

Costly leaf shelters protect moth pupae from parasitoids

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Abstract Many caterpillars construct shelters by folding leaves and feeding from within. Many shelter-constructing species suffer high rates of parasitism as larvae or pupae. In spite of the likely significance, the effects of these shelters on the survival of pupae and the trade-off between feeding and constructing shelters have attracted little experimental attention. In both field and laboratory experiments, fern-feeding caterpillars [*Herpetogramma theseusalis* (Walker) (Lepidoptera: Crambidae)] invested heavily in shelters, losing weight and significantly delaying pupation rather than feeding or pupating in exposed locations. Experimentally thinning the walls of shelters in the field doubled the parasitism rate. Parasitism of pupae exceeded predation by an order of magnitude or more in both seasons of this study. Caterpillars constructed similarly sized shelters regardless of availability of fern fronds, resulting in incomplete shelters on especially small fronds, putting pupae at increased risk of parasitism. We suggest that similar interactions are common and merit more attention.

Keywords Leaf shelters · Host–parasitoid interactions · Indirect interactions · Caterpillars · Pupae

Introduction

Many herbivorous insects construct shelters by rolling up or tying leaves, thereby forming an important and distinctive part of many terrestrial communities. Shelter builders are both abundant and diverse and make up over 20–60 % of the lepidopteran species (moths and butterflies) in many communities (Lill and Marquis 2007; Diniz et al. 2012). Shelter construction takes time and energy for the makers and may put them at increased risk of mortality during construction (reviewed in Greeney et al. 2012). Benefits to shelter builders include protection from certain natural enemies (reviewed in Fukui 2001), amelioration of environmental conditions (Diniz et al. 2012), lessened plant defenses (Sagers 1992) and a protected food source (e.g., Danks 2002; Cerda et al. 2010). More broadly, studies have repeatedly found that leaf shelters increase local diversity by providing habitat for various other arthropods (Lill and Marquis 2003; Martinsen et al. 2000; Nakamura and Ohgushi 2003).

Parasitoids are well known to constitute a major source of mortality of both shelter-inhabiting and open-feeding caterpillars (Hawkins 1994; Hagen et al. 2006; Schott et al. 2010), making them a potentially strong selective force. Gentry and Dyer (2002) found solitary sheltered caterpillars suffered more than twice the parasitoid-induced mortality of solitary exposed caterpillars. In contrast, Hawkins (1994) found that while parasitoids are more diverse per host on shelter constructors than external feeders, they cause similar amounts of mortality on the two guilds, averaging ~40 %. Despite high parasitism, direct predation receives the most

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attention when discussing mortality in leaf shelters; parasitoids have garnered far less attention in the previous work (reviewed in: Greeney et al. 2012; Lill and Marquis 2007, but see Diniz et al. 2012).

Two non-exclusive relationships between the shelter-building guild of caterpillars and their parasitoids have been posited that: (1) shelters must ineffectively defend against parasitoids (Loeffler 1996a) and (2) shelters provide parasitoids with safe space from their enemies (Gentry and Dyer 2002). However, a recent review suggests a need for more studies on these hypotheses (Greeney et al. 2012). We suggest a third hypothesis contrary to the first, based on our field observations: Only well-built shelters protect inhabitants against parasitism.

Larvae of the fern moth *Herpetogramma theseusalis* (Walker) (Lepidoptera: Crambidae) build shelters on ferns, sometimes at high densities, usually constructing two to three shelters per season (N.H. Rose, unpublished data). Fern moth larvae and pupae are heavily parasitized and thus provide an excellent opportunity to test the role of shelters in mediating parasitism, as well as to compare parasitoid impact on this shelter-dwelling lepidopteran with that of predators. Although larval parasitism results in the highest single mortality source of fern moths yearly (Morse 2009, 2011), a large segment of the population survives to pupation.

Pupae constitute an extremely vulnerable stage in the lepidopteran life cycle (Wiklund and Sillén-Tullberg 1985) and often experience the highest mortality of any stage (Hawkins et al. 1997). We hypothesize that shelters play an important role in their defense, since movement and avoidance are limited at this stage and fern moth caterpillars pupate exclusively inside their shelters. Plant characteristics such as leaf area and toughness strongly influence shelter-building behavior (Greeney et al. 2010). Therefore, we examined physical frond characteristics and the effects of these characteristics on shelter architecture in the field and thus indirectly on pupal survival.

