (6–13) depending on the species (see references in Murphy *et al.*, 2011). Larvae were monitored for parasitism throughout development. If an adult parasitoid emerged, the date of emergence was recorded and the parasitoid was placed in a $-20\ ^{\circ}$C freezer and later mounted.

As part of a separate experiment in summer 2008, groups of larvae of one host species, *Euclea*, were reared within sleeve cages (protected from predators and parasitoids) in the field at Little Bennett Regional Park on each of the host plant species listed above with the exception of chestnut oak ($n = 25$ per host plant species). Sleeve cages containing larvae were moved around within the canopy of the host plant as needed to avoid food limitation and when larvae pupated, their

Table 1. Limacodid larval hosts used in this study, the range of sizes (larval lengths) at which they were collected from the field, corresponding to the upper size limit at which they were parasitised, the parasitoid taxa that have been successfully reared from each host, and estimated larval density.

Host species	Size range collected (mm)	Experimentally-exposed (E) or wild-caught (W)	Parasitoid taxa	Mean density ±SE (larvae per 10 m² foliage)
Acharia stimulea	4.0–23.6	E, W	P, T, U, A	0.046 ± 0.041
Adoneta spinuloides	2.0–8.0	W	P, T	0.064 ± 0.031
Euclea delphinii	3.0–21.9	E, W	P, T, U, A	0.019 ± 0.011
Isa textula	1.0–16.0	W	P, T, U	0.623 ± 0.100
Isochaetes beutenmuelleri	10.0	W	U	0^{*}
Natada nasoni	2.1–6.0	W	T	0.451 ± 0.099
Packardia geminata	2.0–6.0	W	P, U	0.007 ± 0.004
Parasa chloris	4.0	W	T	0.010 ± 0.010
Phobetron pithecium	3.5–4.0	W	P	0.019 ± 0.015
Prolimacodes badia	2.0–13.0	W	T, U, A	0.218 ± 0.073
Lithacodes fasciola	1.5–11.0	E, W	P, T, U, A	0.211 ± 0.058

*Never collected during censuses.

Parasitoid taxa: A, *Austrophorocera* sp. (Tachinidae: Diptera); P, *Platyplectrus americana* (Eulophidae: Hymenoptera); T, *Triraphis discoideus* (Braconidae: Hymenoptera), U, *Uramya pristis* (Tachinidae: Diptera).

Host species were either experimentally exposed (E), wild-caught (W), or were a mixture of both (E, W). Larval density is an estimate across six of the primary host plant species (*Prunus serotina*, *Quercus alba*, *Q.rubra*, *Fagus grandifolia*, *Nyssa sylvatica*, and *Carya glabra*) based on manual censuses of over 431 000 leaves over 4 years (2007–2010).

cocoons (pupa + cocoon) were weighed to the nearest 1 mg. The proportion of caterpillars surviving on each host plant in the field was also calculated. These data were used to determine the effect of host plant on caterpillar cocoon mass, survival, and performance. Performance was estimated here as mean cocoon mass × proportion of *Euclea* caterpillars surviving to cocoon formation in the absence of enemies based on cohorts of caterpillars reared in sleeve cages on each host plant in the field. Cocoon mass (cocoon + pupa), a proxy for pupal mass, can be used as a lifetime fitness measure (Slansky & Scriber, 1985; Lill, 2001; Murphy, 2007; Murphy *et al.*, 2011) and cocoon mass × survival captures the full host plant effect on host fitness.

Host and parasitoid phenology

If parasitoid phenology was such that the parasitoid species that attacked early instars (small larvae) only foraged early in the season and those that attacked late instars (large larvae) only foraged late in the season, parasitoids may not be partitioning hosts based on size but instead may simply be attacking host species and developmental stages based on their availability. To isolate host size from the confounding effects of host and parasitoid phenology, we examined graphically the temporal overlap among the focal limacodid host and parasitoid species detailed in this study based on 7 years of data from local study sites. Specifically, we determined (i) the median and range of dates that each wild-caught host species was collected and (ii) the median and range of dates that host larvae were collected that yielded each of the four focal parasitoid species over the 7-year sampling period.

Parasitoid measurements

Mounted parasitoids were photographed using an Olympus SZX16 stereo microscope fitted with a high-resolution digital camera (Olympus, DP71). Parasitoid hind tibia length was measured from these photographs to the nearest 0.01 mm using ImageJ (Rasband, 2008) for the tachinids (*Austrophorocera* and *Uramya*) and for the braconid wasp, *Triraphis*, after calibrating the scale with a slide micrometer. Several studies have demonstrated a positive correlation between hind tibia length and body mass in parasitoid wasps (Harvey & Vet, 1997; Nicol & Mackauer, 1999) and tachinid flies (Reitz & Adler, 1995). *Platyplectrus*, which is minute and gregarious, was not included in parasitoid size measurements or parasitoid sex data because parasitoid clutch size per host may confound these measurements and incomplete emergence complicates sex ratio data for this species. It is important to note that because this study focuses only on emerged parasitoids (realised parasitism), we are investigating the *outcome* of the host–parasitoid interaction. Throughout this paper, 'host size' refers to the body length (mm) of the caterpillar host at the time of collection from the field, a proxy for developmental stage. All parasitoids are deposited in the Smithsonian Institution National Museum of Natural History, Washington, DC.

Data analysis

To test the hypothesis that parasitoids partition their shared limacodid hosts based on size, a Kruskal–Wallis test was performed with host size as the response variable and parasitoid taxon as the predictor (class) variable; this analysis jointly considered all limacodid host species. Because some of the host species are probably too small to support the development of the largest fly species (*Austrophorocera*), a second Kruskal–Wallis test was performed as above with host size as the response variable and parasitoid taxon as the predictor variable but this time excluding the three host species with the smallest maximum larval sizes, *Adoneta*, *Packardia*, and *Isochaetes*.

