Behavioral Ecology
doi:10.1093/beheco/arp166
Advance Access publication 26 November 2009

Stinging spines protect slug caterpillars (Limacodidae) from multiple generalist predators

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Predators have played a significant role in the evolution of herbivorous insects, and we can observe a wide variety of larval defense mechanisms in nature, especially among members of the Lepidoptera. Slug caterpillars (Limacodidae) are known for their unusual morphologies, including various types of protuberances and stinging spines on their dorsal surfaces, which suggest that their evolution has been strongly shaped by their interactions with predators. We tested the hypothesis that limacodid larvae with stinging spines would suffer less predation from generalist predators than larvae that either did not possess stinging spines or were more lightly spined. In a series of behavioral bioassays, we tested the preferences of 2 different invertebrate predators (assassin bugs and paper wasps) for "spined" or "unspined" larvae. We found that all of the predators preferred the unspined or lightly spined prey species over the heavily spined limacodid species *Acharia* (=*Sibine*) *stimulea*. Our results also indicate that at least one of the predators that we tested, the paper wasps, showed a form of aversion learning as indicated by a decreased number of inspections of *A. stimulea* after previous experience. We conclude that limacodid larvae that are heavily armored with stinging spines are well defended against attacks from invertebrate predators and are significantly more likely to survive predator encounters than are unspined or lightly spined larvae. *Key words:* antipredator, behavior, larval defense, Lepidoptera, Limacodidae, *Polistes*, predation, prey choice, Reduviidae. *[Behav Ecol 21:153–160 (2010)]*

Predators have a significant negative effect on the fitness and survival of herbivorous insects in general and have played a strong role in the evolution of members of the Lepidoptera in particular (Strong et al. 1984; Bernays and Graham 1988; Stamp and Casey 1993; Schoonhoven et al. 1998). Lepidopteran larvae, or caterpillars, demonstrate a diverse array of defense mechanisms that are thought to increase larval fitness. Larvae may defend themselves from predators through chemical, behavioral, or morphological means. Chemical defenses are especially important for specialist caterpillars and have been shown to function against a variety of predators (Dyer 1995). Many caterpillars sequester chemicals from their host plants and advertise their distastefulness to predators through aposematic coloration (Bowers 1990; Dyer and Bowers 1996; Grant 2007; Lindstedt et al. 2008). Beyond sequestration, some caterpillars are equipped with defensive glands, such as osmeteria, or use fecal shields, offensive odors, gut regurgitation, or foam barriers to deter predators (Damman 1986; DeVries 1991; Grant 2006, 2007). Behavioral defenses include escape holes, head or tail wagging, suspension on silken threads, aggregation, frass ejection, frass chains, and the construction of leaf shelters among others (Awan 1985; Hunter 2000; Machado and Freitas 2001; Aiello and Solis 2003; Reader and Hochuli 2003; Weiss 2003; Castellanos and Barbosa 2006; Lill and Marquis 2007). Morphological defenses, such as cryptic coloration patterns, enable larvae to blend in with their natural environment and, in doing so, may minimize detection by predators (Greene 1989; Stamp and Wilkens 1993). Other larvae mimic objects in their environment that predators would consider inedible, such as bird droppings or even higher order predators (Lederhouse 1990; Wagner 2005). Morphologically defended larvae may also be equipped with sticky integumental coating, scoli (bristle-bearing outgrowths on the integument), or sclerotized spines or hairs (secondary setae) (Whelan et al. 1989; Dyer and Floyd 1993; Epstein et al. 1994; Deml and Dettner 2003; Lindstedt et al. 2008). All of these defenses presumably prevent attack by either concealing the larva, repelling predators or by protecting the larva directly once the predator initiates an attack. Anyone who has ever been afraid to touch a caterpillar should take comfort in knowing that relatively few caterpillars have antipredator defenses of a magnitude that may be harmful, or even noticeable, to humans. Yet, a larval defense mechanism does exist that can be quite painful, and even deadly, to larger potential predators (including humans): stinging spines.

Spines are common defenses in the animal kingdom (Tollrian and Harvell 1999; Mikolajewski and Rolff 2004; Inbar and Lev-Yadun 2005), and recently, there has been great interest in the evolution of aposematism in association with morphological defense, specifically spines (Inbar and Lev-Yadun 2005; Speed and Ruxton 2005; Halpin et al. 2008). Caterpillars with "stinging" spines (urticating hairs) have hollow quill-like hairs connected to poison sacks and can be found in at least 6 families: Limacodidae, Megalopygidae, Nymphalidae (e.g., *Nymphalis antiopa*, *Hemileuca oliviae*), Noctuidae (e.g., *Acronicta oblineata*), Saturniidae (e.g., *Semileuca maia* and *Automeris io*), and Zygaenidae (*Neoprocis floridana*) (Foot 1922; Gibbons et al. 1990; Deml and Dettner 2003; Wagner 2005). The "sting" of some caterpillars, such as *Lonomia obliqua* (Saturniidae) in Brazil, can even be fatal to humans (Bohrer et al. 2007). Spines are thus often assumed to be a morphological defense against predators, yet

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Received 18 January 2009; revised 4 October 2009; accepted 27 October 2009.

