

Host ontogeny determines parasitoid use of a forest caterpillar

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Abstract

For most organisms, patterns of natural enemy-mediated mortality change over the course of development. Shifts in enemy pressure are particularly relevant for organisms that exhibit exponential growth during development, such as juvenile insects that increase their mass by several orders of magnitude. As one of the dominant groups of insect herbivores in most terrestrial plant communities, larval lepidopterans (caterpillars) are host to a diverse array of parasitoids. Previous research has described how the frequency of herbivore parasitism varies among host plants or habitats, but much less is known about how parasitism pressure changes during host development. To test whether the two major parasitoid taxa, wasps and flies, differentially attack shared hosts based on host developmental stage, we simultaneously exposed early- and late-instar *Euclea delphini* Boisduval (Lepidoptera: Limacodidae) caterpillars to parasitism in the field. We found strong evidence that parasitoids partition hosts by size; adult female wasps preferentially parasitized small caterpillars, whereas adult female flies preferred to attack large caterpillars. Our results demonstrate that host ontogeny is a major determinant of parasitoid host selection. Documenting how shifts in enemy pressure vary with development is important to understanding both the population biology and evolutionary ecology of prey species and their enemies.

Introduction

A central question in evolutionary ecology is how consumers partition shared resources (Schoener, 1974; Amarasekare, 2000; de Roos et al., 2008). Potential axes of resource partitioning include time (Adams & Thibault, 2006), space (Fonseca & Benson, 2003), differences in behavior (Nagamitsu & Inoue, 1997), and complementary tradeoffs in key life-history traits or morphologies related to resource use (Toft, 1985; Brown, 1989; Bonsall et al., 2002; Grant & Grant, 2006). Although often difficult to disentangle empirically, the specific mechanisms of partitioning have important implications for how ecological communities are structured (Bonsall et al., 2002, 2004; Finke & Snyder, 2008).

The ontogeny of both the consumer and its resource organisms (e.g., prey for predators or hosts for parasites or herbivores) is predicted to play a central but often overlooked role in mediating patterns of resource use, competition, and the risk of natural enemy-mediated mortality (Werner & Gilliam, 1984; Relyea, 2005). Many consumers show marked shifts in resource use during development (Werner & Gilliam, 1984). Such ontogenetic dietary shifts have been observed in diverse animal taxa, including insects (Klecka & Boukal, 2012), marine invertebrates (Graham & Kroutil, 2001), frogs (Trakimas et al., 2011), fish (Davis et al., 2011), and sharks (Lowe et al., 1996). A related but less studied phenomenon occurs when consumers stratify their consumption by the ontogenetic stages of their resource organisms (complementary predation; Miller et al., 2010). When confronted with prey or hosts exhibiting ontogenetic variation in their accessibility, quality, or defenses, even fully developed consumers show strong preferences for particular stage(s) of prey/host species generally considered to be a part of their diet (Werner & Gilliam, 1984). Such size- or stage-specific selectivity can have important consequences for the evolutionary

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ecology of resource organisms, which are subject to varying competitor and natural enemy communities over the course of their ontogeny. For example, ontogenetic color change in a variety of animals (Booth, 1990) has been hypothesized to result from stage- and/or size-specific differences in predation pressure.