Five ichneumonid wasps regularly parasitize fern moth pupae at our field site and surrounding areas along the coast of Maine, USA: *Phaeogenes hebrus* (Cresson), *Itopectis conquisitor* (Say), *Vulgichneumon brevicinctor* (Say), *Sinophorus townesorum* Sanborne and *Mesostenus thoracicus* Cresson. *Phaeogenes hebrus* and *V. brevicinctor* have short ovipositors (<2 mm) and thus must enter the shelter to oviposit. *P. hebrus* host-feeds after oviposition (E.F. LoPresti, pers. observ.), a common behavior in long-lived wasps allowing the sequential maturation of eggs (Jervis and Kidd 1986). The other three parasitoids have long ovipositors and oviposit into the pupa while remaining outside the shelter (E.F. LoPresti, pers. observ.). All species occur commonly except *V. brevicinctor*, which only occurred during 1 year of the study (2009), though in

significant numbers. Several other primary parasitoids attack the caterpillars or pupae, but all are rare, and their exact life histories are unknown to us; thus, they are not treated in this paper.

We used manipulative field experiments to test the effectiveness of complete shelters against pupal parasitoids and predators. We evaluated the cost of shelter construction in both the field and laboratory and quantified predation in the field during two seasons. Additionally, we examined what characteristics of shelters put pupae at risk of parasitism and what, if any, effects fern physical characteristics had on this interaction.

Materials and methods

Study area and organisms

We performed all manipulations in a 3.5-ha old field and laboratory at the Darling Marine Center in Walpole, Lincoln County, Maine, USA (43°57'N, 69°33'W), and gathered additional shelters from three nearby roadside patches.

Upon emerging from the litter in the spring, overwintering fern moth caterpillars ascend the newly unfurled fern fronds and construct roughly round shelters (ca. 2–3 cm diameter) on the ferns by rolling the apical end down, notching the rachis (stem) and binding pinnae (leaflets) to the outside of this construction with silk (Online Resource 1). The caterpillars then feed from the inside of the shelters (Morse 2009) and continue construction intermittently during the night for the duration of their late larval period (D.H. Morse, unpublished data), constructing two or more shelters during this time (as described for a congener in Ruehlmann et al. 1988). Though the caterpillars will feed on a variety of ferns, in this study we focused on shelters constructed on sensitive fern *Onoclea sensibilis* (Dryopteridaceae), since it supports the vast majority of the populations in the region.

We performed the fieldwork for this study during the summers of 2009 and 2012. The 2009 summer lagged markedly behind the 2012 summer in overall phenology due to cooler temperatures and heavy rainfall throughout June and early July. However, pupal parasitism rates did not differ greatly (D.H. Morse and E.F. LoPresti, unpublished data). In both 2009 and 2012, *P. hebrus* and *I. conquisitor* were the most common parasitoids and accounted for a majority of pupal mortality.

Short-term shelter construction cost

Caterpillars emerging from the leaf litter in the late spring must construct a shelter before feeding, as they will not feed in open situations (E.F. LoPresti, per observ.).

Additionally, most caterpillars switch ferns 1–2 times during the season (D.H. Morse, unpublished data; for *Herpetogramma aeglealis* Ruehlmann et al. 1988). To simulate the costs that the caterpillar faces at these points in its life, in mid-June 2009, we transplanted sensitive ferns from the field into plastic pots (11.4 cm diameter, 12.4 cm depth) in the laboratory. On the same day we collected caterpillars, weighed them and placed them on the potted ferns, maintaining normal day length and ambient temperature. On the following morning we collected the shelters, recorded the length of the longest axis and the two longest orthogonal axes of the shelter (together the circumscribed prism) and weighed the caterpillars. We then calculated both absolute and percentage change in mass for caterpillars that built a shelter ($n = 26$, out of the 36 caterpillars presented with a fern). We performed a linear regression correlating the resulting shelter size with the absolute change in mass to evaluate whether larger shelters required more investment of energy to construct.

We filmed 16 of these caterpillars using infrared video (3.6 mm cameras with Security Eyes software and Handi-Avis software [AZ Cendant] to time-lapse the video). Eleven of these caterpillars constructed shelters. From the videos, we calculated the amount of time spent constructing shelters.

