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FORUM

Enemy-Free Space for Parasitoids

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ABSTRACT Natural enemies often cause significant levels of mortality for their prey and thus can be important agents of natural selection. It follows, then, that selection should favor traits that enable organisms to escape from their natural enemies into "enemy-free space" (EFS). Natural selection for EFS was originally proposed as a general force in structuring ecological communities, but more recently has become conceptually narrow and is typically only invoked when studying the evolutionary ecology of host plant use by specialized insect herbivores. By confining the application of EFS to specialist herbivores, its potential value to community and evolutionary ecology has been marginalized. As a first step toward exploring the potential explanatory power of EFS in structuring ecological niches of higher trophic-level organisms, we consider host use by parasitoids. Here, we present three distinct mechanisms from our studies of caterpillar host–parasitoid interactions suggesting that parasitoids may be under selection to exploit traits of their hosts and the plants on which those hosts feed to garner EFS for their developing offspring. The neglect of EFS as a top–down selective force on host use by parasitoids may be a serious limitation to basic and applied ecology, given the great diversity of parasitoids and their significance in controlling herbivore populations in both natural and managed ecosystems. Parasitoids and other mesopredators represent excellent candidates for further developments of EFS theory and testing of its broader importance.

KEY WORDS larval defense, natural enemy, parasitoid, predation, tritrophic interaction

Mortality from natural enemies is predicted to favor traits or behaviors that enable organisms to minimize this mortality by exploiting "enemy-free space" (EFS). To the extent that such traits determine associations among species, selection for EFS can act as a general force in structuring ecological communities (Jeffries and Lawton 1984). Jeffries and Lawton (1984) defined EFS as "ways of living that reduce or eliminate a species' vulnerability to one or more species of natural enemies." Their purpose was to challenge ecologists to consider factors other than resource-based competition when studying ecological niches. At the time, the role of competition in structuring assemblages of insect herbivores was being challenged (Strong et al. 1984), and the role of natural enemies was increasingly considered a major determinant of host plant use by insect herbivores (Bernays and Graham 1988). Since then, EFS has been shown to be an important selective force in several different herbivore systems (e.g., Mulatu et al. 2004, Murphy 2004, Diamond and Kingsolver 2010) and may explain enigmatic patterns of plant–herbivore associations in many more (e.g., Wiklund and Friberg 2008, Sendoya et al. 2009). Despite accumulating support for the idea that tritrophic interactions are important determinants of ecological niches of insect herbivores (Singer and Stireman 2005), the concept of EFS has not ascended in prominence in the theory and practice of community ecology. Rather, it has been largely confined to the study of host plant use by herbivorous insects, even though EFS was originally proposed as a general concept that would apply to any species subject to top–down control exerted by natural enemies. By confining the application of EFS to herbivores, its potential value to community and evolutionary ecology has been marginalized.

In their original paper, Jeffries and Lawton (1984) focused primarily on how natural enemies may shape the niches of herbivores, which may have predisposed future studies of EFS to focus on herbivores. Even at this early date, however, the authors mention briefly that EFS may be important for insect parasitoids (Jeffries and Lawton 1984, p. 279), yet they encouraged studies that examine the effects of natural enemies on host plant selection by herbivores, and indeed, research on EFS since then has been restricted to herbivores (Berdegue et al. 1996, Stamp 2001). Historically, herbivore populations have been thought of as controlled by top–down factors whereas predator and parasitoid populations are thought to be controlled largely by bottom–up factors (Hairston et al. 1960). Thus, top–down controls have not figured promi-

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nently in discussions of the ecological factors that affect members of the third trophic level (but see Price 1974, 1975, for a discussion of the evolutionary importance of in-host mortality for parasitoid fecundity). For example, the surge of studies on parasitoid–host interactions and parasitoid community ecology from the late 1980s through the early 2000s (reviewed in Godfray 1994, Hawkins 1994, Hawkins and Sheehan 1994, Sullivan and Volkl 1999, Hochberg and Ives 2000) sought to explain patterns and processes of host use by parasitoids primarily through bottom–up mechanisms. Some of the most comprehensive macroecological analyses (e.g., Hawkins 1994) have used bottom–up factors to explain up to 50% of the variation in response variables such as parasitism rate and the parasitoid species richness per host. This body of work demonstrated that feeding niches of herbivores and other plant and herbivore characteristics can explain variation in parasitism and parasitoid community structure, but the considerable unexplained variation raises the possibility that ignored top–down effects, such as EFS, might also play an important role in structuring parasitoid–host interactions. With increasing recognition that bottom–up and top–down forces typically act in combination (Hunter and Price 1992), new approaches should instead seek to quantify the relative importance of factors such as host-plant quality and EFS for a variety of systems and ecological contexts.