Relative body size is a primary determinant of resource/consumer relationships (Miller & Rudolf, 2011). Fundamental changes in physiology, behavior, and morphology during growth and development alter the quality of resource organisms for consumers (Werner & Gilliam, 1984; Roger et al., 2001; Wilder et al., 2010). For example, ontogeny affects resource allocation to growth and defense in both plants (Boege & Marquis, 2005; Quintero & Bowers, 2012) and animals (Dudycha & Lynch, 2005; Boggs, 2009). Overall natural enemy-mediated mortality risk may decrease as resource organisms grow and develop if the primary consumers are gape-limited (Urban, 2007) or prefer smaller prey (Roger et al., 2001) or may increase if consumers prefer larger prey or only recognize larger prey as potential food items (Li & Jackson, 1996; Quinn & Kinnison, 1999; Westerborn et al., 2006). Alternatively, overall risk of natural enemy-mediated mortality may remain relatively constant if the composition of consumer communities turns over during the growth and development of the resource organism (Miller et al., 2010; Schellekens et al., 2010). Patterns of such ontogenetic succession (Fonseca & Benson, 2003) are perhaps best documented in the plant-herbivore literature, where herbivorous insect communities often shift with the ontogeny of their host plants (Le Corff & Marquis, 1999; Basset, 2001; Thomas et al., 2010). Although there have been several recent reviews and models exploring the role of ontogeny in mediating interspecific interactions (e.g., Boege & Marquis, 2006; Barton & Koricheva, 2010; Thomas et al., 2010; Miller & Rudolf, 2011), there is still a clear need for more empirical investigations, particularly for host-parasite and host-parasitoid systems.

Parasitoids are a dominant component of the natural enemy community for many herbivorous insects (Godfray, 1994) and often specialize on hosts of a particular developmental stage (Waage & Hassell, 1982), resulting in size-structured host use by parasitoid species. Although size- and stage-specific parasitism has been noted for particular species (often insect pests; Neveu et al., 2000; Hegazi & Khafagi, 2005; Chong & Oetting, 2006; Rehman & Powell, 2010), generalizations regarding differential parasitoid host use, and the mechanism(s) underlying it, remain elusive. In addition, most of these agriculturally based studies approach the question from the perspective of the parasitoid (i.e., determining what range of host sizes/stages a particular parasitoid species targets) rather

than that of the host(s). Experimental investigations of how the parasitoid ‘pressure’ exerted by entire assemblages of parasitoids changes over host ontogeny are needed to help interpret natural patterns of ontogenetic shifts in particular morphologies, physiologies, or behaviors hypothesized to be linked to natural enemy avoidance (Relyea, 2005; Grant, 2007; Frankfater et al., 2009).

Through collection and rearing of 11 species of co-occurring slug caterpillars (Limacodidae: Lepidoptera), Stoepler et al. (2011) recently found support for the hypothesis that the two dominant parasitoid taxa, wasps, and flies, partition their shared hosts based on caterpillar size (a proxy for developmental stage) in the field. Specifically, across a range of shared host species, wasp parasitoids were predominantly reared from small caterpillars collected from the field as early instars, whereas fly parasitoids were reared most commonly from larger caterpillars collected as late instars. However, because this study combined data from several limacodid caterpillar species, sampling dates, and seasons, the range of host sizes simultaneously available to the local parasitoid community was uncontrolled, leaving unclear whether parasitism records accurately reflect the true host preferences of these parasitoids.

Here, we report the results of a series of manipulative experiments designed to test the hypothesis that natural enemy taxa differ in their attack rates and/or consumption of a shared resource: caterpillars of the spiny oak slug [*Euclea delphinii* Boisduval (Lepidoptera: Limacodidae)]. By simultaneously exposing small and large *E. delphinii* caterpillars (Figure 1) to natural enemies on a small subset of



Figure 1 A pair of small (early instar) and large (late instar) *Euclea delphinii* caterpillars depicted prior to being exposed to parasitoid attack in the field. Note the difference in color patterning and physical armature (stinging spines) between the instars.