Long-term shelter construction costs

To calculate the longer-term costs of shelter construction in the field, we marked 90 fresh shelters on June 7, 2012, moving around the plot and alternating among shelters to produce three treatments: control (measured circumscribed prism only), handling control (measured and touched for 15 s) and manipulation (measured and opened to the point where every accessible seam was opened). The shelters were then covered with nylon tricot bags, roughly 15×25 cm. We handled and opened the shelters in the handling control and manipulation treatments again on 11 June, 14 June and 17 June and collected all shelters for analysis on 21 June, except three that could not be located. We found two of these the following day and included them in analyses and the other in late July, but it was empty upon collection and thus we excluded it.

Upon collection, we measured the shelters present in the bag and recorded the state and weight of the occupant. We raised all caterpillars and pupae in 7-dram vials to the adult stage under laboratory conditions that mimic field phenology (see Morse 2009) and checked them daily for emergences. We analyzed pupal mass, emergence date and emergence mass using an ANOVA with treatment as the predictor, excluding all parasitized individuals.

Shelter-thinning experiment

To test the effectiveness of shelters in reducing parasitism in pupae, in early July 2009 we manipulated shelters in three plots in different areas of the field (separated by >20 m) by removing the outer layer of shelter material (usually 1–2 pinnae) by peeling it back without breaking the central vein of the pinnae, if possible. In certain sections, we could not remove the entire outer layer, because of the complex weaving of the pinnae; in these cases we removed as much as possible, but not the entire layer. Alternating between thinning and control treatments as we moved around each plot, we measured, handled and tagged the control shelters identically, though we did not manipulate them. Thus, the “control” treatment more accurately represents a handling control; however, our rates of parasitism in the control group were similar to yearly field collections of pupae (Morse 2011); thus, this lack of a true “control” is not a problem in interpretation of the results.

In only the manipulated treatment did we have any prior information on the inhabitant, since the manipulation occasionally produced tiny openings (1–2 mm wide). If we could see inside the shelter without further manipulation and found it empty, we selected a new shelter; if occupied, we noted the stage (e.g., pupa, larva or parasitoid pupa) of the occupant. We needed to secure a sample of fresh pupae, since both *P. hebrus* and *V. brevicinctor* refuse to parasitize pupae over 3 days old in the laboratory (E.F. LoPresti and D.H. Morse pers. observ.), and other studies have also shown more developed pupae to be less desirable hosts for parasitoids (Smith 1932 for a *Phaeogenes* sp., Brault 1991). At the time of manipulation, we had no way of knowing the contents of any other shelters; thus, many fewer shelters were usable than the 606 shelters we marked for the experiment (287 in Plot 1, 182 in Plot 2 and 137 in Plot 3).

We collected the shelters 6 days later, noting whether the occupants had added pinnae to the shelter. We measured and opened the remaining shelters and recorded the occupants, including any predators or preyed-upon pupae. We reared these as detailed in the section on the long-term cost of constructing shelters.

We reared fern moth pupae to determine the length of their pupal period: 12.5 ± 0.15 days SE ($n = 61$). From the time of collection to eclosion, the most common parasitoid, *P. hebrus*, required at least 15 days for larval development (E.F. LoPresti, pers. observ.), which accords well with Smith's (1932) estimate of 15 days (plus 1 day before exiting puparium) for *P. nigridens* under similar conditions. We used these data to determine the age of the pupae from the field experiment. We discarded any emergences that occurred too early for parasitism to have occurred during the study period, since the manipulation

could not have affected this parasitism. Thus, all pupae used were of approximately equal age and vulnerability, except for the shelter manipulation.

We discarded data on empty shelters and shelters that had been added to since the alteration, because shelter building indicated the presence of a caterpillar, not a pupa, during part of the study period. Four shelters disappeared in the control and experimental treatments, likely overlooked when collecting; thus, we discarded these data points from the analysis. Additionally, we discarded individuals that died in the pupal stage, since we could not determine whether they were alive during the field experiment ($n = 57$). We additionally did not include in the analyses the larval parasitoid *Alabagrus texanus* ($n = 126$) or others with undocumented life histories ($n = 12$). Shelters included in analyses for each plot numbered 75, 33 and 38, respectively.

We analyzed these data using G test with a three-way contingency table and the routine outlined in Sokal and Rohlf (1969). We also analyzed the data with a paired t test with plots as pairs and a binomial (logistic) regression. Results for all tests were very similar; for simplicity we report only the G test results.

Predation experiment

To ascertain whether pupal predation occurred that could not be detected in collections due to removal of the pupae by predators, we selected 90 shelters in a separate nearby patch and divided them into control and manipulated treatments. In this experiment we measured, opened and noted the occupants of both control and manipulated shelters, immediately resealing them with small pieces of masking tape. We opened the shelters by prying apart pinnae along seams to minimize structural changes in the size and shape of the shelter.