An important exception to the historical focus on bottom–up effects on parasitoids and predators is the literature on intraguild predation (IGP), which posits that top–down controls may be important for certain mesopredators. However, there presently is very little overlap between IGP and EFS research, and there may be examples of EFS for higher trophic level organisms hidden in the expanding IGP literature. For example, Kester and Jackson (1996) studied an intriguing example of IGP in which the predatory spined stilt bug (*Jalysus wickhami* Van Duzee, 1906, Berytidae) feeds on eggs of the herbivore *Manduca sexta* (L.) (tobacco hornworm, Sphingidae), but also on the hornworm parasitoid, *Cotesia congregata* (Say) (Braconidae), which it consumes in either the prepupal or pupal stage. Generally prepupal and pupal *C. congregata* remain attached to larval *M. sexta* caterpillars, but sometimes they become detached and fall onto the host plant. The authors found that parasitoid pupae that remained attached to *M. sexta* caterpillars suffered reduced predation by stilt bugs compared with pupae that became detached from their host caterpillars. This study was couched in terms of IGP and biocontrol of tobacco hornworms, but could also be seen as an example of EFS when viewed from the parasitoid's perspective; parasitoid pupae that remained attached to their host exploited EFS from predators whereas detached pupae suffered high levels of predation. Similarly, in a study of biocontrol of cotton aphid, Colfer and Rosenheim (2001) focused on the impact of IGP by a ladybird beetle (intraguild predator) upon a parasitoid (intraguild prey) and their combined effects on cotton aphid populations. One intriguing finding was that predatory ladybird beetles preferred to feed on unparasitized aphids over parasitized aphids, which the authors discussed from an IGP perspective. From a different point of view, however, this may be an example of EFS in which the parasitoid alters the attractiveness or repellency of its host's phenotype, reducing its likelihood of predation during development inside the host. Predation of parasitized hosts is common in the IGP literature (Rosenheim 1998 and references therein), and this literature offers other interesting examples of parasitoids that exploit EFS from predators but that were not explicitly discussed or considered from the EFS perspective.

One of the best putative examples of EFS for a third trophic-level organism was reported by Volkl (1992) who studied host use by two parasitoid species that attack the black bean aphid (*Aphis fabae* Scopoli, Aphididae). *A. fabae* is tended by ants that are aggressive toward ovipositing females of one parasitoid species (*Trioxys angelicae* (Haliday), Braconidae), but not the other (*Lysiphlebus cardui* (Marshall), Braconidae). Because *T. angelicae* is prevented from using ant-tended aphids as hosts, it is restricted to non-tended aphid hosts, where hyperparsitism rates are substantial (between 40 and 90%; Volkl 1992). In contrast, *L. cardui* females preferentially oviposit in ant-tended aphids and their offspring exploit EFS from the hyperparasitoid wasps *Alloxysta* sp. (Figitidae), *Pachyneuron aphidis* (Bouche) (Pteromalidae), and *Dendrocerus carpenteri* (Curtis) (Ceraphronidae) through the aggressive protection of the aphid-tending ants. In this study, aphid-tending ants reduce the rate of aphid hyperparasitism by more than threefold (from 70% in untended aphids to 17% in tended aphids); experimental exclusion of tending ants also produced a similar threefold increase in levels of hyperparasitism, highlighting the strong repellant effects of ants on attack by hyperparasitoids. Furthermore, aphids parasitized by *L. cardui* actually increase their honeydew production, which strengthens the protection provided by the ants and further minimizes attack by hyperparasitoids.

Another exciting line of evidence for EFS comes from systems in which parasitoids manipulate their host's behavior, movement, or growth rate to minimize their risk of attack from their own natural enemies during this vulnerable stage. Although this concept is certainly not new (Stamp 1981b, Fritz 1982, Brodeur and McNeil 1989), it has recently experienced a renewed interest and stronger empirical support in the literature (e.g., Grosman et al. 2008, Harvey et al. 2008). Notably, none of these studies refer to the phenomenon that they describe as EFS. Rather, alternative conceptual descriptors are typically used, such as the "usurpation hypothesis," in which developing parasitoids usurp host behavior to protect themselves from their own natural enemies (Brodeur and Vet 1994). We propose that EFS for parasitoids is not limited to instances where the parasitoids manipulate host behavior or physiology, but that there are many other potential avenues for parasitoids to exploit EFS from their own natural enemies. Therefore, a major

Fig. 1. Examples of a physically defended limacodid species, *Acharia stimulea* (upper left, with spines), and a cryptic limacodid species, *Lithacodes fasciola* (lower right, no spines). Photograph by J.T.L. (Online figure in color.)

goal of this forum piece is to encourage a more inclusive approach to thinking about the ways EFS could affect the ecology and evolution of parasitoids and other carnivores.