To test whether parasitoids specialise on certain limacodid host species, the degree of species-level specialisation of each parasitoid species was measured as d', the standardised Kullback–Leibler distance, a network analysis index derived from Shannon diversity (Blüthgen *et al.*, 2006). Here, d' is essentially the deviation from a null model of interaction frequencies that assumes that all parasitoids emerge from hosts in proportion to their estimated abundance. To quantify parasitoid specialisation in this way, a contingency table of the frequency that each of the 4 parasitoid species were reared from the 11 host species was created and this observed table was compared to randomised tables possessing the same row and column totals using Monte Carlo sampling (10^4 randomisations). These calculations were performed online at http://his.biologie.huberlin.de/~nils/stat/(Blüthgen *et al.*, 2006). The standardised Kullback–Leibler distance, d', ranges from 0.0 to 1.0, from extreme generalisation to extreme specialisation, respectively. Using this scheme, a parasitoid would be classified as a specialist if it emerged from hosts more often than expected based on the host's relative abundance but not if it opportunistically parasitised the most abundant host. This method of testing for specialisation in species interactions has several advantages; d' values can be compared across studies and are robust to differences in matrix size and structure (Blüthgen *et al.*, 2006).

In addition, for each of three host species for which sufficient data were available (*Acharia, Euclea* and *Lithacodes*), three separate one-way ANOVAS were performed with host size as the response variable and parasitoid taxon as the predictor variable to test whether host size effects remained after the effect of host species was removed. When significant, these ANOVAS were followed by Tukey's HSD *post hoc* comparisons to determine which pairs differed.

Students' *t*-tests were used to test whether male and female parasitoid hind tibia length differed. A *t*-test was used to test the hypothesis that within one species of solitary wasp, *Triraphis*, females emerge from significantly larger caterpillar hosts than males. All *t*-tests were two-tailed and assumed equal variances (Levene's test was used prior to analyses to test this assumption). Linear regression was also used to test the relationship between *Austrophorocera* (parasitoid fly) mean hind tibia length and *Euclea* (caterpillar host) mean performance when reared from six of the most common host
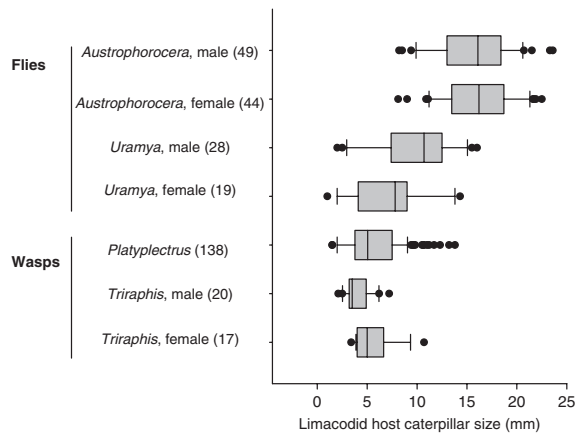
Fig. 1. Parasitoid resource partitioning by host size at time of collection for two tachinid fly species (*Austrophorocera* sp. and *Uramya pristis*) and two wasp species (*Platyplectrus americana* and *Triraphis discoideus*). All solitary parasitoids are separated by sex; *Platyplectrus americana*, the only gregarious species, is not separated by sex. Numbers in parentheses following parasitoid species names are number of reared parasitoids. Hosts include all 11 species of limacodid caterpillars examined in the study. Each box represents the 25th and 75th percentiles of host sizes used; the middle bar is the median. Whiskers contain the lowest and highest data points within 1.5 interquartile range (IQR) of the lowest and highest quartiles, respectively. Filled circles represent outliers.

plant species. All statistical analyses were performed in JMP®, V. 8.0.2 (SAS Institute Inc., 2006).

Results

Caterpillar host size

Parasitoids of different species emerged from hosts of significantly different sizes ($H = 190.847$, d.f. $= 3$, $P < 0.001$), spanning the full range of limacodid larval host sizes available in nature (1.5–23.6 mm, Fig. 1). When the three host species with the smallest maximum sizes (*Adoneta*, *Packardia*, and *Isochaetes*) were removed from the analysis, host size partitioning among parasitoid species was still apparent ($H = 188.70$, d.f. $= 3$, $P < 0.001$) and the relative rankings of the parasitoids in terms of host size ranges (Fig. 1) did not change. The two tachinid fly species differed markedly in that *Uramya* was reared from larvae collected in a wide range of sizes while *Austrophorocera* was only reared from larvae collected as late instars (median host size >15 mm; Fig. 1). By contrast, most of the wasp parasitoids were reared from larvae collected in early instars (median host size ≤ 5 mm; Fig. 1).

Host and parasitoid phenology and host species

The phenology of limacodid larvae and their parasitoids overlapped considerably, with most larvae available from early July until early September, during which parasitoids
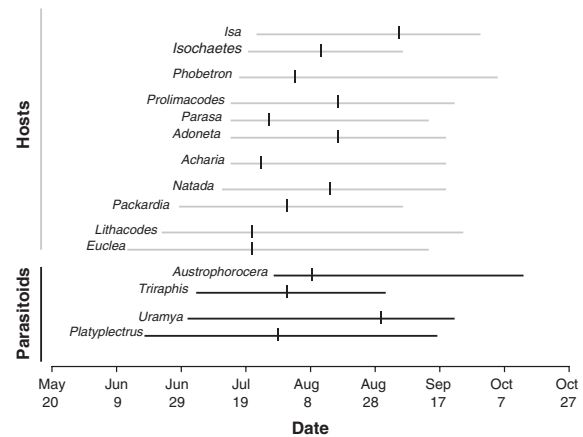

Fig. 2. Limacodid host larvae and parasitoid phenology, 2004–2010. 'Hosts' (in grey): each horizontal line represents the range of dates that each species of wild-collected limacodid larvae has been found on foliage at the study sites during sampling censuses. 'Parasitoids' (in black): each horizontal line represents the range of dates that a limacodid host larva was collected (both wild-caught and experimentally deployed larvae) from which a parasitoid of each species successfully emerged. For both hosts and parasitoids, black tick marks represent the median date of host collection for each species. Genera are used as abbreviations but each represents a single species (listed in Materials and methods). Note that the maximum date of host collection that yielded *Austrophorocera* sp. is later than any of the larval maximum dates because parasitoid data include experimental larvae that were deployed later than they would naturally occur, but were still attacked by *Austrophorocera* sp.