In the laboratory we also presented field-collected fern moth pupae to three potential predators commonly found in the shelters to determine whether they would prey upon pupae: crab spiders *Xysticus emertoni* (Araneae: Thomisidae), jumping spiders *Phidippus clarus* (Araneae: Salticidae) and European earwigs *Forficula auricularia* (Dermaptera: Forficulidae). We collected 10 individuals of each potential predator species from sensitive fern patches (all females in *Xysticus*, since males are very rarely encountered) and then starved them for 3 days in the laboratory before placing them in a clean 9.5-cm-diameter petri dish with a piece of moist paper towel and a fresh pupa. Twenty-four hours later, we recorded the outcome of the trial and released the participants.

Field shelter and patch characteristics

In an effort to determine whether shelter characteristics influenced parasitism in unmanipulated shelters, we

collected midsummer (pupal stage) shelters from four nearby sites: a large patch in our study field (“Field”—152 shelters), two sensitive fern patches off Old Barn Road [“Pondside” ~ 50 and “Center” ~ 75 (empty shelters not completely counted at these two patches)] and one patch near the corner of state Rt. 129 and Clark’s Cove Road (“Roadside” 119 shelters). We recorded volume of each shelter’s circumscribed prism, whether openings existed in the shelter and the contents. We scored an opening if one could see into the shelter from the outside. Openings were unlikely to have been made by parasitoids, as they chew the silk at the junction of pinnae and squeeze into the shelter. We reared all pupae collected following the same protocols detailed above. For the analyses of shelter characteristics, we only used data from completed shelters (i.e., containing a pupa or prepupa at collection) and did not include shelters containing *Alabagrus* pupae, in case the parasitoid larvae changed caterpillar behavior or activity level during construction.

Additionally, we randomly selected ten fronds without shelters in each patch and measured frond area by photographing each frond flat under glass against a white background with a scale bar and then calculating the leaf area using ImageJ (NIH).

Analyses

We performed all statistics and some graphics with R version 2.13 (R Foundation for Statistical Computing 2011) and used Microsoft Excel for tables and other charts. All measures of variance represent one standard error (SE).

Results

Shelter construction costs: short term

Of the subset of 16 caterpillars under video surveillance, 11 constructed shelters, which required from 2:57 to 7:50 h (mean = 5:06 \pm 0:23.5 h). Of the 26 caterpillars that constructed shelters in the laboratory experiment, 24 lost mass and 2 gained mass during the overnight shelter construction period (mean $-12.87\% \pm 1.56$). The linear regression of built shelter size and absolute mass loss (log-transformed) was significant (Fig. 1: $F_{1,23} = 20.23$, $p = 0.0002$, $r^2 = 0.468$) and produced the following formula: change in mass = 7.372–2.44 \times ln(shelter volume).

These 26 shelters averaged 2.27 \pm 0.46 cm³ in circumscribed prism volume. In the field, this measure reached a season maximum of 14.38 \pm 0.83 cm³ (collection on July 23, 2008, $n = 118$). At this time, 32.1 % of shelters were empty (no larva, pupa or pupal cast), implying that some caterpillars built multiple shelters, even before all pupation and shelter construction have occurred.

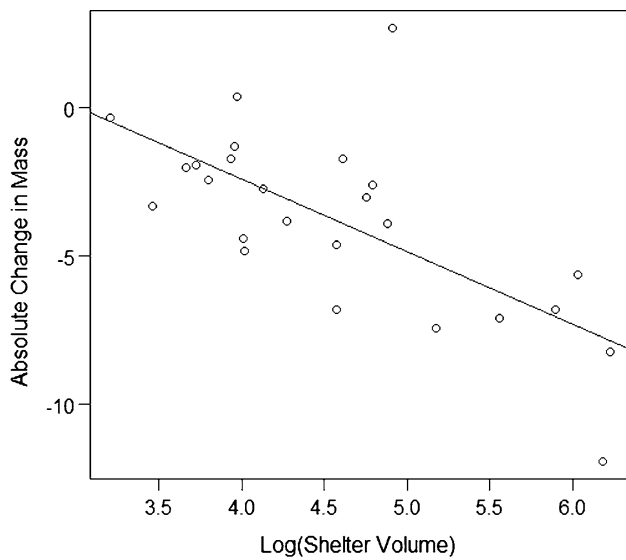


Fig. 1 Relationship between shelter size constructed and the absolute loss of mass in the one-night short-term shelter-cost trials

Shelter construction: long term

Caterpillars experimentally forced to remake shelters pupated 1–2 days later than the control and handling control treatments (one-way ANOVA, $F_{2,46} = 3.36, p = 0.046$). Shelters across treatment groups did not differ significantly in initial circumscribed prism volume (one-way ANOVA, $F_{2,49} = 1.24, p = 0.300$), which suggests no bias in the selection of treatments.