host plants, we test our hypothesis that ontogeny mediates natural enemy attack by the resident limacodid parasitoid community. *Euclea delphinii* caterpillars are used as hosts by a community of parasitoid species that are specific to hosts within the family Limacodidae (Gates et al., 2012) and all of these parasitoid species attack the caterpillar stage (Murphy & Lill, 2010; Murphy et al., 2011; JT Lill & SM Murphy, unpubl.). The period of activity of *E. delphinii* caterpillars overlaps significantly with each of the dominant parasitoids that attack limacodid caterpillars, encompassing the period from mid-June through mid-September (described in detail in Stoepler et al., 2011). Although all of the numerically dominant parasitoid species that attack *E. delphinii* are technically koinobionts that allow the caterpillar host to continue development following oviposition, they differ markedly in the amount of time larvae reside within the caterpillar host before killing it (Stoepler et al., 2011). For instance, the dominant hymenopteran parasitoids that attack *E. delphinii* (Braconidae: *Cotesia empretiae* Viereck and *Triraphis discoideus* Cresson; Eulophidae: *Alveoplectrus lilli* Gates, *Pediobius crassicornis* Thomson; and *Platyplectrus americana* Girault) feed as larvae within the caterpillar host, kill it, and emerge as adult wasps within days to weeks of the initial attack. In contrast, each of the three fly parasitoids that attack *E. delphinii* [*Austrophorocera* spec., *Compsilura concinnata* Meigen, and *Uramya pristis* Walker (all Diptera: Tachinidae)] requires that the parasitized caterpillar host either completes or nearly completes larval development, a process that may take one to many months in these caterpillars. Due to these substantial differences in life history between wasp and fly parasitoid species, we classify the parasitoids that attack *E. delphinii* caterpillars by order, as either wasps (Hymenoptera) or flies (Diptera), to highlight the common biology of members of each group.

Materials and methods

Study system

In temperate regions of eastern North America, *E. delphinii* is reported to be univoltine, but evidence from both adult light-trapping and caterpillar rearing suggests that in most years the species has at least a partial second generation (JT Lill & SM Murphy, unpubl.). Adult flights begin in May and extend through July, resulting in a larval period that spans almost 5 months for this slow-growing species (June–October; Wagner, 2005; Murphy et al., 2011); a range of development stages is thus simultaneously available to natural enemies for much of the summer. *Euclea delphinii* is endemic to deciduous forests in the eastern USA, and the caterpillars are highly polyphagous, with a host plant range that includes more than a dozen trees and

shrubs throughout its range (Epstein, 1988; Wagner, 2005; Lill, 2008; Murphy et al., 2011).

Field experiments

To test whether the natural enemies of *E. delphinii* differentially consume caterpillars of different ontogenetic stages in a natural field setting, we used a paired choice design: caterpillars from each of two size classes (small and large, corresponding with early- and late-instar stages, respectively; Figure 1) were deployed in pairs as ‘sentinel caterpillars’ onto marked understory saplings of common, co-occurring host plants in a series of three separate experiments conducted over 2 years (2009–2010). All experiments were conducted at Little Bennett Regional Park (Clarksburg, MD, USA; 39°15.9′N, 77°16.7′W), a second-growth oak-hickory-beech forest. Sentinel caterpillars originated from our laboratory colony of *E. delphinii* and had been reared (prior to use in experiments) on leaves of one of five common host plants: black cherry [*Prunus serotina* Ehrh. (Rosaceae)], American beech [*Fagus grandifolia* Ehrh. (Fagaceae)], black gum [*Nyssa sylvatica* Marsh (Nyssaceae)], northern red oak [*Quercus rubra* L. (Fagaceae)], or white oak [*Quercus alba* L. (Fagaceae)]. Different sets of host-plant species were used in each of the experiments, depending on availability of laboratory-reared caterpillars of contrasting developmental stages from a given host plant. In each of our experiments, sentinel caterpillars were the offspring of at least 20 mothers that had been haphazardly assigned to host-plant treatments upon hatching; thus, although the genetic background of experimental caterpillars was not explicitly controlled, each experimental tree contained a representative mix of these families. In all experiments, caterpillar diets were the same in both the field and laboratory (i.e., caterpillars were never forced to switch host plants during development).