had a wide range of developmental stages and sizes of host caterpillars to choose from (Fig. 2). The phenology of parasitoids overlapped such that from 27 July through 30 August (34 days), all four parasitoid species were recorded from host collections (Fig. 2). During this period, all 11 species of limacodid larvae are available, including a range of small (e.g. *Isa*, *Isochaetes*), medium (e.g. *Acharia*, *Adoneta*) and large (e.g. *Euclea*, *Lithacodes*) hosts. Of the hosts, *Euclea* and *Lithacodes* larvae were recorded from the greatest range of dates, both representing a 94-day period (Fig. 2). *Platyplectrus* was recorded from the greatest range of host collection dates of all the parasitoids, representing a 91-day period. The median dates of host collection for three of the four parasitoids, *Triraphis*, *Platyplectrus*, and *Austrophorocera*, all fall within an 11-day period between 27 July and 7 August, which roughly corresponds to the peak in limacodid host density in the area (Fig. 2, Murphy *et al.*, 2011).

Host species use also greatly overlapped among parasitoid species. When considering the estimated relative abundance of each host species, the degree of parasitoid specialisation (measured as d') was low, particularly for *Platyplectrus* and *Austrophorocera* (Table 2). In comparison, using the d' metric, *Uramya* and *Triraphis* were relatively more specialised in terms of host species use (Table 2). However, all four parasitoids have been recorded to emerge from larvae of three of the same host species at the study sites, *Euclea*, *Acharia*, and *Lithacodes*, and seven of the 11 host species

Table 2. Species-level specialisation of each of the four parasitoid taxa on 11 limacodid host caterpillars based on standardised Kullback–Leibler distances, d'.

Parasitoid taxon	Parasitoid d'
Tachinid flies:	
Austrophorocera sp.	0.265
Uramya	0.464
Wasps:	
Platyplectrus	0.232
Triraphis	0.405
Weighted average	0.302

Values may range from 0.0 to 1.0, indicating extreme generalisation (0.0) to extreme specialisation (1.0). See Materials and methods for list of host species and a complete description of this metric.

Fig. 3. Sex allocation according to host caterpillar size in *Triraphis discoideus*. Each circle represents an emerged *Triraphis* wasp: black circles represent females ($n = 17$); white circles represent males ($n = 20$). Hosts include eight species of limacodid caterpillars.

are shared by at least two of these four parasitoids (Table 1). Quantified in terms of the total number of host species that each parasitoid species was reared from (irrespective of relative host abundance), *Triraphis* was reared from a total of eight limacodid host species, *Platyplectrus* and *Uramya* were each reared from seven host species, and *Austrophorocera* was reared from 4 of the 11 limacodid host species (Table 1). *Austrophorocera* is the largest parasitoid, so its narrower host size range may partly reflect size constraints imposed by the smaller host species.

Within each of the three host species tested, the species of parasitoid that emerged was significantly affected by host size (*Acharia*: $F_{3,45} = 50.25$, $P < 0.001$; *Euclea*: $F_{3,148} = 139.52$, $P < 0.001$; *Lithacodes*: $F_{3,36} = 14.08$, $P < 0.001$), which indicates a significant effect of host size even after controlling for host species. For all three of these host species, this pattern was driven by *Austrophorocera* emerging from hosts that were on average 2.5–2.7 times larger at the time of collection than the hosts of the other three parasitoid species (Tukey's HSD test, $P < 0.05$). Within both *Acharia* and *Lithacodes*, there was a trend that the wasps, *Triraphis* and *Platyplectrus*, emerged from the smallest hosts while *Uramya* emerged from intermediate-sized hosts, although this pattern was not significant. Within *Euclea, Triraphis*, *Platyplectrus*, and *Uramya* all emerged from hosts collected at similarly small sizes.

Parasitoid sexual dimorphism in size

In both fly species, emerging males were significantly larger than females. In *Austrophorocera*, male hind tibias were 5% longer on average than those of females ($t_{87} = 2.181$, $P = 0.039$, male: 2.42 ± 0.038 mm, female: 2.30 ± 0.040 mm); in *Uramya*, male hind tibias were 10% longer on average than those of females ($t_{49} = 2.239$, $P = 0.03$, male: 2.15 ± 0.059 mm, female: 1.95 ± 0.065 mm). The opposite pattern was found for the wasp, *Triraphis*; female hind tibias were 46% longer on average than those of males ($t_{47} = -4.944$, $P < 0.001$, female mean hind tibia length: 1.08 ± 0.050 mm, male: 0.738 ± 0.049 mm).
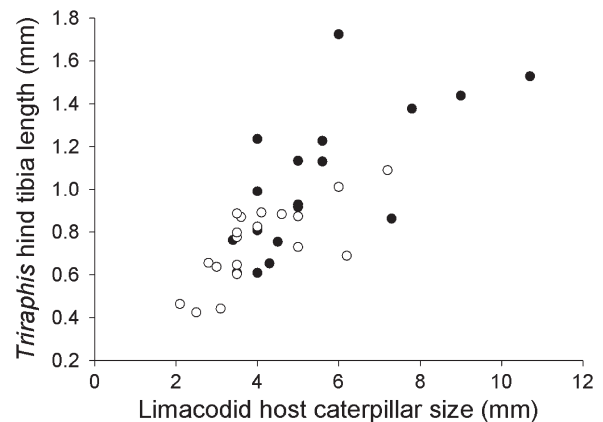
Parasitoid sex allocation

When considering all host caterpillar species together, caterpillar host size affected sex allocation by the solitary braconid wasp, *Triraphis. Triraphis* females emerged from hosts that were 40% larger on average than the hosts of emerged males and this difference was highly significant ($t_{35} = -2.90$, $P = 0.006$; female hosts: 5.60 ± 0.40 mm, male hosts: 4.01 ± 0.37 mm; Fig. 3). The caterpillar hosts from which *Triraphis* wasps successfully emerged ranged from 3.4 to 10.7 mm in length for females and 2.1 to 7.2 mm for males (Fig. 3), which suggests a lower threshold in host size below which female eggs are either not laid or do not survive to emergence and an upper threshold above which male eggs are either not laid or do not survive to emergence. Although our sample sizes were not sufficient to statistically test for an effect of host size on sex allocation within a host species, the pattern we found for all caterpillar species combined appears to hold even within host species. For example, of the nine wasps that emerged from *Euclea*, the host species that has the potential to grow the largest of those that produced *Triraphis* wasps, eight wasps were female and only one was male. Similarly, the host caterpillar species that do not have the potential to grow as large (e.g. *Lithacodes*, *Natada*, and *Isa*) produced mostly male wasps (19 out of 30 wasps reared from these three hosts were male).