Three Possible Mechanisms of EFS for Parasitoids

As a first step toward exploring the potential explanatory power of EFS in structuring ecological niches of higher trophic-level organisms, we consider host use by parasitoids, which, like insect herbivores, are diverse and abundant members of many terrestrial communities. We predict that female parasitoids should seek EFS for their offspring through the evolutionary ecology of host choice and host manipulation. Here we suggest three distinct mechanisms by which EFS may structure parasitoid–host interactions, along with some preliminary evidence for each case. These examples are neither exhaustive nor definitive, but rather are intended to broaden the purview of EFS to incorporate a wider range of organisms and trophic interactions than has been investigated to date.

EFS in Physically Defended Hosts. Caterpillars in the moth families Limacodidae and Megalopygidae are well known for their unusual morphologies and appear to employ a wide variety of defense mechanisms to protect themselves from their predators. Some caterpillars are morphologically and behaviorally cryptic, adaptations that presumably enable them to minimize detection by natural enemies, while other caterpillars appear warningly colored and are physically defended with stinging spines (Fig. 1). These stinging spines have been demonstrated to be an effective defense against a variety of generalist predators such as assassin bugs and paper wasps (Murphy et al. 2010). Although stinging spines may protect limacodid larvae from generalist predators, they do not appear to protect these caterpillars from parasitoids; indeed, quite the opposite. We propose that ovipositing parasitoids may target physically defended hosts because their offspring benefit from the physical defenses of their herbivorous host; these defenses, while no longer offering a fitness advantage to the parasitized herbivore, which will inevitably die, may yet protect immature parasitoids from attack by generalist predators. Thus, in a nonintuitive twist, physical defenses of the caterpillars no longer provide EFS to the herbivore that posses them, but instead offer EFS to the parasitoid(s) that reside inside the host caterpillar against attack from generalist predators.

Over four years (2006–2009), Lill and Murphy collected >1,100 caterpillars of 14 limacodid and megalopygid species from seven host plant species at four sites near Washington, D.C. We found that "defended" caterpillar species that possess various protuberances and spines (*Acharia stimulea* (Clemens), *Adoneta spinuloides* (Herrich-Schäffer), *Euclea delphinii* (Boisduval), *Isa textula* (Herrich-Schäffer), *Isochaetes beutenmuelleri* (Henry Edwards), *Megalopyge crispata* (Packard), *Natada nasoni* (Grote), *Parasa chloris* (Herrich-Schäffer), *Phobetron pithecium* (Smith)) are significantly more likely to be parasitized by a shared community of relatively specialized parasitoids than are "undefended" cryptic species (*Apoda y-inversum* (Packard), *Lithacodes fasciola* (Herrich-Schäffer), *Packardia geminata* (Packard), *Prolimacodes badia* (Hubner), *Tortricidia flexuosa* (Grote)/*pallida* (Herrich-Schäffer); hereafter referred to only by genus; $\chi^2 = 17.16$, df = 1, $P < 0.0001$; Fig. 2A). Notably, when we compared sources of mortality among limacodid species, we found that parasitism increased with increasing levels of physical defense ($\chi^2 = 41.57$, df = 3, $P < 0.0001$; Fig. 2B), which suggests that parasitoids may preferentially target caterpillars with higher levels of physical defense because their protective phenotypes offer EFS to the developing parasitoid larvae. In this system, because primary parasitoids appear undeterred by the hosts' physical defenses, EFS is more likely to be effective with regard to generalist predators than hyperparasitoids (which were in very low numbers, prohibiting meaningful comparisons).

Parasitoid species differ tremendously in how long they reside in their host before they kill it. Idiobiont parasitoids immediately paralyze their hosts, which arrests the host's development; after their host is paralyzed, idiobiont parasitoids rapidly commence feeding, which causes host death. In contrast, koinobiont parasitoids allow their host to continue development for at least a few days (and often weeks to months), postponing host death until the host reaches a larger size. Although most of the parasitoids that we studied in the limacodid system are technically koinobionts, they vary markedly in the amount of time their larvae reside within the caterpillar host before killing it (Stoepler et al. 2011). For instance, most primary hymenopteran parasitoids that attack limacodid caterpillars (e.g., Braconidae: *Cotesia empretiae* (Viereck) and *Triraphis discoideus* (Cresson); Eulophidae: *Alveoplectrus lilli* Gates, *Pediobius crassicornis* (Thomson), and *Platyplectrus americana* (Girault)) feed as larvae within the host, kill the host while it is still an early- to mid-instar caterpillar, and then emerge as adult wasps within a few weeks of the initial attack. In