On each experimental sapling, *E. delphinii* caterpillars were placed in pairs, with each pair consisting of a ‘small’ or early-instar caterpillar and a ‘large’ or late-instar caterpillar; replicate pairs were haphazardly placed on individual leaves distributed throughout the tree. Although natural understory densities of *E. delphinii* caterpillars are <1 caterpillar/10 m² foliage (Stoepler et al., 2011), we experimentally increased caterpillar densities to increase parasitoid encounter rate with caterpillars of both size categories, allowing a more accurate assessment of parasitoid host choice based on size. Saplings were located in the interior of the park in shaded understory forest and measured ca. 1.8–2.5 m high with an average (\pm SE) total leaf area of 1.96 ± 0.16 m². Prior to deploying experimental caterpillars, each tree was searched, and any ‘wild’ *E. delphinii* caterpillars were removed. Once deployed, caterpillars were free to move about and redistribute themselves on

the plant. At the time sentinel caterpillars were deployed, we measured their body lengths with calipers (to the nearest 0.1 mm). We used body length as a proxy for development stage and mass because *E. delphinii* caterpillars cannot be readily assigned to instar based on head capsule widths as is performed in other caterpillar species (standard curves relating body length to mass for *E. delphinii* have been generated previously; Murphy et al., 2011). In all of our experiments, caterpillars remained exposed to natural enemy attack in the field for 1 week, after which we exhaustively searched each sapling for remaining *E. delphinii* caterpillars. Caterpillars that were missing from the host plant were presumed to have been depredated as limacodid caterpillars only leave their host plant to pupate (JT Lill, pers. obs.). In addition, experimental caterpillars were selected to avoid penultimate instars that could complete development during the week-long exposure period. We brought all recovered caterpillars from the field back to the laboratory to complete development on their assigned host plant and monitored them for parasitoid emergence (for rearing methods, see Murphy et al., 2011).

For each caterpillar that we retrieved from the field, we recorded whether it was parasitized (yes/no), survived to adulthood, or died during development prior to adult emergence. Caterpillars that died prior to adult emergence may have been parasitized, but to be conservative, we did not consider them parasitized unless we could positively identify the parasitoid taxa (wasp or fly) due to the presence of characteristic parasitoid eggs, larvae, or pupae in or on the host. If a caterpillar was parasitized, we recorded whether it was attacked by a wasp or fly; these categories were not mutually exclusive and a single caterpillar could be parasitized by both types of parasitoid (i.e., multiparasitized). For the most commonly reared parasitoid fly, *Austrophorocera* spec., eggs are laid externally on the caterpillar's cuticle allowing us to estimate attack rates. For the wasps and the less common flies, however, successful parasitism was the only response we could measure (i.e., parasitoid larvae emerged and pupated, killing the host; parasitoid eggs/larvae that died in the host could not be detected). Parasitoid voucher specimens are deposited at the Smithsonian National Museum of Natural History in Washington, DC, USA.

Recognizing that natural enemy attack is often highly spatially variable, each experiment focused on within-plant comparisons of parasitism or predation of the two size classes concurrently presented to the local natural enemy community (i.e., these experiments were not designed to test for variation among host plant species in parasitism). We note that all of the common parasitoids of *E. delphinii* have been reared from caterpillars feeding on

each of the five host plant species, suggesting that parasitoids routinely search for limacodid hosts on this set of plants (Gates et al., 2012).

2009 field experiment. On July 23, 2009, we deployed 4–5 pairs of small and large *E. delphinii* caterpillars onto a total of 21 saplings (3–5 trees of each of the five host-plant species) for a total of 208 caterpillars. The mean (\pm SE) initial lengths for small and large caterpillars were 4.4 ± 0.2 and 10.1 ± 0.2 mm, a significant difference ($t = 452.9$, d.f. = 205, $P < 0.0001$).