Host plant species

Host plant species indirectly affected parasitoid size through direct effects on host caterpillar size and performance. The relationship between *Euclea* mean cocoon mass and *Austrophorocera* mean hind tibia length on each host plant was positive, although not significant ($R^2 = 0.391$, d.f. $= 5$, $P = 0.18$). This result was primarily driven by white oak and hickory, which appear to be outliers on which *Austrophorocera* flies attain a larger size than predicted from the mean cocoon mass of their hosts. When white oak and
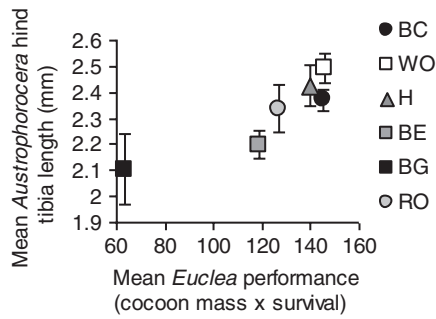
Fig. 4. Correlation between *Euclea delphinii* host caterpillar mean performance in the absence of enemies and *Austrophorocera* sp. adult mean hind tibia length for parasitoids emerged from exposed hosts reared on six different host plants (BC, black cherry; BE, American beech; BG, black gum; H, pignut hickory; RO, red oak; WO, white oak). Error bars represent ±1 SE for hind tibia length.

hickory are removed, the positive relationship between *Euclea* mean cocoon mass and *Austrophorocera* mean hind tibia length is significant ($R^2 = 0.948$, d.f. = 3, $P = 0.027$). The relationship between *Euclea* performance, considering cocoon mass and survival together, and *Austrophorocera* mean hind tibia length on each host plant was stronger. The host plants on which *Euclea* caterpillars performed best in the absence of enemies (black cherry, white oak, and pignut hickory) yielded the largest *Austrophorocera* flies ($R^2 = 0.799$, d.f. = 5, $P = 0.016$; Fig. 4).

Discussion

Caterpillar host size

The limacodid parasitoid community appears to partition their hosts based on size. The solitary wasp, *Triraphis*, attacked the smallest hosts (early instars), the gregarious wasp, *Platyplectrus*, and the tachinid fly, *Uramya*, attacked hosts of intermediate size and the tachinid fly, *Austrophorocera*, attacked the largest hosts (late instars). It is important to note that these results were based on the size of the host when it was collected from the field, but the exact size of the host at the time of attack was unknown (equal to or less than the size at collection). In particular, for the tachinid flies that exhibit prolonged periods of host association during development, the lower limits or minimum host sizes at collection that yielded a fly are more meaningful than the upper limits or maximum host sizes because the fly could have attacked the host at any time prior to host collection. In contrast, for the wasps that develop and emerge relatively quickly, both the lower and upper limits of host size are important. Because using host size at time of collection provides a conservative estimate of actual size differences at the time of attack, the fact that we still see strong differences in host sizes that are attacked by these four parasitoids is compelling evidence for host partitioning based on size.

Given that all four of these parasitoids are specialists on caterpillars in the family Limacodidae, interspecific competition for host resources in this system may select for partitioning of development stages among parasitoid species (Price, 1972; Briggs, 1993; Harvey, 2005). Limacodid larvae naturally occur at our study site at low densities (<1 larva per 10 m² foliage; Table 1) so foraging parasitoids that share these hosts should be selected to minimise multiparasitism by specialising on a specific developmental size range. Early-attacking parasitoids effectively remove host individuals from the population and thereby reduce the availability of potential hosts for later-attacking parasitoid species, a form of interference competition. However, if late-attacking tachinid flies avoid small hosts, tachinids can minimise the risk of choosing a host that has already been parasitised by an early-attacking wasp. Captive trials with both wasp and tachinid fly parasitoid colonies are needed to differentiate between parasitoid host preference and host suitability.

Host and parasitoid phenology and host species

These four parasitoid species do not appear to partition their hosts based on host species or phenology. It is clear that hosts are available in a range of sizes during the time these parasitoid species are foraging and that the phenology of all four parasitoids overlaps considerably. Notably, three of the parasitoids that attack the smallest (*Triraphis*, *Platyplectrus*) and largest (*Austrophorocera*) hosts have been reared from hosts collected within a median date range spanning only 11 days, suggesting that each parasitoid has a range of host sizes and species available to attack. The parasitoid species reared latest in the season (based on median date of host collection), *Uramya*, attacks intermediate-sized hosts even though many larger/later instar hosts are available at this time. *Uramya* may preferentially attack smaller hosts than *Austrophorocera* as these hosts may provide more time for *Uramya* larvae to complete development before the host reaches the prepupal stage. Similarly, *Platyplectrus* has been reared from small hosts collected in mid-September when most hosts are large, which suggests that this wasp species either avoids large hosts or unsuccessfully parasitises them.

All four parasitoids were relatively generalised in terms of their host use within the Limacodidae, with d' values similar to those recorded for generalised seed dispersal mutualisms between various animal and plant species (Blüthgen *et al.*, 2007). When controlling for host species and when excluding the host species with the smallest maximum larval size ranges, host partitioning based on size was still apparent. The lack of *Austrophorocera* rearings from the three smallest host species may reflect adaptive avoidance of these hosts, which are unlikely to provide sufficient resources for a single fly to complete development. Alternatively, *Austrophorocera* may attack other limacodid species but their offspring are unable, or rarely able, to survive to emerge from smaller host species. However, the three species that we tested for a host species effect, and that make up a significant proportion of the data, are large enough to support *Austrophorocera* development. Together, these results suggest that parasitoids do not simply attack the hosts that are available at the time they are foraging,

but rather that they are either actively choosing or differentially surviving to emerge from hosts of different sizes. Planned experiments that simultaneously expose hosts of different sizes to parasitoid attack on shared host plants will help clarify these empirical findings.

