Contact with caterpillar hairs triggers predator-specific defensive responses

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Organisms have evolved morphological and behavioral traits that reduce their susceptibility to predation. However, few studies have considered how morphological structures work to provide informational inputs necessary for effective behavioral responses to predation risk. In this study, we demonstrate that the hairs of Orgyia leucostigma (Lymantridae) caterpillars not only function as physical barriers that deter predators but also act to provide sensory inputs triggering behavior that reduces predation risk. In particular, the way in which caterpillars respond when their hairs are touched is predator specific. Mechanical parameters of the interaction determining the response were identified correlatively and confirmed manipulatively. Caterpillars predominantly dropped in response to high hair bending velocities and predominantly walked away in response to low hair bending velocities. These stimulus-specific responses appear to be adaptive as they led to increased survival. Our results demonstrate a functional link between morphology and behavior. The ability to respond effectively only after the initiation of a predator attack should reduce the costs associated with antipredator behaviors. Key words: behavior, costs, defense, host, Lepidoptera, morphology, Orgyia leucostigma, predation risk, signals.

INTRODUCTION

Animals have evolved different ways to reduce mortality risks when exposed to predation, including physiological, morphological, and behavioral defenses (Endler 1986; Sih 1987; Lima and Dill 1990). Behavioral defenses that reduce susceptibility to predation involve reducing activity levels, fleeing, and hiding (Lima and Dill 1990; Lima 1998). Morphological defenses include a range of structures such as protective spines and armor (Edmunds 1974; Gross 1993). Different types of antipredator defenses often function at different stages in the predation sequence: primarily, either before or after detection of the prey by a predator (Endler 1986; Sih 1987; Lima and Dill 1990).

Studies on antipredator defenses have often focused on either behavior or morphology taken separately, even if antipredator adaptations may frequently be complex in their nature (e.g., Dewitt et al. 1999; Mikolajewski and Johannson 2004; Lind and Gresswell 2005; Arendt 2009). Moreover, the effectiveness of prey morphological defenses is rarely evaluated against different predators (Murphy et al. 2010), while not all predators are equally deterred by direct morphological defenses of the prey (Eisner and Eisner 2000; Sandre et al. 2008). In such cases, the interplay between behavioral and morphological defenses is far from straightforward, and the attention on the phenotype as a whole must therefore be the most fruitful way to understand predator avoidance.

In arthropods, specialized structures such as hairs, spines, and thick sclerotized cuticles are thought to primarily function as physical barriers that dissuade both predators and parasitoids from attacking or reduce prey handling-success after capture (e.g., Dyer and Floyd 1993; Gerling et al. 1998; Riessen and Young 2003; Lindstedt et al. 2008; Rezak et al. 2008; Flennor et al. 2009; Murphy et al. 2010). However, because these structures, when associated with sensory neurons, often act as cuticular mechanoreceptors that sense stimuli from outside the organism (McIver 1975; Zacharuk and Shields 1991; Barth and Dechant 2003; Casas and Dangles 2010) they may also serve to provide information that triggers behaviors necessary to evade, escape, or confront predators.

We investigated the role of defensive hairs of Orgyia leucostigma J.E. Smith (Lepidoptera: Lymantriidae) larvae as touch detectors under predator attack. We tested the hypothesis that the physically defensive hairs also provide sensory input triggering behavior that reduces predation and examined the possibility that the response is predator specific, or more accurately, if it is specific to the type of stimulus produced by different predators. Accordingly, we 1) analyzed whether antipredator behaviors of larvae occur in response to stimulating their hairs, 2) determined if predators that produce different rates of hair displacement elicit different behavioral responses, 3) manipulated the possible antipredator behavioral responses of larvae to determine whether the differential responses against different predators may be adaptive, and 4) determined if hair removal reduces the possibility that caterpillars escape from predation.

MATERIALS AND METHODS

Study organisms

Prey species

Larvae of the nearctic O. leucostigma are covered with conspicuous defensive hairs (Figure 1), with some of the tufts...
Figure 1
An *Orgyia leucostigma* caterpillar. Photograph by D.L. Wagner, with permission.

exceeding in length the diameter of the body (Payne 1917). The polyphagous larvae are external solitary feeders on the foliage of deciduous trees. *Orgyia leucostigma* larvae used in the experiments originated from a laboratory colony established from larvae collected from *Acer negundo* L. (Aceraceae) (box elder) trees in Patuxent Wildlife Refuge Research Center, MD, USA. Larvae eclosing from egg masses were reared individually in 237-ml plastic containers, being fed box elder foliage.

**Predator species**

The predators used in this study had been identified as common members of the insect community in tree canopies in Patuxent (Barbosa P, Castellanos I, Segarra AE, unpublished data). The 3 invertebrate predators of caterpillars included in the study were the adults of the wasp *Polistes fuscatus* Fabricius (Vespidae), the stink bug *Podisus maculiventris* Say (Pentatomidae), and the spined assassin bug *Sinea diadema* Fabricius (Reduviidae). These predators were chosen to represent different modes of action.

Adults of *P. fuscatus* capture and process caterpillars to feed their larvae (Michener and Michener 1951; Rabbé 1960; Gould and Jeanne 1984; Raveret Richter 2000). Hunting *Polistes* wasps appear to detect prey items from some distance when hovering, either visually or chemically, and they pounce upon prey, either visually or chemically, and they pounce upon prey within a few millimeters or after perching. *Podisus maculiventris* typically employs a sit wait strategy for capturing prey with its raptorial forelegs (Taylor and Schmidt 1994; Freund and Olmstead 2000). On encountering prey, *P. maculiventris* extend their beak and slowly attempt to insert it into the nearest part of the prey (Evans 1982).