2010 field experiments. On July 22, 2010, we deployed 5 pairs of small and large *E. delphinii* caterpillars onto 20 saplings (10 trees each of black cherry and beech), in total 200 caterpillars. The mean (\pm SE) initial length for small and large caterpillars was 6.7 ± 0.1 and 11.5 ± 0.2 mm, a significant difference ($t = 24.3$, d.f. = 198, $P < 0.0001$). We refer to this experiment as 2010–1. On August 11, 2010, we deployed a second cohort of 5 *E. delphinii* caterpillar pairs onto each of 13 trees (black cherry, beech, and white oak), in total 130 caterpillars. The mean initial length for small and large caterpillars was 8.7 ± 0.3 and 15.5 ± 0.2 mm, again a significant difference ($t = 18.94$, d.f. = 128, $P < 0.0001$). We refer to this experiment as 2010–2. We note that the average caterpillar size in each treatment varied slightly among experiments, particularly for the 2010–2 experiment, where both small and large caterpillars had larger initial sizes than in 2009. Because we were trying to deploy larvae during the peak activity periods of the two dominant parasitoids (wasps and flies, during 2010–1 and 2010–2, respectively), some variation in development stage was unavoidable. For this reason, direct comparisons among experiments need to be interpreted cautiously. We also note, however, that all of the ‘small’ caterpillars exposed in both of the 2010 experiments were within the size range accessible to wasps based on previous rearing data (e.g., body length < 12.8 mm; JT Lill & SM Murphy, unpubl.).

Data analysis

All statistical analyses were conducted using SAS 9.3 or JMP pro 9 (SAS Institute, Cary, NC, USA). To test for size treatment effects on predation of sentinel caterpillars (recovery rate following field exposure), we scored each caterpillar as missing or recovered (0 or 1) and conducted a generalized linear mixed model (GLMM) with ‘size treatment’ as a fixed effect and ‘tree’ as a random effect to account for the non-independence of caterpillars on shared experimental trees (Proc GLIMMIX, logit link, binary distribution). Similarly, for recovered caterpillars reared for parasitism, we used a GLMM as above to

compare the incidence of parasitism of small and large caterpillars by wasps and flies. Trees in which only small or only large caterpillars were recovered were excluded from analyses because the objective of the experiments was to test for relative differences in attack (2009: 1 tree; 2010–1: 2 trees; 2010–2: 2 trees excluded). Finally, because the dominant tachinid fly, *Austrophorocera* spec., often lays multiple eggs on individual hosts, we tested whether the number of tachinid eggs on a caterpillar was related to initial host size with linear regression.

Results

Recovery rate

Over all three experiments, we deployed a total of 538 *E. delphinii* caterpillars and recovered 255 (47.4%) of them from the field. The proportion of caterpillars that were missing and presumed predated ranged from 35 to 57% of deployed caterpillars but did not differ significantly between the small and large treatments for any of the three experiments (2009: $F_{1,186} = 0.48$, $P = 0.49$; 2010–1: $F_{1,179} = 1.00$, $P = 0.32$; 2010–2: $F_{1,116} = 1.55$, $P = 0.22$).

Parasitoids

In each of the individual experiments, the two parasitoid orders (wasps or flies) differentially attacked *E. delphinii* caterpillars according to their developmental stage (Figure 2). In the 2009 experiment, wasp parasitism of small *E. delphinii* caterpillars (51%) was more than triple that of large caterpillars (14%; $F_{1,80} = 14.08$, $P = 0.0003$; Figure 2A). All of the wasp parasitoids that we reared in 2009 belonged to a single eulophid species, *P. americana*. We found that *P. americana* parasitized caterpillars on all five host plant species and the initial body size of the parasitized caterpillars was small (median = 3.5 mm) but had a wide range (all caterpillars but one were between 1.6 and 8.6 mm; however, one outlier had an initial length of 14.2 mm). Our estimate of peak flight period for our two most common tachinid fly parasitoids, *Austrophorocera* spec. and *U. pristis*, during the 2009 field season unfortunately preceded their actual flight period by about 3 weeks. Thus, in 2009, we deployed our experimental caterpillars prior to the peak tachinid flight period and as a result, only a single caterpillar was parasitized by a tachinid fly; this individual was parasitized by *C. concinnata* and belonged to the large treatment (initial length = 10.1 cm).