Potential selective pressures favouring the use of small hosts over larger ones include the higher abundance of early-stage larvae in nature (Price, 1972) and less-developed innate immune responses in smaller hosts (Strand & Pech, 1995). Previous studies have shown that host defences against wasp parasitoids, including encapsulation, are heightened in late instars compared to early instars in some species (e.g. Brodeur & Vet, 1995). Wasps may avoid late instar hosts due to increased risk of encapsulation. In contrast, flies may minimise direct competition for hosts and multiparasitism by foraging slightly later in the season when the late instar hosts they find have presumably avoided or survived wasp parasitism. Unlike many wasps whose eggs are injected into the host's haemolymph, tachinid fly eggs are laid externally and hatch quickly (Stireman *et al.*, 2006). Tachinid larvae must develop on the host cuticle before entering the host (Stireman *et al.*, 2006), so smaller host caterpillars which moult more often (Dyar, 1890) may be more likely to moult newly laid eggs or larvae from the cuticle. Mobile tachinid fly larvae are likely to be less subject to encapsulation and other host immune responses than static was eggs because they often reside in specific tissues within the host, instead of circulating in the haemocoel (Belshaw, 1994). Indeed, many tachinid species including *Austrophorocera*, co-opt the host's encapsulation response to construct a breathing tube ('respiratory funnel') that provides continuous outside oxygen flow (Vinson, 1990; Stireman *et al.*, 2006). Selective pressures likely to favour the use of larger or later instar hosts include greater apparency to visually oriented parasitoids such as tachinids, as well as the greater degree of plant damage caused by large hosts that may serve as a cue in host location (Turlings *et al.*, 1990), and a significant reduction in the risk of intraguild predation and multiparasitism subsequent to attack (Price, 1972).

Parasitoid sexual dimorphism in size

Our results for *Triraphis* corroborate other studies that show that female wasps are larger and emerge from larger hosts than do males of the same species (reviewed in King, 1989). Host size-dependent sex allocation is predicted to be more common in parasitoids that attack non-growing host stages (idiobionts) but has also been shown to occur in some koinobionts (King, 1989). We argue that although *Triraphis* is technically a koinobiont, because it attacks and kills the host quickly leaving little time for the host to continue to grow, the size of the host at the time of attack remains a strong predictor of the host's final size. Size in female wasps has been positively correlated with several measures of parasitoid fitness including egg number, egg size, fecundity, search efficiency, and longevity (King, 1987; Godfray, 1994). However, this is the first study to our knowledge to show this pattern for a rogadine braconid wasp. This is also one of only a few studies

to show the opposite pattern for two species of tachinid flies: both *Austrophorocera* and *Uramya* males were larger than females in terms of hind tibia length. Several studies have reported larger tachinid body size in females compared to males (McLain *et al.*, 1990; Pintureau & Grenier, 1992; Gross *et al.*, 1996), while others have reported larger body size in males (Adamo *et al.*, 1995; Allen & Hunt, 2001). Although not recorded for the two tachinid species used in the current study, in other tachinids that form leks, large males have been shown to gain a mating advantage (Lederhouse *et al.*, 1976; Alcock & Kemp, 2006), suggesting that male size may be under greater selection pressure than female size in some tachinid species.

Sex allocation

In at least one parasitoid, the wasp *Triraphis*, caterpillar host size influences sex allocation of offspring by females. *Triraphis* females emerged from significantly larger hosts than males, suggesting one of two possibilities: (i) foraging wasps chose significantly larger hosts for their daughters than for their sons (primary sex ratio alteration), or (ii) female offspring suffer differential mortality within small hosts (secondary sex ratio alteration). Because this study focused on emerged parasitoids only, we are unable to separate these two alternative mechanisms, however, our results corroborate many studies that have shown that females emerge from larger hosts than males (e.g. Jones, 1982; Seidl & King, 1993; Croft & Copland, 1995; Ueno, 1998; Wang *et al.*, 2008).

Host plant species

Host plant quality indirectly affects parasitoid size, and presumably parasitoid fitness, through its direct effects on the size and performance of the caterpillar host (Ode, 2006). These differences could contribute to host plant-specific differences in parasitoid foraging if parasitoids with prolonged host association periods can use host plant traits as reliable indicators of the future growth potential of their herbivorous hosts. Host plant effects are most likely to 'cascade up' in parasitoids with prolonged host association periods (e.g. *Austrophorocera* and other larval–prepupal or larval–pupal parasitoids) where host plant effects on host caterpillars are fully realised. Host plant effects on parasitoid fitness have been found in other studies (e.g. Barbosa *et al.*, 1991; Teder & Tammaru, 2002; Holton *et al.*, 2003; Campan *et al.*, 2005; Caron *et al.*, 2008); however, these studies either focused on intraspecific host plant differences or compared only two species of host plants. Ours is one of the first studies to demonstrate host plant effects across multiple plant species on parasitoid fitness (see also Lampert & Bowers, 2010).

Significant host plant effects on parasitism frequencies are evident in this system (J. T. Lill and S. M. Murphy, in prep.), however, it is unclear whether foraging parasitoids use host plant or host caterpillar cues (or both) to locate and select hosts. Given that host plant species affects the adult size attained by at least one parasitoid species, parasitoids may be able to use the host plant as an indicator of host abundance, host quality,

or potential host quality. If this is the case, parasitoids should be selected to preferentially search the host plants on which host caterpillars perform best or occur on most frequently. However, host plant nutritional quality has also been shown to affect encapsulation ability, an important immune defence against developing parasitoids (Ojala *et al.*, 2005; Lee *et al.*, 2006; Srygley *et al.*, 2009). If hosts feeding on higher quality host plants have greater immune defences, foraging parasitoids may face a trade-off between high quality host resources and the risk of host immune responses.