We hypothesized that the pupal mass would be lower for caterpillars forced to rebuild shelters, yet treatments did not differ significantly in this metric (Table 1: one-way ANOVA, $F_{2,46} = 0.46, p = 0.63$). Treatment did not affect pupal period, suggesting no quicker development to compensate for the later pupation date in the manipulation group (Table 1: one-way ANOVA, $F_{2,46} = 2.15, p = 0.13$).

We also hypothesized that the manipulation and the resulting higher energy expenditure would result in smaller adults for those forced to remake shelters. We found no significant differences in mass at emergence (Fig. 2: one-way ANOVA: males: $F_{2,25} = 0.15, p = 0.86$, females: $F_{2,18} = 1.04, p = 0.37$). Females in the manipulated

treatment averaged ~ 10 % less in mass than females in the other two treatments, though this result was not significant.

Therefore, increased shelter building likely had little effect on adult fecundity (which usually correlates well with female size), yet it slowed development. All of these data strongly suggest a major expenditure of time and resources in shelter production and maintenance.

Shelter-thinning experiment

Pupae in experimentally thinned shelters experienced approximately twice the rate of parasitism as the control treatment in all three plots (Fig. 3: overall G test, $G = 19.139, df = 7, p = 0.002$). This overall G test only tests the independence of the plot, treatment and fates, and a more specific g test found that treatment and fate were highly significant non-independent ($G = 17.173, df = 1, p < 0.001$), while no other terms (plot and treatment, plot and fate) were significant ($G = 1.462, df = 2, p = 0.241, G = 0.632, df = 2, p = 0.365$, respectively).

Both short- and long-ovipositor parasitoids exploited the experimentally thinned shelters more heavily than the unmanipulated shelters. Short-ovipositor species occurred in all plots in each treatment and accounted for significantly more mortality in thinned shelters (G test of independence of treatment and fate, $G = 12.931, df = 1, p < 0.001$). All but one ($n = 13$) of the long-ovipositor species occurred in Plot 1 (control/thinned 2:10) significantly higher in the thinned group than control in that plot (Fisher’s exact test, $p = 0.007$).

Predation

The frequency of predation did not differ between the control and thinned shelters. One instance of predation occurred in 45 control shelters, and another in the 44 manipulated shelters. In the laboratory predation trials, two of ten jumping spiders, three of ten crab spiders and one of ten earwigs consumed the pupae presented. The spiders that consumed pupae made conspicuous marks on the pupal surface and left the pupae wrinkled, whereas the earwig opened a large hole in the side of the pupa and consumed almost all of the contents. In shelter collections, we

Table 1 Pupation dates, pupal masses and pupal periods of moth pupae in the field shelter construction experiment

	Control	Handling control	Manipulation
Average pupation date	21 Jun (17, 0.49)	21 Jun (20, 0.52)	23 Jun (17, 0.52)
Pupal mass (mg)	82.98 (13, 2.81)	80.42 (14, 2.33)	78.94 (16, 4.01)
Pupal period (days)	9.53 (15, 0.27)	9 (18, 0.08)	9.06 (16, 0.21)