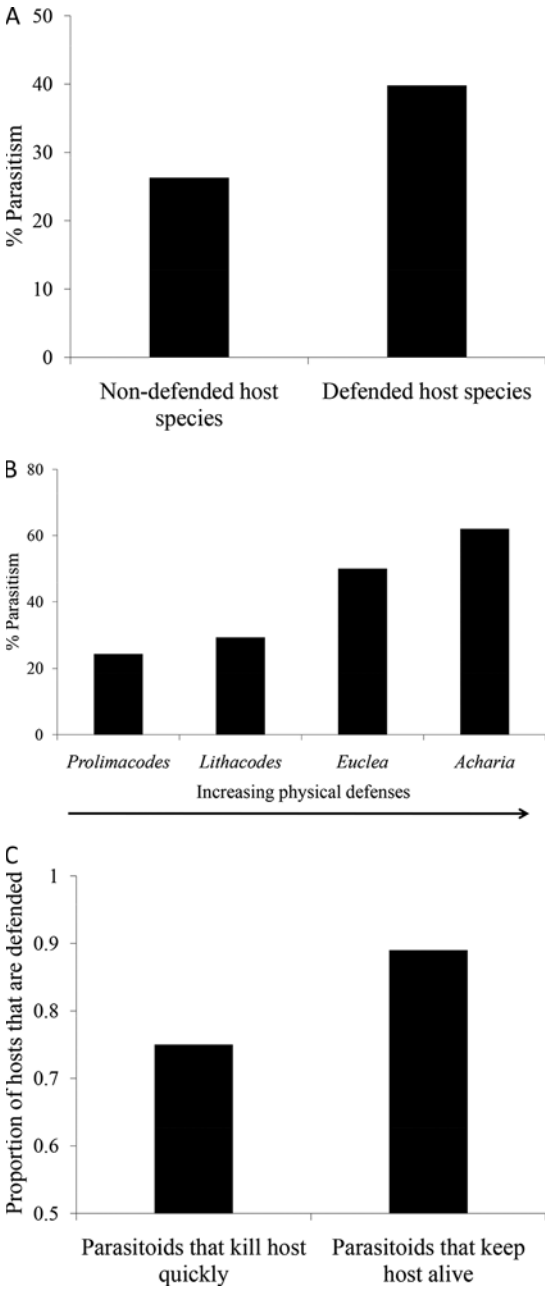

Fig. 2. (A) The percentage of wild-caught caterpillars that were parasitized for all nondefended (cryptic, no stinging spines; $n = 300$) and defended (possess stinging spines; $n = 791$) species combined. (B) The percentage parasitism of wild-caught caterpillars of four limacodid species: *Prolimacodes badia* ($n = 103$), *Lithacodes fasciola* ($n = 147$), *Euclea delphinii* ($n = 44$), and *Acharia stimulea* ($n = 108$). *Prolimacodes* and *Lithacodes* do not possess stinging spines. Both *Euclea* and *Acharia* do possess stinging spines, and *Acharia* is more heavily defended with spines than is *Euclea*. (C) For parasitoid species that either kill their caterpillar host quickly (<1 wk) or keep their host alive (until just before pupation) before killing it, the proportion of hosts that are defended limacodid species compared with undefended limacodid species.

contrast, all of the tachinid fly parasitoids that attack limacodid caterpillars (e.g., Tachinidae: *Austrophorocera* sp., *Compsilura concinnata* (Meigen), and *Uramya pristis* (Walker)) require that the parasitized host either completes or nearly completes larval development, a process that may require one to several months in these slow-growing caterpillars. Thus, we predicted that although all parasitoids could potentially benefit from attacking defended caterpillars in preference to nondefended caterpillars, tachinid parasitoids should be under greater selective pressure to attack defended caterpillars than the majority of the hymenopteran parasitoids, which have a much shorter window of vulnerability within their herbivorous host than do tachinids. In support of this prediction, we found that parasitoid species with a long duration of host association (i.e., tachinids) use a greater proportion of defended host species than do parasitoid species that kill their host relatively quickly ($\chi^2 = 9.99$, df = 1, $P = 0.0015$; Fig. 2C). Consistent with these findings, Jervis and Ferns (2011) recently argued that one advantage of postponing host death is the additional protection provided to developing koinobiont parasitoids via the hosts' pupation retreat. Thus, parasitoids appear to disproportionately attack physically defended hosts, which is consistent with the hypothesis that parasitoids may select caterpillar hosts that offer their offspring EFS from their own natural enemies.

EFS in Chemically Defended Hosts. Dyer and Gentry (1999) noted that in surveys of host–parasitoid interactions, caterpillar hosts that are chemically defended often suffer a higher incidence of parasitism, which suggests that these defended hosts offer "safe havens" for developing parasitoids (see also Lampert et al. 2010). There are two primary ways in which chemically defended hosts may represent EFS for parasitoids. First, because the chemically defended host is protected from being eaten by predators, the parasitoids are protected from such predation as well (Ode 2006 and references therein). Second, if the parasitoids themselves are able to store chemical compounds from their hosts (e.g., Bowers 2003, van Nouhuys et al. 2012), then inhabiting a chemically defended host may provide a direct defense for the parasitoid against its own enemies (hyperparasitoids and predators).