Conclusions

In conclusion, we have shown that tachinid fly and wasp parasitoids partition their shared host caterpillar resources based on host size and that host size can mediate adult parasitoid size and influence sex allocation in at least one wasp species. Host plant species has strong effects on host caterpillar performance, which in turn can affect the size and presumably the fitness of emerging tachinid flies. Although most tritrophic interaction studies have looked at just one host plant or have focused on agricultural systems with one host and one parasitoid, our study is unique in including seven host plant species, 11 host caterpillar species, two parasitic wasps, and two parasitic flies, all of which naturally co-occur. Only through these kinds of community studies of hosts and their parasitoids can we begin to understand and separate the complex interactions that shape host choice, such as competition with other parasitoids and host plant quality effects on host immune response and parasitoid fitness. Future studies should focus on how parasitoid communities partition host resources by detailing which host stages are attacked and how host plants mediate parasitoid choice.

References

Adamo, S.A., Robert, D., Perez, J. & Hoy, R.R. (1995) The response of an insect parasitoid, *Ormia ocheacea* (Tachinidae), to the uncertainty of larval success during infestation. *Behavioral Ecology and Sociobiology*, **36**, 111–118.

Alcock, J. & Kemp, D. (2006) The hilltopping mating system of *Leschenaultia adusta* (Loew) (Diptera: Tachinidae). *Journal of Insect Behavior*, **19**, 645–656.

Allen, G.R. & Hunt, J. (2001) Larval competition, adult fitness, and reproductive strategies in the acoustically orienting ormiine *Homotrixa alleni* (Diptera: Tachinidae). *Journal of Insect Behavior*, **14**, 283–297.

Askew, R.R. & Shaw, M.R. (1986) Parasitoid communities: their size, structure and development. *Insect Parasitoids* (ed. by J. Waage and D. Greathead), pp. 225–264. Academic Press, London, U.K.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.

Barbosa, P. & Greenblatt, J. (1979) Suitability, digestibility and assimilation of various host plants of the gypsy moth *Lymantria dispar* L. *Oecologia*, **43**, 111–119.

Barbosa, P., Gross, P. & Kemper, J. (1991) Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology*, **72**, 1567–1575.

Barbosa, P., Saunders, J.A., Kemper, J., Trumble, R., Olechno, J. & Martinat, P. (1986) Plant allelochemicals and insect parasitoids: effects of nicotine on *Cotesia congregata* (Say) (Hymenoptera: Braconidae) and *Hyposoter annulipes* (Cressen) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology*, **12**, 1319–1328.

Belshaw, R. (1994) Life history characteristics of Tachinidae (Diptera) and their effect on polyphagy. *Parasitoid Community Ecology* (ed. by W. Hawkins and B. A. Sheehan), pp. 145–162. Oxford University Press, Oxford, U.K.

Blüthgen, N., Menzel, F. & Blüthgen, N. (2006) Measuring specialization in species interaction networks. *BioMed Central Ecology*, **6**, 9.

Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B. & Blüthgen, N. (2007) Specialization, constraints, and conflicting interests in mutualistic networks. *Current Biology*, **17**, 341–346.

Bokonon-Ganta, A.H., Van Alphen, J.J.M. & Neuenschwander, P. (1996) Competition between *Gyranusoidea tebygi* and *Anagyrus mangicola*, parasitoids of the mango mealybug, *Rastrococcus invadens*: interspecific host discrimination and larval competition. *Entomologia Experimentalis et Applicata*, **79**, 179–185.

Bowers, M.D. (2003) Host plant suitability and defensive chemistry of the *Catalpa* sphinx, *Ceratomia catalpae*. *Journal of Chemical Ecology*, **29**, 2359–2367.

Briggs, C.J. (1993) Competition among parasitoid species on a stage-structured host and its effect on host suppression. *American Naturalist*, **141**, 372–397.

Brodeur, J. & Vet, L.E.M. (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.

Bukovinszky, T., Poelman, E.H., Gols, R., Prekatsakis, G., Vet, L.E.M., Harvey, J.A. *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.

Campan, E.D.M., Callejas, A., Rahier, M. & Benrey, B. (2005) Inter-population variation in a larval parasitoid of bruchids, *Stenocorse bruchivora* (Hymenoptera: Braconidae): host plant effects. *Environmental Entomology*, **34**, 457–465.

Campbell, B.C. & Duffey, S.F. (1979) Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. *Science*, **205**, 700–702.

Caron, V., Myers, J.H. & Gillespie, D.R. (2008) Fitness-related traits in a parasitoid fly are mediated by effects of plants on its host. *Journal of Applied Entomology*, **132**, 663–667.

Caron, V., Myers, J.H. & Gillespie, D.R. (2010) The failure to discriminate: superparasitism of *Trichoplusia ni* Hubner by a generalist tachinid parasitoid. *Bulletin of Entomological Research*, **100**, 255–261.

Charnov, E.L. (1982) *The Theory of Sex Allocation*. Princeton University Press, Princeton, New Jersey.

Charnov, E.L. & Skinner, S.W. (1985) Complementary approaches to the understanding of parasitoid oviposition decisions. *Environmental Entomology*, **14**, 383–391.

Charnov, E.L., Los-den Hartogh, Z.L., Jones, W.T. & van den Assem, J. (1981) Sex ratio evolution in a variable environment. *Nature*, **289**, 27–33.

Croft, P. & Copland, M. (1995) The effect of host instar on the size and sex ratio of the endoparasitoid *Dacnusa sibirica*. *Entomologia Experimentalis et Applicata*, **74**, 121–124.

Dyar, H.G. (1890) The number of molts of lepidopterous larvae. *Psyche*, **5**, 420–422.

Fidgen, J.G., Eveleigh, E.S. & Quiring, D.T. (2000) Influence of host size on oviposition behaviour and fitness of *Elachertus cacoeciae* attacking a low-density population of spruce budworm *Choristoneura fumiferana* larvae. *Ecological Entomology*, **25**, 156–164.