Numbers in parentheses are N and standard error

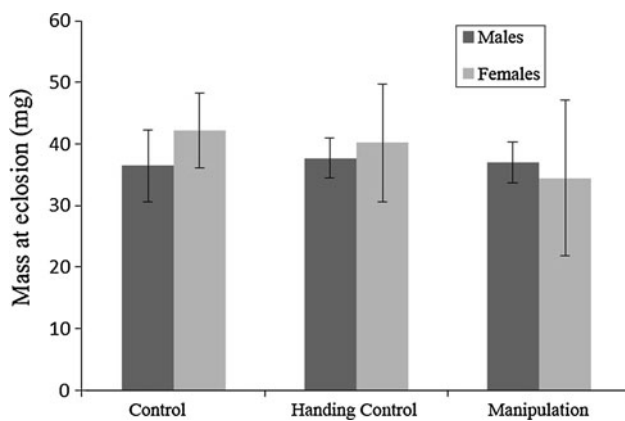


Fig. 2 Mass at eclosion of moths in the long-term shelter-cost experiment

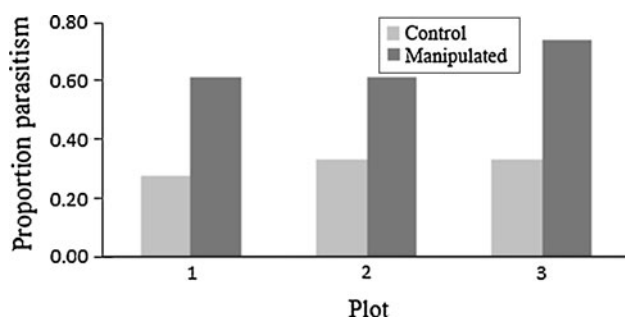


Fig. 3 Percentage of moth pupae parasitized in each plot in thinned and control treatments. Sample sizes of control treatments 1, 2, 3 = 35, 15, 15; sample sizes of thinned treatments 1, 2, 3 = 39, 18, 23

observed these three species inside shelters in each patch, yet none of the predation events were consistent with spider predation, and we only noted one event consistent with earwig predation. Likely, pupae are an inferior resource or not easily recognized as prey by these predators.

Very little predation occurred during the shelter-thinning experiment. Only 2 of 277 pupae in the control (unmanipulated) shelters (0.7 %) and 7 of 281 pupae in altered shelters (2.5 %) suffered possible predation (plots pooled). This measure may be an overestimate since host-feeding by parasitoids and desiccation may have been included as predation. The level of pupal parasitism in the shelters exceeded that of pupal predation by over an order of magnitude (65.5 vs. 2.5 % in altered shelters; 31.5 vs. 0.7 % in control shelters). In the field shelter collections, we only found evidence of two instances of pupal predation, one consistent with an earwig and the other with host-feeding, resulting in an overall rate of 2.1 % (0–5 % per plot) compared to an overall parasitism rate of 55.8 % (18–70 % per plot). These disparate data demonstrate that pupae rarely are preyed upon in this system.

Field shelter and patch characteristics

Patches differed significantly in average frond area of sensitive fern (Fig. 4, top: one-way ANOVA, $F_{3,36} = 20.02$ $p = 1.03 \times 10^{-8}$), though they did not differ in the volume of shelters built by the caterpillars (Fig. 4, bottom: one-way ANOVA, $F_{3,65} = 0.419$, $p = 0.740$). Moths eclosed from complete shelters more frequently than expected, while *P. hebrus*, the most common parasitoid (93.0 % of parasitism events: $n = 43$), enclosed more frequently than expected in shelters with openings (*G* test: $G = 6.04$, $df = 1$, $p = 0.014$). The openings in shelters at the roadside site were due to the small frond area of this patch; almost every shelter involved an entire frond, which often did not form a shelter with complete walls. We never observed this phenomenon at the other three sites; all other openings were broken junctions or pinnae not completely bound in.