In the first of the 2010 experiments (2010–1), we recovered cohorts of both small and large *E. delphinii* caterpillars, which were compared for parasitism by the tachinid *Austrophorocera* spec. In this experiment, the incidence of parasitism by wasps was very low and highly spatially clumped; caterpillars from only 4 of the 17 trees yielded

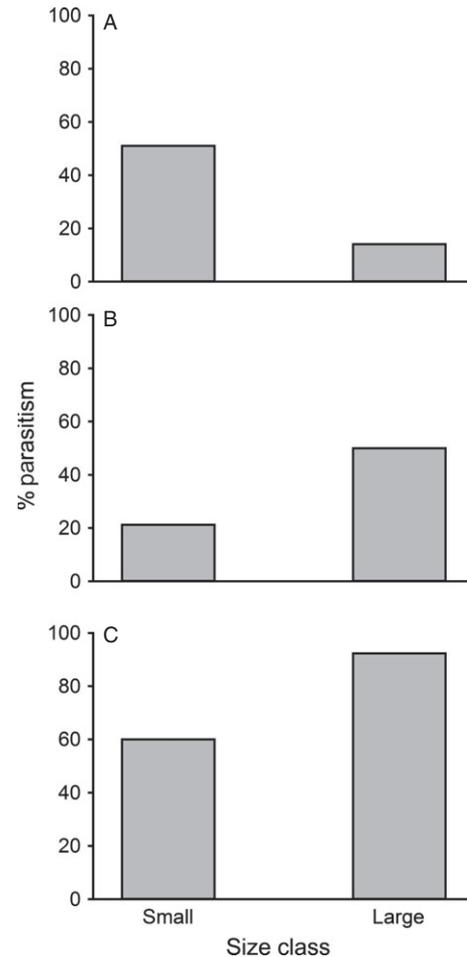


Figure 2 Mean percentage of exposed small and large *Euclea delphinii* caterpillars that were parasitized by (A) wasps in the 2009 experiment ($P = 0.0003$), (B) flies in the first 2010 field experiment, 2010–1 ($P = 0.005$), and (C), flies in the second 2010 field experiment, 2010–2 ($P = 0.012$). Data were analyzed using general linear mixed models with fixed treatment effects and a random block ('tree') effect to account for non-independence of individual caterpillars.

any wasp parasitoids and only 11 total caterpillars were parasitized by wasps, which precluded any meaningful statistical comparisons. Reared wasps included *P. americana* and the gregarious micrograstine braconid *C. empretiae*. By contrast, the proportion of large caterpillars parasitized by flies (50%) was more than double that of small caterpillars (21.2%) ($F_{1,80} = 8.38$, $P = 0.0049$; Figure 2B). Only a single recovered *E. delphinii* caterpillar was parasitized by the tachinid *U. pristis*, whereas all the remaining records of fly parasitism by *Austrophorocera* spec.

In the second 2010 experiment (2010–2), parasitism by wasps was again very low (only two small caterpillars

yielded wasps). *Austrophorocera* spec. parasitism, however, was once again extremely high in this experiment, and flies again showed a strong preference for attacking large caterpillars (92% of large caterpillars vs. 60% of small caterpillars; $F_{1,44} = 6.84$, $P = 0.012$; Figure 2C). No other species of tachinid flies were reared from *E. delphinii* caterpillars in this experiment. There was no effect of ‘tree’ (random effect) on wasp parasitism in the 2009 experiment ($Z = 1.18$, d.f. = 17, $P = 0.12$) or on fly parasitism in 2010–2 ($Z = 0.49$, d.f. = 10, $P = 0.31$), but the ‘tree’ effect was significant in the 2010–1 experiment ($Z = 1.42$, d.f. = 15, $P = 0.043$), indicating that fly attacks were more spatially patchy in 2010–1.

Across both the 2010 experiments, *Austrophorocera* spec. attacked caterpillars of 4.9–17.1 mm body length (as evidenced by at least one egg visible on the cuticle), but the median size was strongly biased towards later instars (2010–1: 10.5 mm; 2010–2: 13.6 mm). The number of *Austrophorocera* spec. eggs laid on each caterpillar ranged from 1 to 8 eggs (mean \pm SE = 2.2 ± 0.2 ; $n = 77$). We found that caterpillar size was a significant predictor of the number of tachinid eggs laid on each caterpillar ($r^2 = 0.12$, d.f. = 1, $P < 0.0001$) with the number of eggs laid increasing with caterpillar size; all caterpillars with more than five tachinid eggs had body sizes greater than 12 mm.