Caterpillars that sequester one group of plant allelochemicals, the iridoid glycosides, provide a system with which to investigate both these possibilities. Caterpillars in at least five different families (Sphingidae, Nymphalidae, Erebidae, Noctuidae, and Geometridae) are chemically defended by sequestering iridoid glycosides from the plants on which they feed (Nishida 2002 and references therein). Sequestration of iridoid glycosides serves as a defense against predators; caterpillars or adult butterflies containing high amounts of these compounds are avoided by both vertebrate and invertebrate predators (Bowers 1991, and references therein). Although caterpillars that sequester iridoid glycosides may gain protection from predators, in some species these benefits appear to be

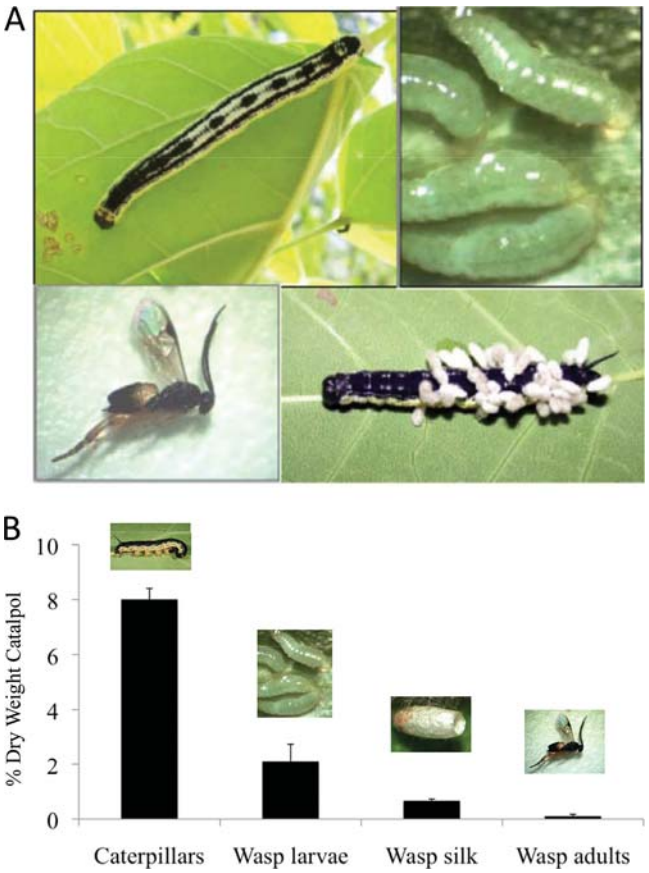

Fig. 3. (A) The catalpa sphinx, *Ce. catalpae*, and its parasitoid, *C. congregata.* Clockwise from upper left: *Ce. catalpae* last instar larva, *C. congregata* parasitoid larvae, *Ce. catalpae* caterpillar parasitized by *C. congregata*, and *C. congregata* adult (Photographs: 1, 2, and 4 by E. Lampert, 3 by D. Bowers). (B) Comparative iridoid glycoside content of last instar *Ce. catalpae* caterpillars (mean ±SE of 102 wild-collected larvae, range 1.5–15.7%), *C. congregata* parasitoid larvae (mean ±SE of 30 broods, range 0.3–5.0%), cocoons and meconium (mean ±SE of broods of 17–25 cocoons from four individual caterpillars), and adults (mean ±SE of broods of 20–25 individuals from four individual caterpillars). (Online figure in color.)

offset by high levels of parasitism. For example, collections of iridoid glycoside sequestering larvae of the sphingid, *Ceratomia catalpae* Boisduval (Fig. 3A), a specialist on species of *Catalpa* (Bignoniaceae), from six populations in the eastern United States, showed that parasitism by the gregarious parasitoid, *C. congregata* (Braconidae), ranged from 15 to 80% of the caterpillars collected (Lampert et al. 2010). In other species of iridoid glycoside-sequestering melitaeine butterflies, parasitism rates also vary substantially (Stamp 1981a, van Nouhuys and Hanski 2004 and references therein). To the degree that this temporal and spatial variation in parasitism reflects differential responses of these parasitoid populations to levels of host sequestration, these patterns provide a template to investigate EFS for the parasitoids.

If toxin-sequestering caterpillar hosts provide EFS for parasitoids, then the success or performance of parasitoids should be better in hosts with higher levels of sequestered compounds compared with those with lower levels when enemies are present. In field collections of *Ce. catalpae* (Fig. 3A), Bowers and colleagues found a weak positive, but not significant, relationship ($r = 0.20$, $P = 0.09$) between sequestered iridoid glycosides and clutch size of the parasitoid *C. congregata*, and survivorship was >90% for all parasitoid broods, regardless of caterpillar iridoid glycoside content (Lampert et al. 2010). These data indicate no negative relationship between host-sequestered iridoid glycosides and parasitoid success and suggest a positive effect of increasing levels of sequestered host plant compounds on parasitoid host choice.