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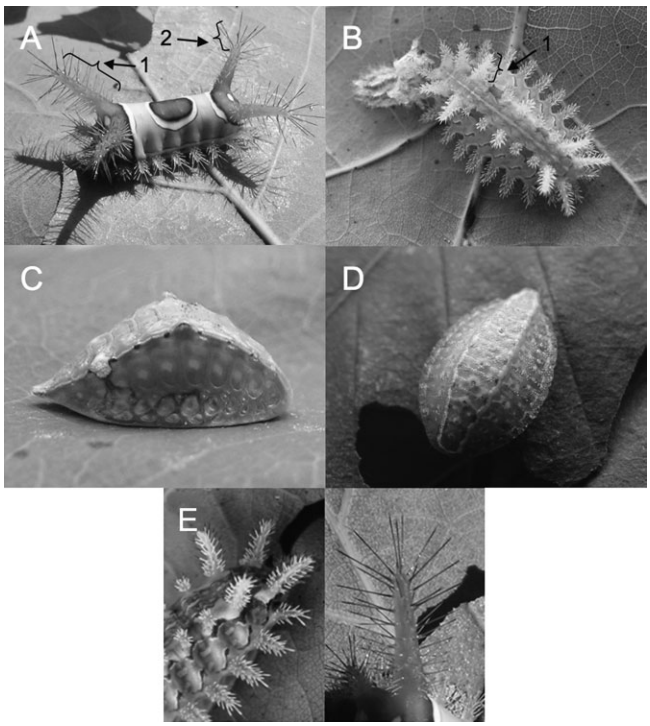

Figure 1
Slug caterpillars are well known for unusual morphologies, which often include intricate color patterning and various types of protuberances on their dorsal surfaces. Limacodid larvae also vary greatly in their defensive strategies: (A) An *Acharia stimulea* (heavily spined) individual with stinging spines. Arrow #1, on the left, is pointing to a dorsal scolus that is covered with long spines and arrow #2, on the right, is pointing to a single spine on another dorsal scolus; (B) *Euclea delphinii* (lightly spined) also has stinging spines, but they are shorter than those of *A. stimulea*. Arrow #1 is pointing to a dorsal scolus that is covered with short spines; (C) *Prolimacodes badia* (unspined) is cryptic but with an unidentified chemical defense that appears to be excreted through dorsal glands (the authors' personal observations); (D) *Lithacodes fasciola* (unspined) is cryptic; and (E) Close-ups of an *E. delphinii* scolus (left) and an *A. stimulea* scolus (right).

demonstrations of the effectiveness of these defenses against specific predators are rare. For example, some slug caterpillars in the family Limacodidae are equipped with stinging spines (Figure 1), but whether these spines function to defend larvae, as they are commonly assumed to do, has never been tested. Here, we present evidence that the stinging spines of slug caterpillars (Limacodidae) are highly deterrent to generalist predators and thus function as an effective morphological defense.

In eastern North America, the larvae of approximately 20 species of moths in the family Limacodidae feed during late summer and early autumn in deciduous forests (Covell 1984). Their common name, slug caterpillars, derives from their unusual locomotory habit, characterized by a high degree of ventral contact with the substrate, the use of abdominal "sucker" appendages in movement and the secretion of semifluid silk that serves to enhance substrate contact (Epstein 1995). The larvae are also highly polyphagous, feeding on trees and shrubs in well over a dozen plant families (Epstein 1988; Wagner 2005; Lill et al. 2006; Lill 2008). Limacodid larvae are perhaps best known for their unusual morphologies, which often include intricate color patterning and various types of protuberances on their dorsal surfaces (Figure 1). Similar to other caterpillar species, limacodid larvae are subject to intense predation in the field (unpublished data) and vary greatly in their putative defensive strategies. Many species, such as *Acharia* (=*Sibine*) *stimulea* (Figure 1A) and *Euclea delphinii* (Figure 1B), possess stinging setae (commonly referred to as spines) for all or a portion of their larval development (Dyar 1899) and can be quite painful to touch. Among the limacodid species that possess spines, there are gradations of armoring. For instance, *E. delphinii* has many dorsal scoli, but each scolus is relatively small, and the spines are much shorter than those of *A. stimulea* (Figure 1E); we thus refer to *E. delphinii* as being "lightly spined," which is a relative classification. *Acharia stimulea*, by contrast, has only 4 prominent dorsal scoli, but each is covered with long spines that are considerably more urticating than the spines of *E. delphinii*; thus, *A. stimulea* bestows a much more powerful sting than that received by touching *E. delphinii* (the authors' and many field assistants' personal experiences), and we refer to *A. stimulea* as being "heavily spined." Although the 4 scoli on the dorsal surface of *A. stimulea* larvae are located on either end of the body and not in the middle (as in *E. delphinii*), the larvae actively flex their anterior and posterior spines toward each other in order to protect the unspined portion of their dorsum when threatened (Supplementary Material, video one). This defensive "posturing" is one part of a suite of traits observed in *A. stimulea* that serves to integrate morphology, defensive chemistry, and behavior into a single putatively defensive phenotype. The biochemistry of the caterpillar's venom is not well understood, but the toxin is thought to be a protein (Foot 1922). Other limacodid species, such as *Prolimacodes badia* (Figure 1C) and *Lithacodes fasciola* (Figure 1D) do not have spines and presumably rely on crypsis to avoid their predators.