Discussion

Our results clearly demonstrate that the two dominant parasitoid groups that attack *E. delphinii* caterpillars show distinct and opposing host use preferences for either early (wasps) or late (flies) ontogenetic stages of their prey. Although adult female wasp and fly parasitoids varied substantially in relative abundance during our 3-week-long field experiments, the larval stages of these parasitoid taxa overlap for most of the juvenile stage of their limacodid hosts, which extends for weeks to months in the field (Murphy et al., 2011; Stoepler et al., 2011; Gates et al., 2012). Thus, we predict strong selection on adult female wasps and flies to reduce competition between their offspring by differentiating their host preferences. Although the experimental exposure periods appeared to differ markedly in their overlap with peak activity periods of the dominant parasitoid guilds (wasps vs. flies), hosts of appropriate sizes were potentially available to both guilds during all experiments.

Together, the results of our manipulative choice experiments presented here in conjunction with our previous observational results from a much larger set of limacodid caterpillar host species and parasitoid taxa (Stoepler et al., 2011) demonstrate that wasp and fly parasitoids stratify

their choice of shared limacodid hosts by caterpillar body size. Furthermore, our results suggest that passive sampling and rearing efforts, when conducted intensely or over many years, may reasonably reflect parasitoid host preferences.

Several potential advantages and disadvantages are associated with specialization on hosts that vary in size/stage (Stoepler et al., 2011). In multiparasitoid food webs, early-attacking parasitoids often enjoy a competitive advantage by making parasitized hosts unavailable to later-attacking species. Other advantages of attacking small (or early developmental stage) hosts include the greater abundance of small hosts in nature (Price, 1973, 1974, 1975), and their often reduced physical and immunological defenses (Brodeur & Vet, 1995; Strand & Pech, 1995; Stoepler et al., 2013). In contrast, large (or late developmental stage) hosts may be easier to locate via feeding-related volatile and/or visual cues (Turlings et al., 1990) and may provide greater resource quantity. Additionally, because the dominant fly parasitoid, *Austrophorocera* spec., lays eggs externally on the host cuticle, host molting prior to egg hatch could enable hosts to ‘escape’ parasitism from these flies. By focusing on larger, later instars that have longer intermolt intervals, flies may thus increase the likelihood of successful parasitism by their offspring. Attacking late-stage hosts may also reduce the risk of parasitoid larvae being predated or outcompeted within the host prior to completing development (Price, 1972). This latter phenomenon is highly germane to the *E. delphinii* host-parasitoid system because in instances of multiparasitism observed for Limacodidae, both in this experiment and in our long-term rearing efforts, the parasitoid wasps (and even other tachinid fly species), which exclusively kill larvae prior to the completion of larval development, always ‘win’ in direct competition with *Austrophorocera* spec. flies. For instance, in the set of experiments described here, 10% of the caterpillars that were attacked by *Austrophorocera* spec. flies were also attacked by a wasp, which ultimately killed both the host caterpillar and any developing fly larvae. Similarly, over 2 years (2009–2010) of rearing efforts of larval Limacodidae from the same area, we recorded all instances where we found *Austrophorocera* spec. eggs laid on limacodid caterpillars ($n = 159$ caterpillars) and of these, 14% were multiparasitized (i.e., caterpillars were attacked by both *Austrophorocera* spec. and either a wasp or another tachinid fly species, such as *U. pristin*). Our estimate of multiparasitism is likely highly conservative as we could only include caterpillars on which we observed *Austrophorocera* spec. eggs, which means that any caterpillars that were attacked by *Austrophorocera* spec. more than a few days before collection (after the eggs hatched) may not have been included in this estimate.