Parasitoids that reside in sequestering hosts may be protected from their own hyperparasitoids in two ways. They may be directly protected if they have the ability to sequester defensive compounds from their hosts and if these sequestered compounds are deterrent or toxic to their hyperparasitoids. In addition, they may be indirectly protected if sequestered compounds in, for example, the caterpillar host cuticle or hemolymph are deterrent to hyperparasitoids. There are a few reports of parasitoids sequestering compounds from their caterpillar hosts (e.g., Bowers 2003, Talsma 2007, Lampert et al. 2011), and sequestration

of iridoid glycosides by parasitoids has been reported in two different host–parasitoid systems: *Melitaea cinxia* (Nymphalidae) and its parasitoids (Talsma 2007) and *Ce. catalpae* and its braconid parasitoid, *C. congregata* (Bowers 2003, Lampert et al. 2011; Fig. 3B).

Very few studies have directly examined the effects of host sequestration on hyperparasitoids; however, van Nouhuys et al. (2012) investigated this using two species of hyperparasitoid (*Lysibia nana* Gravenhorst and *Gelis agilis* F., both Ichneumonidae), with wide host ranges. These hyperparasitoids were reared on a chemically defensed wasp host, *Cotesia melitaearum* Wilkinson reared on *Melitaea cinxia* L., and on a nondefended wasp host, *Cotesia glomerata* L. reared on *Pieris brassicae* L. (van Nouhuys et al. 2012). *C. melitaearum* can sequester iridoid glycosides from its caterpillar host (Talsma 2007). Results showed that one hyperparasitoid, *G. agilis*, did sequester iridoid glycosides, but the other species contained only trace amounts. Furthermore, they found that in a set of laboratory experiments, both hyperparasitoids performed equally well on the chemically defended and the nondefended hosts (van Nouhuys et al. 2012), indicating that in this system, sequestration by the primary parasitoid does not protect against hyperparasitoids, but may protect against generalist predators.

A last instar larva of *Ce. catalpae* may contain as much as 23 mg of the iridoid glycoside catalpol (Lampert et al. 2011), and the hemolymph contains 50% dry weight of this compound (Bowers 2003). Thus, *C. congregata* parasitoids that develop in these caterpillars are exposed to very high levels of iridoid glycosides. Chemical analyses of parasitoid larvae and adults of *C. congregata* parasitoids show that they do indeed sequester catalpol, but at levels substantially lower than those found in the host caterpillars (Bowers 2003, Lampert et al. 2011, Fig. 3B). The levels of catalpol found in the parasitoid larvae (mean of 2% dry weight) could be deterrent to hyperparasitoids, although this has not been tested. There are several hyperparasitoids that have been recorded from *C. congregata* parasitizing *Ce. catalpae* caterpillars (Lampert et al. 2011); however, experimental investigation of the importance of parasitoid chemical content on the responses of hyperparasitoids is still needed (but see van Nouhuys et al. 2012). Similar levels of iridoid glycosides in larvae of the beetle, *Longitarsus melanocephalus* (Geer) (Chrysomelidae), were effective against an entomopathogenic bacterium, *Bacillus thuringiensis* (Baden and Dobler 2009), which suggests that sequestered iridoids could also serve an antipathogenic function. Furthermore, ants prey on *C. congregata* pupae from *Ce. catalpae* caterpillars (Ness 2003), raising the possibility that variation in catalpol sequestration by parasitoids could influence the risk of ant predation.

Data from at least one system, that of plants containing iridoid glycosides, sequestering caterpillars feeding on those plants, and the parasitoids of those caterpillars, suggest that sequestering caterpillars may indeed provide chemically mediated EFS for primary parasitoids. Future experimental work needs to address: 1) the importance of caterpillar host chemistry in protecting parasitoids by protecting the caterpillar hosts, 2) the efficacy of chemical compounds sequestered by parasitoids in protecting them from hyperparasitoids and their generalist predators, and 3) both the direct and indirect effects of host caterpillar chemistry on oviposition decisions by both parasitoids and hyperparasitoids.