The objective of our study was to examine the response of a suite of generalist predators to ostensibly well-defended slug caterpillars. Notably, studies of the effectiveness of caterpillar defenses against predators generally focus on the responses of insectivorous birds and ants (e.g., Coppinger 1970; Bernays and Cornelius 1989; Epstein et al. 1994; Dyer 1995; Lindstedt et al. 2008, but see Grant 2007). Although birds and ants are undoubtedly important predators, we chose to study the responses of other common predators to determine whether stinging spines are an effective defense against a range of generalist invertebrate predators, such as assassin bugs and paper wasps. Moreover, the responses of invertebrate predators to larval defenses may not necessarily be the same as those of vertebrate predators due to differences in learning or physiology (Montllor and Bernays 1993). The stinging spines of limacodids appear to be quite effective against vertebrates (the authors' own painful personal experiences), but whether these spines deter invertebrate predators as well was previously unknown. We hypothesized that limacodid larvae with stinging spines as a physical defense would suffer less predation from invertebrate predators than would unspined larvae.

MATERIALS AND METHODS

Origins of study organisms

Prey

All of the limacodid larvae that we used in our bioassays are from our laboratory colonies. These colonies were started in 2004 with individuals collected as larvae or adults from 3 field sites in the Washington, DC metropolitan area: Patuxent National Wildlife Refuge (Beltsville, MD), Little Bennett Regional Park (Clarksburg, MD), and Rock Creek Park (Washington, DC). New individuals are added yearly to maintain the genetic diversity within colonies. Adults are collected by light trapping, and larvae are found by manually searching the foliage of 6 focal tree species: American beech (*Fagus grandifolia* Ehrh.),

white oak (*Quercus alba* L.), northern red oak (*Quercus rubra* L.), black cherry (*Prunus serotina* Ehrh.), black gum (*Nyssa sylvatica* Marsh), and pignut hickory (*Carya glabra* Mill.).

In the laboratory, limacodid adults are placed in flight chambers (60-cm^3 BugDorm-2, BioQuip, Rancho Dominguez, CA) and allowed to mate. Once mated, females are placed in individual 32-oz deli containers (Fabri-Kal, Kalamazoo, MI) where they are allowed to lay their eggs. The eggs are misted daily until they hatch; after the larvae molt to the second instar, they are moved to smaller 16-oz deli containers (Fabri-Kal, Kalamazoo, MI). These larval containers are provisioned with a moistened filter paper disc (7.5-cm diameter; VWR, West Chester, PA) and excised foliage from 1 of the 6 focal tree species, which is replaced as needed, at least every 2–3 days. All of the limacodid larvae used in the experiments described below were reared on foliage from red oak or beech and within each bioassay all of the larvae were reared on the same host plant (i.e., all of them were reared on red oak or all of them were reared on beech).

The unspined prey species used in our wasp bioassays, beet army worm (*Spodoptera exigua*), was obtained from breeding colonies at Benzon Research Inc. (Carlisle, PA). These larvae were fed the artificial diet with which they were shipped and were stored in a refrigerator (~5 °C) in order to retard their growth until they were used in experimental trials.

Predators

Assassin bugs (Reduviidae: *Pselliopus* sp.) were collected at Little Bennett Regional Park in July and August 2007. These predators were kept in individual 16-oz deli containers that were provisioned with a moistened filter paper disc and excised red oak leaves. Prior to experimental trials, the predators were fed 3 *Drosophila melanogaster* larvae (~3–4 mm; from an existing colony at George Washington University) every 48–72 h.

Ten paper wasp nests were collected from the Patuxent National Wildlife Refuge on 12 June 2008. Of these 10 nests, 8 were *Polistes fuscatus*, a native of North America, and 2 were *Polistes dominulus*, an invasive species from Europe. Only nests that consisted of a few cells and a single foundress were collected, so the foundress was the only individual of each nest that had any foraging experience prior to the bioassays. The nests were carefully cut down from wooden overhangs with a razor, while the foundress was present, and placed in individual 16-oz deli containers. The nests were transported to George Washington University where they were cooled in a refrigerator (~5 °C) for up to 30 min, so that the foundresses could be isolated from the nest for a few minutes while the pedicel of the nest was glued to a new wooden frame (6 cm × 25 cm × 36 cm) contained within a flight cage (60-cm^3 BugDorm-2, BioQuip, Rancho Dominguez, CA). Flight cages were housed in the laboratory (~22–24 °C) and were provisioned with nest-building materials (colored construction paper), water, and honey. Each day, wax worms (*Achroia grisella*; Pyralidae) or fall webworms (*Hyphantria cunea*; Arctiidae) were cut into pieces, so they would not resemble prey items offered during the bioassays and placed on excised red oak leaves in aquapiks that were positioned in the center of the cages; through these repeated offerings, foragers from 5 of the 8 *P. fuscatus* colonies and both of the *P. dominulus* colonies were conditioned to fly down from their nests to forage for new prey items when the leaves were placed in their cages. Wasps were individually marked on the dorsal part of their abdomen with white correction fluid (BIC USA Inc., Milford, CT) and/or colored gel pens (Gelly Roll Metallic, Sakura, Japan) to keep track of each individual's experience; wasps had no experience with larval species used in the bioassays prior to testing.