Notably, *Austrophorocera* spec. never successfully emerged from these multiparasitized hosts, likely due to their much longer residence time within the host compared with the other parasitoid species.

As expected, use of these sentinel caterpillars by many of the parasitoid species was often patchy, particularly for uncommon species. When a female of these species (e.g., *C. emprethiae*) located a 'patch' of caterpillars, it appears that she often attacked multiple caterpillars on the sapling, suggesting that these parasitoids show patch fidelity when they find suitable hosts. These findings also suggest that foraging parasitoids were likely to encounter multiple potential hosts on experimental plants and may often have been able to 'choose' among potential hosts. Yet, our finding that larger caterpillars were more likely to have a greater number of tachinid eggs laid upon them than were smaller caterpillars may indicate that *Austrophorocera* spec. females prefer to superparasitize large hosts rather than relegate their offspring to smaller, unparasitized hosts, despite the fact that only a single adult *Austrophorocera* fly ever emerges from a superparasitized host (SM Murphy, JT Lill & TM Stoepler, pers. obs.). Our findings of consistently strong patterns of size-selective parasitism underscore the importance of host ontogenetic stage in parasitoid host selection.

Shifting enemy pressures are often invoked to explain programmed ontogenetic color change in a great variety of animals (Booth, 1990) and may play a similar role in this system, where caterpillars progressively increase in both defensive armature and aposematism as they grow (e.g., compare early and late instar *E. delphinii* caterpillars in Figure 1). Such a shift may play an adaptive role in *E. delphinii* defense as consumer pressure from visually oriented predators and/or parasitoids (e.g., predatory wasps and many tachinids) increases. We note, however, that the most common specialist tachinid, *Austrophorocera* spec., appears little deterred by these advertised defenses, which may be expected to be more effective in deterring generalist enemies (Murphy et al., 2010).

Numerous species of caterpillars have been reported to mimic entirely different sets of models in different instars (e.g., North American caterpillars in the genus *Acronicta*; Wagner, 2005). Such dramatic ontogenetic shifts in color patterning within a particular life stage can be considered a form of hypermetamorphosis that is thought to evolve because the frequency of size-matched models changes as the mimic grows, forcing the mimic to adopt an alternative defense strategy in later instars ('transformational mimicry'; Booth, 1990; McIver & Stonedahl, 1993). Yet, it is also possible that these ontogenetic color changes represent adaptations

to the different visual acuities or perceptions of different sets of predators that forage for small vs. large prey. Even among these relatively well-studied systems, there is clearly a dearth of investigations examining how consumer communities change over prey ontogeny.

In conclusion, we argue that changes in the magnitude and/or species identity of consumer pressure over the ontogeny of the resource species are generally not captured in traditional food web-type models of species interactions in terrestrial systems. When estimating the strength of interaction pathways in these interaction webs, multiple developmental stages need to be taken into consideration and data need to be collected across a wide temporal window due to the idiosyncratic nature of consumer-resource phenologies (Relyea, 2005). Moreover, an important repercussion of our findings is that many of the large-scale community rearing projects that have been conducted (e.g., Stireman & Singer, 2003; Stireman et al., 2005) and/or that are ongoing (e.g., the exhaustive caterpillar and parasitoid inventory of the Area Conservación de Guanacaste in Costa Rica led by Dan Janzen), that focus efforts on collecting and rearing ultimate or penultimate instar caterpillars, are likely to be highly skewed toward tachinid parasitism. In fact, these studies have all found a very high diversity and intensity of tachinid parasitism, a finding that surely would have been reflected in this study as well had we only exposed late-instar caterpillars in our field experiments. Whereas the rationale for focusing on later developmental stages is practical (i.e., they are easier to locate and identify to species, and require less rearing), we suggest that these later stages may often represent those caterpillars that have already escaped wasp parasitism in early instars. We call for a more balanced approach to rearing hosts from a variety of ontogenetic stages and representing many different host lineages to help clarify the generality of this observation.

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