Gates, M.W., Lill, J.T., Kula, R.R., O'Hara, J.E., Smith, D.R., Whitfield, J.B. *et al.* (2012) Review of parasitoid wasps and flies (Hymenoptera, Diptera) associated with Limacodidae (Lepidoptera) in North America, with a key to genera. *Proceedings of the Entomological Society of Washington*, in press.

Gentry, G.L. & Dyer, L.A. (2002) On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology*, **83**, 3108–3119.

Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, New Jersey.

Gols, R., Bukovinszky, T., van Dam, N.M., Dicke, M., Bullock, J.M. & Harvey, J.A. (2008) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild *Brassica* populations. *Journal of Chemical Ecology*, **34**, 132–143.

Gross, H.R., Rogers, C.E. & Carpenter, J.E. (1996) Development of *Archytas marmoratus* (Diptera: Tachinidae) reared in *Galleria mellonella* larvae (Lepidoptera: Pyralidae) feeding on selected diets. *Biological Control*, **6**, 158–163.

Hågver, E.B. (1988) Multiparasitism of the green peach aphid, *Myzus persicae*: competition in the egg stage between *Aphidius matricariae* and *Ephedrus cerasicola*. *Entomologia Experimentalis et Applicata*, **47**, 275–282.

Harvey, J.A. (1996) *Venturia canescens* parasitizing *Galleria mellonella* and *Anagasta kuehniella*: is the parasitoid a conformer or regulator? *Journal of Insect Physiology*, **42**, 1017–1025.

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

Harvey, J.A. & Vet, L.E.M. (1997) *Venturia canescens* parasitizing *Galleria mellonella* and *Anagasta kuehniella*; differing suitability of two hosts with highly variable growth potential. *Entomologia Experimentalis et Applicata*, **84**, 93–100.

Harvey, J.A., Harvey, I.F. & Thompson, D.J. (1994) Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. *Ecology*, **75**, 1420–1428.

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

Heimpel, G.E. & de Boer, J.G. (2008) Sex determination in the Hymenoptera. *Annual Review of Entomology*, **53**, 209–30.

Henry, L.M., May, N., Acheampong, S., Gillespie, D.R. & Roitberg, B.D. (2010) Host-adapted parasitoids in biological control: does source matter? *Ecological Applications*, **20**, 242–250.

Holton, M.K., Lindroth, R.L. & Nordheim, E.V. (2003) Foliar quality influences tree–herbivore–parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype. *Oecologia*, **137**, 233–244.

Jones, W.T. (1982) Sex ratio and host size in a parasitoid wasp. *Behavioral Ecology and Sociobiology*, **10**, 207–210.

Kato, M. (1994) Structure, organization, and response of a species-rich parasitoid community to host leafminer population dynamics. *Oecologia*, **97**, 17–25.

King, B.H. (1987) Offspring sex ratios in parasitoid wasps. *Quarterly Review of Biology*, **62**, 367–396.

King, B.H. (1989) Host-size-dependent sex-ratios among parasitoid wasps – does host growth matter? *Oecologia*, **78**, 420–426.

King, B.H. & Napoleon, M.E. (2006) Using effects of parasitoid size on fitness to test a host quality model assumption with the parasitoid wasp *Spalangia endius*. *Canadian Journal of Zoology – Revue Canadienne De Zoologie*, **84**, 1678–1682.

Kouamé, K.L. & Mackauer, M. (1991) Influence of aphid size, age and behaviour on host choice by the parasitoid wasp *Ephedrus californicus*: A test of host-size models. *Oecologia*, **88**, 197–203.

Kula, R.R., Lill, J.T., Murphy, S.M. & Stoepler, T. (2009) First host records for the nearctic species *Triraphis discoideus* (Hymenoptera: Braconidae: Rogadinae). *Entomological News*, **120**, 380–386.

Lampert, E.C. & Bowers, M.D. (2010) Host-plant species affects the quality of the generalist *Trichoplusia ni* as a host for the polyembryonic parasitoid *Copidosoma floridanum*. *Entomologia Experimentalis et Applicata*, **134**, 287–295.

Lampson, L.J., Morse, J.G. & Luck, R.F. (1996) Host selection, sex allocation, and host feeding by *Metaphycus helvolus* (Hymenoptera: Encyrtidae) on *Saissetia oleae* (Homoptera: Coccidae) and its effect on parasitoid size, sex, and quality. *Environmental Entomology*, **25**, 283–294.

Lederhouse, R.C., Morse, R.A., Ambrose, J.T., Burgett, D.M., Conner, W.E., Edwards, L. *et al.* (1976) Crepuscular mating aggregations in certain *Ormia* and *Sitophaga*. *Annals of the Entomological Society of America*, **69**, 656–658.

Lee, J.H. & Pemberton, R.W. (2007) Seasonal phenology and stage-specific parasitism of the apple ermine moth, *Yponomeuta malinellus* Zeller, in Korea. *Entomological Research*, **37**, 54–59.

Lee, K.P., Cory, J.S., Wilson, K., Raubenheimer, D. & Simpson, S.J. (2006) Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 823–829.

Li, B. & Mills, N. (2004) The influence of temperature on size as an indicator of host quality for the development of a solitary koinobiont parasitoid. *Entomologia Experimentalis et Applicata*, **110**, 249–256.

Lill, J.T. (1999) Structure and dynamics of a parasitoid community attacking larvae of *Psilocorsis quercicella* (Lepidoptera: Oecophoridae). *Environmental Entomology*, **28**, 1114–1123.

Lill, J.T. (2001) Selection on herbivore life-history traits by the first and third trophic levels: the devil and the deep blue sea revisited. *Evolution*, **55**, 2236–2247.

Mackauer, M. & Sequeira, R. (1993) Patterns of development in insect parasites. *Parasites and pathogens of insects* (ed. by N. Beckage, S. N. Thompson and B. A. Federici), Vol. 1, pp. 1–23. Academic Press, New York, New York.

McLain, D.K., Marsh, N.B., Lopez, J.R. & Drawdy, J.A. (1990) Intravernal changes in the level of parasitization of the southern green stink bug (Hemiptera, Pentatomidae), by the feather-legged fly (Diptera, Tachinidae) – host sex, mating status, and body size as correlated factors. *Journal of Entomological Science*, **25**, 501–509.