Bioassays

Bioassays were conducted to determine whether heavily spined limacodid larvae are less preferred by generalist predators than are unspined or relatively lightly spined larvae. All bioassays were choice trials, and the 2 prey items that were offered to the predators were approximately matched for size (in mm) as size has been demonstrated to be important in prey choice by solitary foragers (Dyer 1997). Although a range of sizes was used in the bioassays, all larvae were in mid-late instars; it is difficult to assess the specific instar of a limacodid larva because head capsules are effectively concealed and larvae tend to immediately eat their molt, which makes it difficult to observe or record their passage through developmental stages without constant surveillance. Moreover, larvae in this family are characterized by a large and variable number of instars, even within a species (Epstein 1988). Within bioassays, all prey items were offered to the predators on a similar background. All predators were deprived of prey for 18–30 h before the trials. Bioassays with assassin bugs occurred from 25 July to 24 August 2007, whereas bioassays with *Polistes* wasps occurred from 30 July to 20 August 2008. All bioassays occurred in the laboratory under ambient light conditions.

Assassin bug bioassays

In each trial, the predator was offered a choice between a spined limacodid larva and an equally sized unspined or lightly spined limacodid larva. Two of the limacodid species that were used in the trials were considered to be spined: *A. stimulea*, which is heavily spined with stinging spines, and *E. delphinii*, which also possesses stinging spines, but is relatively lightly spined compared with *A. stimulea* (Figure 1). The other 2 limacodid species used in the trials were considered to be unspined because they are both cryptic and neither possesses stinging spines: *P. badia* and *L. fasciola*. Two types of bioassays were conducted. In the first bioassay, assassin bugs ($n = 17$) were offered a choice of a spined larva (*A. stimulea* or *E. delphinii*) and an unspined larva (*P. badia* or *L. fasciola*). All prey were offered on beech leaves in this bioassay. In the second bioassay, assassin bugs ($n = 6$) were offered a choice between the 2 spined species, *A. stimulea* and *E. delphinii*, the latter of which has shorter and less urticating spines than the former. All prey were offered on red oak leaves in this bioassay. Preliminary trials were run until both larvae were consumed, usually overnight, to verify that the predators were capable of killing all of the test species.

Trials were conducted in a 9.5-cm plastic Petri dish containing a moistened sponge and a single red oak leaf. The 2 limacodid larvae were placed in the Petri dish at opposite ends of the leaf. Once the larvae were established, the predator was placed into the center of the Petri dish, equidistant from both prey species, and the Petri dish was closed. Predators were observed and their behaviors noted until one of the larvae was consumed or until 60 min had elapsed, whichever happened first. Prey that moved to the underside of the leaf during the trial were moved back to the top of the leaf where they were accessible to the predator. Chi square goodness-of-fit tests (Conover 1999) were used to test whether predators chose the spined or unspined larva first and also to test whether they discriminated between the heavily spined and lightly spined larvae.

Wasp bioassays

In each choice trial, a wasp was offered a choice between an *A. stimulea* larva, which possesses stinging spines, and an unspined *S. exigua* larva. Although we would have ideally paired *A. stimulea* larvae with their unspined limacodid counterparts,

as in the assassin bug trials above, sufficient numbers of these larvae were not available in 2008. Before a bioassay began, all foraging wasps except the focal test wasp were captured and isolated in 30-ml plastic cups. Wasps were not held in the cups for longer than 45 min and appeared to forage normally on their return to the colony. The trial began when the aquapik with a single red oak leaf containing the 2 prey species was placed in the cage and ended when the wasp killed one of the larvae or when 20 min had elapsed, whichever happened first. During each trial, we noted the time elapsed until the first larva was killed, the number of times that each larva was contacted by the wasp, and how long these inspections lasted. Wasps usually contacted the larvae with their antennae, but sometimes also with their legs. Once the wasp began to kill the prey, she would use her mouthparts to process the prey into a ball and then carry it to the nest where she would feed it to the colony's larvae (further details on *Polistes* foraging and processing behavior can be found in Raveret Richter 2000). Fresh leaves and larvae were used for each trial and the positions of the spined and unspined larvae on the leaves were alternated.