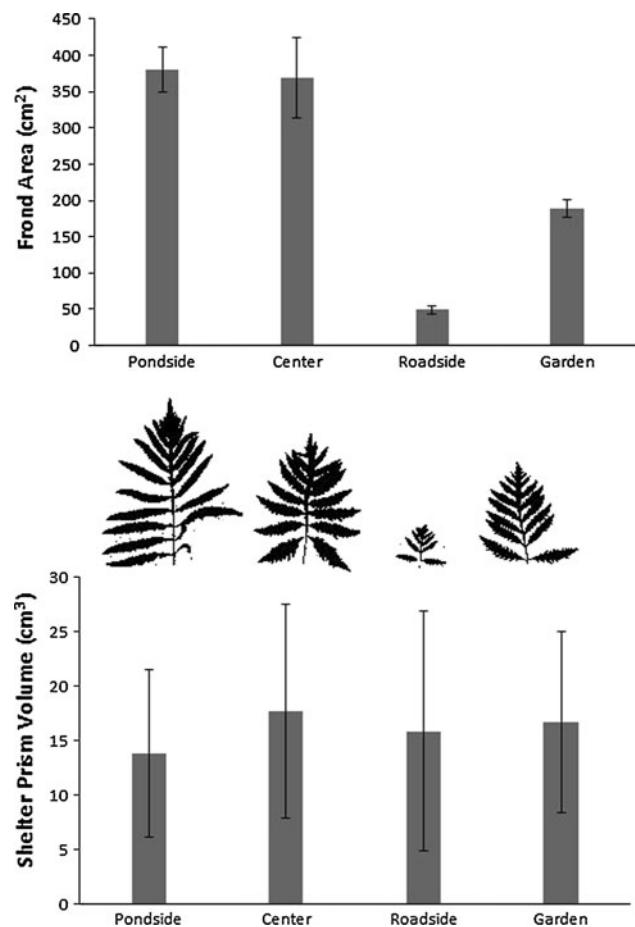


Fig. 4 Top average frond area (cm²) of ten randomly sampled fronds at each of four collection sites. Middle outline of median frond at each location (either 5th or 6th). Bottom volume of the shelter circumscribed prism at each of the four collection sites. Sample sizes are $n = 21, 13, 15$ and 38 for the four sites, respectively

Discussion

In this system, parasitoid-induced mortality was high in both years, even with well-built shelters. However, alteration in a shelter—the equivalent of building only a thin-walled shelter or an incomplete shelter—more than doubled the rate of pupal parasitism, a result supported by collections of shelters in widely spaced patches. These results support the hypothesis that pupating inside a well-built shelter confers a degree of protection for the fern moth caterpillar despite the significant time and energy investment of building substantial shelters. Coupled with very low predation on pupae, this result suggests that parasitism, not predation, provides a strong top-down selective pressure operating on the shelter-constructing behavior.

Other studies have characterized the parasitoid species hosted by caterpillars that construct leaf shelters (e.g., Ruehlmann et al. 1988; Loeffler 1994; Gentry and Dyer 2002), but have not tested whether the shelters provide protection to their inhabitants against parasitoids. Because of this high parasitoid-inflicted mortality, Loeffler (1996b) speculated that shelters probably did not provide protection from parasitoids. In our system, total parasitoid mortality can reach >50 %, yet pupae in thinned shelters suffered even more parasitism; thus, Loeffler's conclusion that high parasitism equates to ineffective defense does not hold in our system and demands testing in other systems. However, our system certainly is dissimilar to others and our conclusions may not hold for other systems as high parasitism rates do not occur in every shelter-constructing species (e.g., Abarca and Boege 2011) and high predation rates are common in others (e.g., Jones et al. 2002). Additionally, we did not test the hypothesis that parasitoids may prefer sheltered hosts because shelters may represent relatively enemy-free space for the parasitoids (Gentry and Dyer 2002); however, our data suggest that well-sheltered hosts may be less detectable or accessible to parasitoids.

We cannot directly address the mechanisms behind the increase in parasitism in thinned shelters with our experimental design. Our manipulation likely decreased both distance from the exterior to the pupa and the structural integrity of the shelter. However, it is possible that thinning increased escape of volatile cues from chewed leaves or frass inside the shelter which may attract parasitoids (e.g., De Moraes et al. 1998). Our handling of the shelters might have caused a similar release of cues; however, we avoided breaking pinnae whenever possible to minimize this risk and handled the control group as well while measuring and tagging it; the control group parasitism rates were similar to those of field collections (Morse 2009, D.H. Morse, unpublished data), and thus, we believe this possibility is unlikely. As both guilds of parasitoids (short and long

ovipositors) increased with shelter thinning, we cannot differentiate between the first two mechanisms (distance and structural integrity), and any increase is consistent with increased cues escaping. All of these mechanisms may have played a role; further experimentation into these mechanisms may be able to address the exact one responsible for the shelter's defensive role in this and other systems.

Although other studies have compared sheltered caterpillars to those held outside shelters (Loeffler 1996b; Jones et al. 2002; Abarca and Boege 2011), we believe this manipulation would introduce confounding factors into the experiment as behaviors may differ in unnatural situations and in our system fern moth caterpillars never pupate in the open, nor leave the shelters during daylight. Therefore, comparing parasitism of sheltered and exposed pupae is not biologically meaningful and introduces the confounding factors of exposure and dislodgment.

The short-term loss in caterpillar mass while constructing shelters, as well as the delayed pupation observed when caterpillars were forced to remake shelters in the field, supports this high cost to construct a shelter. The immediate loss of weight likely explains the delayed pupation time observed in the field experiment. As pupal parasitism increases throughout the season (D.H. Morse and E.F. LoPresti, pers. observ.), this delay puts pupae at a greater risk, though pupating at a smaller size or pupating with a less-complete or smaller shelter may represent a more risky action for the caterpillar. Additionally, as a protandrous species in which males are not immediately reproductively mature (D.H. Morse and E.F. LoPresti, pers. observ.), delayed pupation and emergence may lead to a decreased number of mating opportunities for males.