Murphy, S.M. (2007) The effect of host plant on larval survivorship of the Alaskan swallowtail butterfly (*Papilio machaon aliaska*). *Entomologia Experimentalis et Applicata*, **122**, 109–115.

Murphy, S.M., Lill, J.T. & Smith, D.R. (2009) A scattershot approach to host location: uncovering the unique life history of the trigonalid hyperparasitoid *Orthogonalys pulchella* (Cresson). *American Entomologist*, **55**, 82–87.

Murphy, S.M., Leahy, S.M., Williams, L.S. & Lill, J.T. (2010) Stinging spines protect slug caterpillars (Limacodidae) from multiple generalist predators. *Behavioral Ecology*, **21**, 153–160.

Murphy, S.M., Lill, J.T. & Epstein, M.E. (2011) Natural history of limacodid moths (Zygaenoidea) in the environs of Washington, D.C. *Journal of the Lepidopterists' Society*, **65**, 137–152.

Nakamura, S. (1995) Optimal clutch size for maximizing reproductive success in a parasitoid fly, *Exorista japonica* (Diptera: Tachinidae). *Applied Entomology and Zoology*, **30**, 425–431.

Nicol, C.M.Y. & Mackauer, M. (1999) The scaling of body size and mass in a host–parasitoid association: influence of host species and stage. *Entomologia Experimentalis et Applicata*, **90**, 83–92.

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

Ojala, K., Julkunen-Tiitto, R., Lindstrom, L. & Mappes, J. (2005) Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. *Evolutionary Ecology Research*, **7**, 1153–1170.

Opitz, S.E.W. & Müller, C. (2009) Plant chemistry and insect sequestration. *Chemoecology*, **19**, 117–154.

Parry, D. & Goyer, R.A. (2004) Variation in the suitability of host tree species for geographically discrete populations of forest tent caterpillar. *Environmental Entomology*, **33**, 1477–1487.

Pintureau, B. & Grenier, S. (1992) Variability in morphological and biological characteristics in two strains of the parasitoid *Lixophaga diatraeae* (Diptera: Tachinidae). *Biological Control*, **2**, 176–180.

Price, P.W. (1972) Parasitoids utilizing the same host: adaptive nature of differences in size and form. *Ecology*, **53**, 190–195.

Rajapakse, C. & Walter, G. (2007) Polyphagy and primary host plants: oviposition preference versus larval performance in the lepidopteran pest *Helicoverpa armigera*. *Arthropod–Plant Interactions*, **1**, 17–26.

Rasband, W.S. (2008) *ImageJ*. National Institutes of Health, Bethesda, Maryland. URL http://imagej.nih.gov/ij; 1997–2011.

Reitz, S. & Adler, P. (1995) Fecundity and oviposition of *Eucelatoria bryani*, a gregarious parasitoid of *Helicoverpa zea* and *Heliothis virescens*. *Entomologia Experimentalis et Applicata*, **75**, 175–181.

Reudler Talsma, J.H. (2007) *Costs and benefits of iridoid glycosides in multitrophic systems*. PhD thesis, Wageningen University, the Netherlands.

Sarfraz, M., Dosdall, L.M. & Keddie, B.A. (2009) Host plant nutritional quality affects the performance of the parasitoid *Diadegma insulare*. *Biological Control*, **51**, 34–41.

SAS Institute Inc. (2006) *JMP® Version 8.0.2*. SAS Institute Inc., Cary, North Carolina.

Seidl, S.E. & King, B.H. (1993) Sex ratio response to host size in the parasitoid wasp *Muscidifurax raptor*. *Evolution*, **47**, 1876–1882.

Singer, M.S., Rodrigues, D., Stireman, J.O. & Carriere, Y. (2004) Roles of food quality and enemy-free space in host use by a generalist insect herbivore. *Ecology*, **85**, 2747–2753.

Slansky, F. & Scriber, J.M. (1985) Food consumption and utilization. *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (ed. by G. A. Kerkut and L. I. Gilbert), pp. 87–163. Pergamon, Oxford, U.K.

Srygley, R.B., Lorch, P.D., Simpson, S.J. & Sword, G.A. (2009) Immediate protein dietary effects on movement and the generalised immunocompetence of migrating Mormon crickets *Anabrus simplex* (Orthoptera: Tettigoniidae). *Ecological Entomology*, **34**, 663–668.

Stireman, J.O., O'Hara, J.E. & Wood, D.M. (2006) Tachinidae: Evolution, behavior, and ecology. *Annual Review of Entomology*, **51**, 525–555.

Strand, M.R. & Pech, L.L. (1995) Immunological basis for compatibility in host–parasitoid relationships. *Annual Review of Entomology*, **40**, 31–56.

Teder, T. & Tammaru, T. (2002) Cascading effects of variation in plant vigour on the relative performance of insect herbivores and their parasitoids. *Ecological Entomology*, **27**, 94–104.

Tillman, P.G. & Cate, J.R. (1993) Effect of host size on adult size and sex ratio of *Bracon mellitor* (Hymenoptera: Braconidae). *Biological Control*, **22**, 1161–1165.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by herbivore-seeking parasitoid wasps. *Science*, **250**, 1251–1253.

Ueno, T. (1998) Sex allocation by a parasitoid wasp (Hymenoptera: Ichneumonidae) to different host species: a question for the mechanism of host size estimation. *Journal of Insect Behavior*, **11**, 811–821.

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

Visser, M.E. (1994) The importance of being large – the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology*, **63**, 963–978.

Wagner, D.L. (2005) *Caterpillars of Eastern North America*. Princeton University Press, Princeton, New Jersey.

Wang, X.-Y., Yang, Z.-Q., Wu, H. & Gould, J.R. (2008) Effects of host size on the sex ratio, clutch size, and size of adult *Spathius agrili*, an ectoparasitoid of emerald ash borer. *Biological Control*, **44**, 7–12.

Zalucki, M.P., Clarke, A.R. & Malcolm, S.B. (2002) Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology*, **47**, 361–393.