Only trials where a wasp killed one of the prey items, and had therefore made a choice between the 2 prey items, were used in the analyses. None of the wasps ever killed both larvae; a total of 11 *P. fuscatus* (4 foundresses and 7 daughters from 5 colonies) and 9 *P. dominulus* (1 foundress and 8 daughters from 2 colonies) were tested in both "naïve" and "experienced" trials. In the naïve trials, all of the wasps were assumed to have had no previous experience with the focal caterpillar species, whereas in experienced trials, they all had exactly one prior experience with the focal caterpillar species. Because the foundresses had previous foraging experience in the wild before they were brought into the laboratory they may have encountered the focal species before; this is unlikely, however, as limacodids are quite uncommon at the site where the wasps were collected (Lill JT, Murphy SM, unpublished data). As there were too few foundresses to test their responses separately from the daughters, our analyses combined these 2 groups; in general, their behaviors were similar, but any differences in their responses during the bioassays were noted. Chi square goodness-of-fit tests (Conover 1999) were used to test whether wasps tended to choose either the spined or unspined larvae first. Within each type of trial (naïve or experienced), whether wasps stayed with their first choice of larval prey or switched from spined to unspined prey (or vice versa) was tested with the McNemar test for significance of changes (Conover 1999), but as the expected frequencies bordered on being too small (most of our expected frequencies >5, but one expected frequency was 3 and thus <5), the data were also analyzed with the binomial test as advised by Siegel and Castellan (1988). Naïve and experienced trials were analyzed separately.

To test whether wasps learn to avoid *A. stimulea* larvae with experience, we compared the responses of *P. fuscatus* ($n = 7$) and *P. dominulus* ($n = 5$) daughters in the naïve trials with their responses in the experienced trials. All of these daughters had emerged in the laboratory, which allowed us to control their entire foraging history. None of the foundresses were included because their foraging history before they entered the laboratory was uncertain. If the wasps contacted the *A. stimulea* larva fewer times in the experienced trial than in the naïve trial, then that would suggest that the wasps needed fewer interactions with the spined *A. stimulea* larva to assess its suitability as prey and would therefore have learned to avoid *A. stimulea* with experience. The wasps were not predicted to touch the unspined larva fewer times in the experienced trial than the naïve trial because there was no apparent reason for them to learn to avoid this type of prey. Differences in the number of times the wasps touched spined and unspined larvae between the naïve and experienced trials were analyzed with Wilcoxon signed-ranks tests (Conover 1999).

For each trial in which the spined *A. stimulea* larva was killed by wasps (*P. fuscatus* $n = 1$; *P. dominulus* $n = 3$) and a subset of the trials where the unspined larva was killed by wasps (*P. fuscatus* $n = 8$; *P. dominulus* $n = 3$), we also noted how long it took the wasp to process the prey item and return to the nest with it. This "handling time" began when the larva was first attacked and ended when the wasp left the leaf with the prey. For 3 of the wasps that killed *A. stimulea* larvae, we were able to time how long they took to process the spined *A. stimulea* larva as well as an unspined *S. exigua* larva. Differences in the time it took wasps to process spined and unspined larvae were analyzed with Wilcoxon signed-ranks test (Conover 1999).

RESULTS

Assassin bug bioassays

Unspined limacodid larvae (*P. badia* or *L. fasciola*) were preferred as prey significantly more than spined limacodid larvae (*A. stimulea* or *E. delphinii*) by assassin bugs (Figure 2A; $\chi^2 = 9.94$, df $= 1$, $P = 0.0016$). These predators also preferentially attacked and killed lightly spined *E. delphinii* over heavily

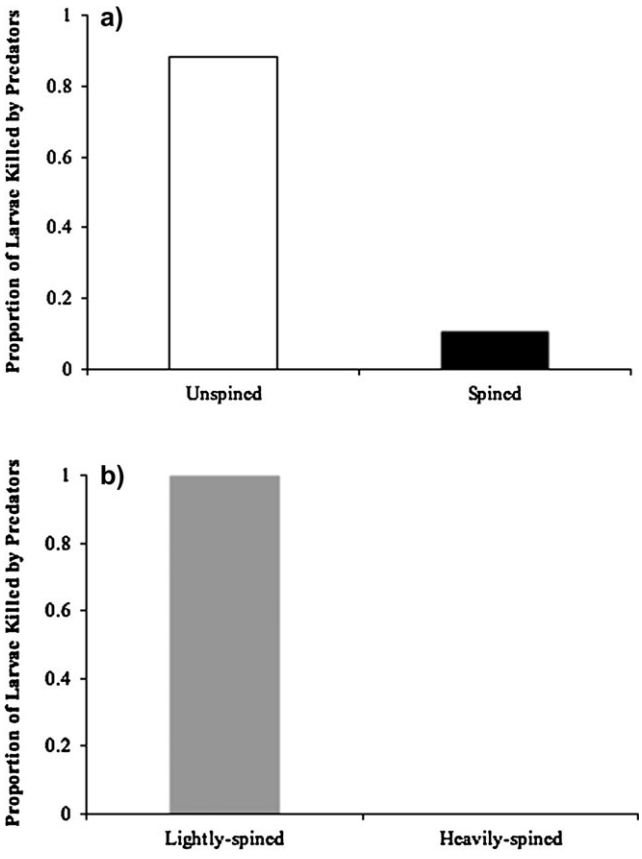

Figure 2
(A) The proportion of unspined (*Prolimacodes badia* and *Lithacodes fasciola*) and spined (*Acharia stimulea* and *Euclea delphinii*) limacodid larvae killed by predators (assassin bugs) during the bioassays ($n = 17$). (B) The proportion of lightly spined (*E. delphinii*) and heavily spined (*A. stimulea*) limacodid larvae killed by predators (assassin bugs) during the bioassays ($n = 6$).

spined *A. stimulea* (Figure 2B; $\chi^2 = 6$, df $= 1$, $P = 0.0143$). In one of the 2 trials where an assassin bug first attacked *A. stimulea*, it did so by inserting its beak through the bottom of the leaf directly into the ventrum of the larva, thereby avoiding the spined dorsum entirely.