Host Plant-Related EFS. Because parasitoids spend their immature lives in their hosts, some of the same factors that can generate EFS for hosts can secondarily generate EFS for parasitoids. In studies of EFS for herbivores, the host plant plays a prominent role (Price et al. 1980, Gratton and Welter 1999, Murphy 2004). Aside from host plant-derived chemical defense (described above), different host plant species or phenotypes may expose herbivores and their parasitoids to different levels of predation risk due to variation in predator encounter rate with prey or hosts. Consistent exposure to predation on or in their hosts suggests that parasitoids are likely to experience selection to preferentially oviposit in hosts living in microenvironments offering EFS. If predators forage in a density-dependent manner, then avoidance of predation by parasitoids might often (but not always) lead to negatively spatial, density-dependent parasitism (Tscharntke 1992), a mechanism that has generally been neglected in the study of density-dependent parasitism (Hassell 2000). The focus here, however, is the effect of host plant species or phenotypes on EFS for parasitoids of herbivores. Host plant-related EFS for parasitoids may be associated with plant variation in food quality for the herbivore (e.g., slow-growth and high-mortality hypothesis), variation in herbivore density, or both. Several studies have shown host plant-related variation in parasitism of herbivores at a community level (e.g., Barbosa et al. 2001, Lill et al. 2002, Farkas and Singer 2013), but the underlying mechanisms remain elusive. Furthermore, the possibility of EFS for parasitoids has rarely been discussed in this context.

To provide an initial test of host plant-related EFS for parasitoids at a community level, Singer and colleagues compared the frequency of parasitism of an assemblage of dietary generalist caterpillars among tree species upon which the caterpillars are subject to a gradient in the risk of bird predation per caterpillar (Singer et al. 2012). The EFS for parasitoids hypothesis predicts a negative relationship between the frequency of parasitism and the risk of bird predation. As discussed above (EFS in physically defended hosts), this hypothesis additionally predicts the strongest negative relationship for parasitoids that, as larvae, occupy host life stages most vulnerable to predation. We therefore make the additional empirical prediction that this negative relationship should be strongest for tachinid fly parasitoids of caterpillars, which, as larvae, typically remain in the host until the caterpillar's final instar or beyond (Stireman et al. 2006). In contrast, the larvae of many hymenopteran parasitoids, such as braconid wasps, exit the host during earlier caterpillar instars (Godfray 1994), thus reduc-

Fig. 4. Generalist caterpillars are attacked by both predators (e.g., black-capped chickadee feeding on a caterpillar, on left) and parasitoids (e.g., generalist noctuid *Himella intractata* Morrison (currently, *H. fidelis* Grote) parasitized by a hymenopteran parasitoid, on right). Photographs: left by C. Skorik, right by M.S.S. (Online figure in color.)

ing the larval parasitoid's exposure to bird predation while in the host.

To test these predictions, we examined data from a forest food web constructed by Singer and colleagues in Middlesex County, CT, in late spring–early summer (May–June; Singer et al. 2012). An assemblage of dietary generalist caterpillars occupying eight different tree taxa (*Acer rubrum* L., *Betula lenta* L., *Carya* spp., *Fagus grandifolia* Ehrhart, *Hamamelis virginiana* L., *Prunus serotina* Ehrhart, *Quercus alba* L., and *Quercus rubra* L.) served as the hosts for parasitoids, with caterpillars and parasitoids subjected to concomitant predation by insectivorous birds (Fig. 4). To determine the frequency of parasitism of each caterpillar species on each tree species, we systematically collected caterpillars from haphazardly chosen branches, reared the caterpillars in the laboratory on the same tree species from which they were collected, and tallied the proportion of caterpillars that yielded parasitoid flies and wasps (see full methods in Farkas and Singer 2013). These data were collected over five field seasons (2004–2008) at three forest sites separated by >10 km (Cockaponset State Forest, Haddam; Hurd State Park, East Hampton; Miller's Pond State Park, Durham, CT).

To determine the magnitude of bird predation of caterpillars on each tree species, we collected caterpillars from tree branches with bird exclosures along with caterpillars from paired control branches lacking exclosures (for full methods, see Singer et al. 2012). This same general methodology of bird exclusion has been used in >50 previous studies to quantify the effect of bird predation on arthropods (Mooney et al. 2010). In our study, bird exclosures were in place over a 3-wk period before caterpillar collection; these exclosures permit access by insects (including parasitoids) but keep out birds and most other vertebrate predators. Caterpillar densities on each branch were calculated as the number of caterpillars/total leaf area of the branch. Total leaf area for each branch was estimated as the number of leaves multiplied by the average leaf area (mean area of 10 undamaged leaves randomly sampled from each branch). The magnitude of bird predation was expressed per pair of branches as a log response ratio: ln(caterpillar density without birds/caterpillar density with birds). The mean log response ratio per tree species was our measure of the magnitude or effect size of bird predation of caterpillars on each tree species. The bird predation data were collected over two field seasons (2008–2009) at the same time of year and sites used for measuring parasitism in earlier years.