As a further cost, caterpillars generally build more than one shelter and add to them on a regular basis during the short larval period in the spring. Ruehlmann et al. (1998), working on another fern-feeding *Herpetogramma* species, suggested that multiple shelter construction represents an energetic trade-off between construction and avoiding parasitoids and predators by having many shelters in a patch unoccupied at any point. Like Ruehlmann et al., we did not test the efficiency of this strategy in preventing parasitism; however, *P. hebrus*, the most common parasitoid in the system, does respond to frass and spent larval skin cues in the laboratory (E.F. LoPresti, pers. observ.). Further testing in similar systems by removing unoccupied shelters could differentiate between shelter switching for an increase in food quality due to fresher plant material (e.g., Loeffler 1996b; Abarca and Boege 2011) or this cue-spreading hypothesis.

The demonstrated cost of shelter construction contrasts with other published studies quantifying the costs of shelter construction, where no cost to the caterpillar was found

(Loeffler 1996b; Lill et al. 2007, in field; Abarca and Boege 2011, in laboratory). Although a small expenditure of time and energy may have a negligible fitness cost, our results suggest that the investment in shelter construction in this species likely has a high fitness cost. Thus, the benefits from reduced parasitism and reduction in bottom-up stressors likely balance these high costs and maintain this behavior in the population.

We did not test any bottom-up benefits the shelters may have provided to the inhabitant due to better-quality food or lessening of environmental stressors. Although shading as a result of shelter construction can reduce plant defenses (Sagers 1992), in our system, this effect varied through the season and at certain times chemical defenses are greater in shelter material than in unsheltered pinnae (N.H. Rose and D.H. Morse, in prep). In addition to building costs, fern moth caterpillars are constrained to feed within the shelter, reducing plant availability in comparison with more mobile species; thus, they must deal with induced plant defenses physiologically, since they do not move often. However, this may be responsible for some amount of shelter switching; shaded plants could be initially more nutritious and then, due to induction, become less so and force the caterpillar to move and construct a new shelter.

Plant leaf or frond characteristics play a large role in shelter construction behavior and final structure. First-instar hesperiid caterpillars altered their shelter construction behavior and shelter architecture on a host species with thick leaves that were difficult to bend, correlating with an oviposition preference on an alternate, thinner-leafed host (Greeney et al. 2010). Similarly, the more easily manipulated leaves of certain hybrid cottonwoods hosted increased densities of shelters (Martinsen et al. 2000). In our system caterpillars across plots built shelters with similar volumes despite different leaf areas, which produced shelters in one plot with large numbers of openings. As predicted, this plot had the highest rate of parasitism. Thus, a fern phenotype had effects on overall parasitism rates, though this pronounced result is likely uncommon in this system; the roadside plot was mown early in the season, regrowing into tiny fronds and thus thin shelters with holes which resulted in elevated parasitism.

Herpetogramma theseusalis represents a good system in which to study shelter architecture, as its host range of ferns includes species with vastly different frond morphologies. Although it experiences similar success on several fern species in the laboratory and in nature, we expect shelter-building behavior of caterpillars to differ between fern species because of the ferns' physical characteristics. *Herpetogramma aeglealis* shelters on Christmas fern (*Polystichum acrostichoides*), as described by Ruehlmann et al. (1988), differ greatly from the structures we observed on sensitive fern, likely a result of the different

shape of the fronds and the waxy tough leaves of Christmas fern in comparison with sensitive fern.

Since parasitoids may constitute 20 % of all insect species (Hochberg and Hawkins 1992; Godfray 1994), and shelter-forming Lepidoptera constitute a substantial portion of total lepidopteran diversity (reviewed in Lill and Marquis 2007), the interaction between the two taxa is likely to have important implications at the community level. We have experimentally demonstrated that shelters provide significant protection from parasitoids at a vulnerable stage in the life cycle and that the shelters represent a significant investment of time and resources. Parasitoids clearly play an important role in the welfare of these specialized herbivores, and in our system their impact greatly exceeds that of predators. If similar results occur commonly among shelter-forming lepidopterans, parasitoids provide a strong, top-down force in terrestrial communities.

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