Wasp bioassays

Polistes fuscatus and *P. dominulus* wasps were equally likely to approach either the spined *A. stimulea* larva or the unspined *S. exigua* larva first, regardless of whether they were in the naïve bioassay (*P. fuscatus*: $\chi^2 = 0.091$, df $= 1$, $P = 0.76$; *P. dominulus*: $\chi^2 = 0.11$, df $= 1$, $P = 0.74$) or the experienced bioassay (*P. fuscatus*: $\chi^2 = 0.091$, df $= 1$, $P = 0.76$; *P. dominulus*: $\chi^2 = 0.66$, df $= 1$, $P = 0.41$). These results indicate that the wasps did not have an innate attraction to either of the prey species at the beginning of the bioassays.

When offered a choice between a heavily spined *A. stimulea* larva and an unspined larva, 9 of the 11 *P. fuscatus* and 3 of the 9 *P. dominulus* wasps touched both prey species during the naïve and experienced trials (i.e., made a legitimate choice); the wasps that did not make a legitimate choice touched only one of the prey items during either the naïve or experienced trial. The 12 wasps that made legitimate choices during both the naïve and experienced trials are the only ones used in the statistical analyses that follow. In the naïve trial, all 12 of the wasps chose the unspined prey over the spined *A. stimulea* larva. Of these, 7 wasps initially contacted the *A. stimulea* larva, but ultimately chose to kill the unspined larva. The other 5 wasps initially contacted the unspined larva and ultimately chose to kill the unspined larva rather than the spined *A. stimulea* larva (Figure 3). Thus, together *P. fuscatus* and *P. dominulus* wasps showed a significant tendency to change their choice of prey in the naïve trial, but only when they first attacked *A. stimulea* larvae (McNemar: $T_2 = 7$, df $= 1$, $P = 0.0082$; Binomial: $x = 0$, $N = 7$, $P = 0.008$). In the experienced trial, the 8 wasps that first encountered the spined *A. stimulea* larva elected instead to kill the unspined larva. Of the 4 wasps that first encountered the unspined larva, however, only 3 of them ended up killing it; the other wasp, a *P. fuscatus* foundress, chose instead to kill the *A. stimulea* larva. Therefore, as in the naïve trials, *P. fuscatus* and *P. dominulus* wasps in the experienced trial also showed a significant tendency to change their choice of prey after first touching *A. stimulea* larvae but not after touching the unspined larvae (McNemar: $T_2 = 8$, df $= 1$, $P = 0.0047$; Binomial: $x = 1$, $N = 8$, $P = 0.035$).

Although the bioassay was designed as a choice test, not all wasps made a legitimate choice by contacting both prey items; some interacted with only one of the prey species during the trial and either killed that prey or flew back to the nest without attacking either prey. Two *P. fuscatus* wasps did not make a legitimate choice in either the naïve or experienced trials. In both trials, the wasp that touched only the unspined larva killed it, whereas the wasp that touched only the spined *A. stimulea* larva did not kill it and returned to the nest without any prey; these results are consistent with those from the choice tests. Six *P. dominulus* wasps did not make a legitimate choice in either the naïve or experienced trials. All of the wasps that touched only the unspined larva killed it. Inconsistent with the results from the choice trials, however, 2 *P. dominulus* wasps touched only *A. stimulea* larvae and killed them.

By comparing the responses of *P. fuscatus* and *P. dominulus* wasps in the experienced trial to their responses in the naïve trial, we found that the wasps' response to *A. stimulea* larvae changed over time (Figure 4). As predicted, wasps inspected the spined *A. stimulea* larva significantly more times in the naïve trial than in the experienced trial ($N = 8$, $T = 6$, $P = 0.05$), but did not differ in the number of times that they inspected the unspined larva between the naïve and experienced trials ($N = 7$, $T = 12.5$, $P = 0.4$).

A minority of the wasps was able to kill *A. stimulea* larvae (*P. fuscatus*: $n = 1$; *P. dominulus*: $n = 3$). The only *P. fuscatus* wasp that killed an *A. stimulea* larva, was a foundress and thus may have had prior experience attacking and killing spined prey. None of the *P. dominulus* wasps, however, were foundresses, and they came from both of the *P. dominulus* colonies that we had in the laboratory. In general, the *P. dominulus* colonies were more aggressive. Three of the wasps that killed *A. stimulea* larvae did so by first cutting off the stinging spines before trying to process the prey into a food bolus (Supplementary Material, video two). The fourth wasp did not cut off the spines but instead flipped the *A. stimulea* larva over and attacked the larva from its unspined ventral side. On average, it took wasps of both species 187% longer to attack and process spined *A. stimulea* larvae than unspined *S. exigua* larvae (Figure 5). For 3 of the 4 wasps that killed *A. stimulea* larvae, we have data on the amount of time it took the same wasp to kill and process both the spined *A. stimulea* larva and an unspined *S. exigua* larva; although our sample size is very small and should therefore be interpreted cautiously, we did find that it took these wasps significantly longer to kill and process *A. stimulea* than *S. exigua* ($n = 3$, $T = 0$, $P < 0.05$). Notably,