We observed that the frequency of parasitism of generalist caterpillars varied among tree species (Farkas and Singer 2013). As observed in similar studies (Barbosa et al. 2001, Lill et al. 2002), these differences in parasitism among tree species were consistent over the 5-yr period (i.e., no tree species × year interaction). To address the EFS for parasitoids hypothesis, we separately tested the effect size of bird predation per tree species and the effect of generalist caterpillar density per tree species on the total mortality of generalist caterpillars from parasitoids per tree species, pooled over all years. In these analyses, we used mul-

Table 1. Multiple regression analyses of variation in parasitism of the generalist caterpillar assemblage among tree species

Model	Tachinid parasitism			Hymenopteran parasitism		
	R^2	Parameter estimate	P	R^2	Parameter estimate	P
LRR bird predation caterpillar species richness	0.67	−11.40 0.76	0.025 0.077	0.083	−0.56 0.16	0.85 0.59
Caterpillar density caterpillar species richness	0.49	−4.15	0.081 0.57	0.82	−2.49 0.21	0.0059

LRR, log response ratio.

tiple regression models that also included the species richness of generalist caterpillars per tree species because previous, unpublished work on this system identified this variable as a predictor of parasitism frequency. As predicted by the EFS hypothesis, variation among tree species in the frequency of generalist caterpillar mortality from tachinid parasitoids was negatively associated with variation in the effect size of bird predation among trees ($P = 0.025$, Table 1), with a similar negative trend in relation to generalist caterpillar density ($P = 0.081$, Table 1). Thus, plant species on which caterpillars tended to be at low densities and faced consistently lower levels of predation by birds (e.g., *Fagus grandifolia*) subjected these caterpillars to higher levels of attack by tachinid flies. By contrast, no such pattern was observed for caterpillar mortality by hymenopteran parasitoids ($P = 0.85$, Table 1). However, caterpillar mortality inflicted by wasps was negatively associated with generalist caterpillar density among tree species ($P = 0.006$, Table 1). It is possible that EFS still plays a role in host use by hymenopteran parasitoids, though in this case invertebrate predation (not measured here) might be a stronger selective force than avian predation because invertebrate predation is especially intense on the earliest life stages of insect herbivores (e.g., Feeny et al. 1985, Bernays and Montllor 1993, Mira and Bernays 2002, Remmel et al. 2011), which is when hymenopteran attack also tends to be highest. We encourage more refined tests of the hypothesis that parasitoids and other carnivores might preferentially choose hosts or habitats as an adaptive response to avoid host plant- or habitat-related predation risk.

Conclusions

Here we have presented three case studies of possible mechanisms for the role of EFS in parasitoid–host interactions. We believe that these examples illustrate some of the various ways parasitoids may garner EFS by preferentially attacking physically and chemically defended hosts deterrent to generalist predators and hyperparasitoids or by displaying host-selection tactics that minimize IGP by vertebrates. Although two of our three case studies focus on the top–down forces of generalist predators, we hypothesize that hyperparasitism may be an equally important force in selection for EFS for primary parasitoids. In highlighting these case studies, we hope to spur additional research on EFS in primary parasitoids as well as other guilds of insect predators and hyperparasitoids to highlight the important role that EFS may play in shaping ecological niches of a much wider array of species than herbivores. We propose that any species with natural enemies could be subject to EFS, even those at higher trophic levels.

Traditionally, in terrestrial systems, bottom–up effects have received the most attention when considering the forces that structure population dynamics at higher trophic levels. In contrast, the importance of top–down control in aquatic systems has been recognized for a long time (Strong 1992). Incorporation of the EFS concept at higher trophic levels could explain variation in population and community dynamics that is currently unexplained by bottom–up processes alone. Furthermore, this perspective could improve our understanding of natural selection on traits of higher trophic-level organisms. Although the case studies we present here all concern interactions between parasitoids and their natural enemies, predators are probably also subject to the selective pressures of EFS. For instance, refuges are often thought to be used by predators to enhance their hunting ability by reducing their visibility to prey. Experiments by Manicorn et al. (2008) demonstrated that predators also construct refuges for protection against their own predators, which we suggest is a type of EFS. Alternatively, predators may alter their distribution in response to higher trophic-level threats, as seen in the cheetahs of Tanzania that preferentially occupy the fringes of national parks where predation of their cubs by lions is minimized (Kelly and Durant 2000). Smaller-bodied or less aggressive predators, occupying certain niches, including a variety of mesopredators, are likely targeted by stronger top–down pressures than higher order carnivores, but by failing to consider that carnivores in general are subject to the same top–down pressures as organisms at lower trophic levels, we limit our understanding of the role that natural enemies play in natural communities. Rather than dichotomize top–down and bottom–up effects, the most productive approach would be to investigate the simultaneous effects of top–down and bottom–up factors on parasitoids and other higher trophic-level consumers, similar to the numerous studies that study their joint effects on herbivores. The interplay between top–down and bottom–up effects on multiple trophic levels could be a fruitful area of research and would present a new role for studies of EFS in terrestrial systems.

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