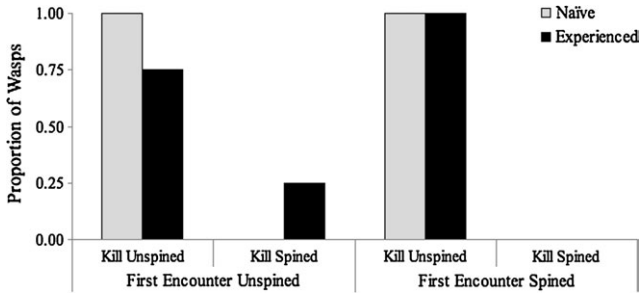

Figure 3
The proportion of wasps that killed either unspined *Spodoptera exigua* larvae or spined *Acharia stimulea* larvae as a function of the first prey item they encountered during the bioassay. Data are given for 1) *Polistes fuscatus* and *Polistes dominulus* wasps in the naïve trial (gray bars) whose first encounter was with the unspined larva ($n = 5$) or the spined larva ($n = 7$); 2) *P. fuscatus* and *P. dominulus* wasps in the experienced trial (black bars) whose first encounter was with the unspined larva ($n = 4$) or the spined larva ($n = 8$).

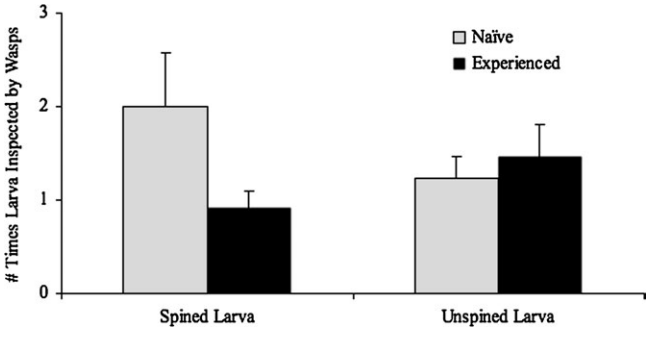

Figure 4
The number of times that *Polistes fuscatus* and *Polistes dominulus* wasps inspected the heavily spined *Acharia stimulea* larva and the unspined *Spodoptera exigua* larva in both the naïve (gray bars) and experienced (black bars) bioassays ($n = 12$; 7 *P. fuscatus* and 5 *P. dominulus*).

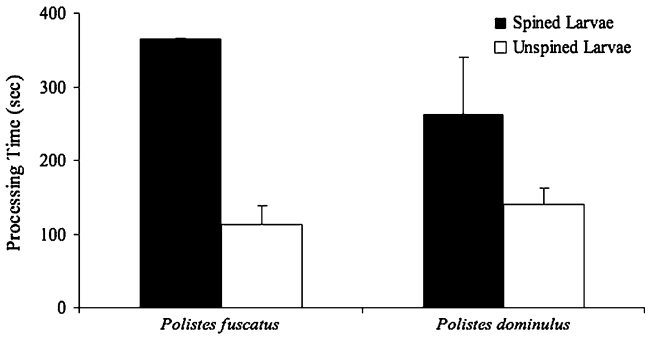

Figure 5
The length of time required for *Polistes fuscatus* and *Polistes dominulus* wasps to process heavily spined *Acharia stimulea* larvae (black bars; *P. fuscatus* $n = 1$, *P. dominulus* $n = 3$) and unspined *Spodoptera exigua* larvae (white bars; *P. fuscatus* $n = 9$, *P. dominulus* $n = 3$); this is the amount of time from when the wasp killed the prey until she returned to the nest with the prey.

none of the wasps that killed an *A. stimulea* larva ever attempted to kill another *A. stimulea* larva in subsequent trials.

DISCUSSION

Our hypothesis that spined limacodid larvae would suffer less predation from invertebrate predators than unspined larvae was supported. Our bioassays demonstrated that assassin bugs prefer the unspined limacodid species *P. badia* and *L. fasciola* as prey over the limacodid species *A. stimulea* or *E. delphinii*, which both possess stinging spines. Furthermore, assassin bugs were able to distinguish between the 2 spined larval species and preyed on lightly spined *E. delphinii* larvae in preference to heavily spined *A. stimulea* larvae; indeed, none of the assassin bugs attacked *A. stimulea* when given a choice between these 2 spined larval species. We also tested a single lacewing larva (Chrysopidae: *Chrysopa* sp.) in the same bioassay design as the assassin bugs. Similar to our results for the assassin bugs, the lacewing larva also preferred the unspined limacodid species *P. badia* and *L. fasciola* as prey, compared with the spined species *A. stimulea* and *E. delphinii*. Furthermore, the lacewing larva also preferred to attack *E. delphinii* instead of the heavily spined *A. stimulea* when given a choice between these 2 spined limacodids. Our hypothesis was further supported by the results of our wasp bioassays. Both *P. fuscatus* and *P. dominulus* wasps preferred to attack unspined larvae over spined *A. stimulea* larvae in both naïve and experienced choice trials, and were likely to change their initial prey choice only when they first encountered *A. stimulea*. Thus, our results support the conclusion that limacodid larvae with stinging spines are well defended against generalist invertebrate predators.

Our bioassays were designed to test predator preferences for different levels of physical defense and whether limacodid larvae with stinging spines were less preferred than larvae that did not possess spines. Although controlled bioassays are never completely natural, these types of studies are justified because they are the only way to control for the foraging history of the predators. Additionally, limacodid caterpillars are relatively rare, and it is thus difficult to observe their interactions with predators in the wild. It is possible that prey items differed in some features other than morphological defense (e.g., nutritional quality or palatability), but it should be noted that all of the predators that we tested found both prey items to be acceptable food items, and all of the choices in the naïve trials were made in the absence of prior information regarding nutrition or palatability. Rayor et al. (2007) found that *P. dominulus* wasps may selectively remove a caterpillar's gut while they process the prey that has fed on host plants containing deterrent chemical compounds. By contrast, the wasps in our study never selectively removed any of their prey's gut, which suggests that the host plant chemistry did not compromise our bioassay of physical defenses. In the future, we plan to test predator preferences for freshly killed larvae with their spines intact or removed to further control for these factors as well as for potential differences in larval behavior.

Our results also suggest that at least one of the predator genera that we studied, *Polistes* wasps, may learn to avoid heavily spined *A. stimulea* larvae. *Polistes fuscatus* and *P. dominulus* wasps rejected *A. stimulea* larvae as potential prey after fewer inspections during the experienced trial than in the naïve trial. We had thought that wasps would avoid *A. stimulea* larvae altogether in the experienced trial, but instead, rather unexpectedly, we found that wasps were equally likely to first approach either the spined larva or unspined larva. Although this result suggests that the wasps in our experienced bioassay had not learned to negatively associate the colorful patterning of *A. stimulea* larvae with stinging spines before they approached the defended prey, we did find that they touched the *A. stimulea* larvae fewer times, which suggests that they had learned to assess prey quality with fewer encounters than were necessary during the naïve trial. These results are notable because although there are numerous studies on the ability of insects to learn, most of these are studies of herbivores and parasitoids, rather than arthropod predators (but see Berenbaum and Miliczky 1984; Montllor and Bernays 1993; Weiss et al. 2004).

Four of the wasps in our bioassays were able to kill defended *A. stimulea* larvae; 3 of these wasps methodically chewed off the stinging spines with their mandibles before trying to kill the *A. stimulea* larva. The fourth wasp was able to pry the *A. stimulea* larva from the leaf, which is difficult with slug caterpillars as they adhere relatively well to substrates, and then flip the larva over, so that its unspined underside was vulnerable, before killing it. It is possible that other wasps in the colony may have been able to learn to attack *A. stimulea* larvae by observing successful predation events by more experienced females, but we minimized the likelihood of this by testing our wasps in isolation (placing other foragers under small opaque cups) so none of the other wasps were immediately present the few times that an *A. stimulea* larva was killed. We found that it took significantly longer for wasps to attack and process defended limacodid larvae than undefended larvae. We also observed that the venom from *A. stimulea* spines could elicit a dramatic response by wasps that were "stung." On at least a dozen occasions, we observed wasps that were stung in their mouthparts recoil from the prey, fly off, and spend several minutes grooming their mouthparts with their front tarsi. Perhaps foraging wasps might learn to avoid spined prey in the wild not only because of the difficulty in subduing such a prey item and possibility of self injury but also because of the significant amount of time required to process the prey and return with it to the nest; time that might be better spent searching for less hazardous prey if such prey is common in the environment.

Stinging spines on caterpillars are generally thought to be defensive against vertebrate predators such as birds and mammalian insectivores or even herbivores such as browsing deer (Foot 1922). Rarely has the effectiveness of these morphological and chemical defenses been tested with generalist invertebrate predators and never, to our knowledge, with invertebrate predators that are not ants. Our results suggest that these defenses provide some level of protection for limacodid larvae against some of the most common arthropod predators encountered within tree canopies.

FUNDING

National Science Foundation (grant number NSF-DEB 0642438 and REU Supplement awarded to J.T.L.) and a George Washington University Luther Rice Fellowship awarded to S.M.L.

We thank V. Fiorentino, R. Liebson, J. Moore, L. Power, T. Stoepler, and N. Trager for their assistance in both the field and laboratory. M. Weiss, D. Uma, and A. Caldas provided essential advice and guidance in establishing the captive wasp colonies, and we thank M. Buffington for confirming identifications of our wasp species. D. Johnson kindly provided *D. melanogaster* larvae. This manuscript was greatly improved by comments by M. Epstein, members of the DC Plant-Insect Group (DC-PIG) and 3 anonymous reviewers.

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