*Sinea diadema* is a generalist predator that attacks a diverse range of arthropod species (Readio 1924). A foraging adult typically employs a sit wait strategy for capturing prey with its raptorial forelegs (Taylor and Schmidt 1996). It locates and approaches prey items using antennal olfaction and extends its raptorial forelegs that are used for grasping prey (Taylor and Schmidt 1994; Freund and Olmstead 2000).

All predator individuals used to start laboratory colonies were collected from the Patuxent Wildlife Refuge Research Center. Colonies of all predator species were maintained in the laboratory and were provided with larvae of various insects.

**Experimental protocol**

**Behavioral responses to predator attacks**

An individual trial consisted in exposing a larva of *O. leucostigma* to a predator in an experimental setting, in the laboratory at room temperature. For each trial, we recorded the behavioral responses of the larvae after predator contact with prepectic hairs. Three types of response were observed: dropping off the leaf, walking away from the predator, or no reaction at all. We also noted the predator body part (i.e., antennae, mouthpart, or leg) that came in contact with larval when a response was observed. *Orgyia leucostigma* larvae were considered survivors when the predator left the experimental leaf with the larva unharmed. The predators were starved before the experiments to increase their motivation to attack larvae.

A total of 23 trials were conducted with *P. fuscatus*, 21 with adult *P. maculiventris*, and 20 with adult *S. diadema*. In each trial, a third-instar *O. leucostigma* larva was placed on the leaf of a 15-cm *A. negundo* branch positioned 0.5 m above the ground and was allowed to acclimate for half an hour. The branch had been placed inside a vial with water and fixed with a clamp to a vibration-isolating table (Newport LW3048B-OPT). Different predator and prey individuals were used for each behavioral trial. In experiments, *P. fuscatus* wasps flew from their nest to the experimental leaf, whereas *P. maculiventris* and *S. diadema* individuals were placed on a vertical stem in contact with the experimental leaf. All trials were conducted in the laboratory at room temperature of about 25 ± 2 °C.

**Caterpillar hair displacement by predators**

To quantitatively characterize the interaction of the predators with larval hairs, the encounters of the insects were videotaped. Encounters between predators and caterpillars were filmed at 30 frames per second. Hair displacement velocities were calculated from replayed video sequences. This was facilitated by a 1-mm² grid having been placed behind the leaf on which predators and prey interacted. Six attacks by wasps, and 9 attacks each by stink bugs, and spined assassin bugs were analyzed.

**Responses to controlled stimuli**

Hair displacement was mechanically reproduced to mimic velocities produced by the different predators. Hair displacement was then compared with the real predators. Hairs were bent with a single sinusoidal wave of either 1.0 or 0.01 Hz. The 1-Hz stimulus resulted in a velocity within the range produced by both *P. fuscatus* and *S. diadema* during contact with hairs (maximum hair tip velocity of 30 mm/s), and the 0.01-Hz stimuli resulted in a velocity within the range produced by *P. maculiventris* (maximum hair tip velocity of 0.75 mm/s). Both stimuli were adjusted to have the same displacement.

The sinusoidal waves were produced with a waveform generator (Agilent Technology, Santa Clara, CA/Hewlett Packard 33120A), amplified with a power amplifier (Labworks PA-119) and connected to an electromechanical shaker (Labworks ET-152-2). Contact with larval hairs was achieved using an excised antenna of a *P. fuscatus* wasp that was glued to a base attached to, and extending 7 cm from, the armature of the shaker. The mechanical displacement was longitudinal with respect to the caterpillar in order to bend dorsal hairs perpendicularly to their base because most of the attacks by the predators had been observed to occur along this axis. Hair displacement was applied approximately 1 mm from the base of dorsal hairs, and the stimuli were not strong enough to mechanically knock down larvae. We exposed 42 caterpillars to the 1-Hz stimulus and 35 caterpillars to the 0.01-Hz stimulus. The setup of the experiment was identical to that used with the real predators.

**Consequences of responses to predators**

Once we had determined the differences in behavioral responses of larvae to each predator, we also recorded the consequences of the responses to larval survival by manipulatively...
restricting the set of possible reactions. Third-instar *O. leucostigma* were 1) allowed to respond by dropping or walking (larvae were placed on a leaf of a 15-cm box elder branch positioned 0.5 m above the ground), 2) the larvae were able to walk but not drop (larvae were placed on a box elder leaf on the ground), or 3) the larvae were unable to either drop or walk (larvae were tied to a leaflet of a 15-cm box elder branch positioned 0.5 m above the ground). Larvae were then exposed to individual foraging wasps, stink bugs, or spined assassin bugs. A caterpillar was recorded as a survivor when the predator or the larva left the leaf with the larva remaining unharmed.

Sample sizes for larvae allowed to respond by dropping or walking were 23 for *P. fuscatus*, 20 for *S. diadema*, and 21 for *P. maculiventris*; for larvae allowed to respond by walking or not allowed to respond by dropping or walking, the sample size was 16 for each predator. All trials were conducted in the laboratory at an ambient temperature of about 25 ± 2 °C.

**Hair removal and caterpillar survival**

The hairs of 45 third-instar *O. leucostigma* larvae were cut with curved dissecting scissors, leaving hair shafts approximately 0.5 mm long (the hair removal treatment, hereafter). Of these 45 caterpillars, 15 were exposed to each of the 3 predators (wasps, spined assassin bugs, and stink bugs). In order to control for the possible effect of the procedure of hair cutting per se, we only cut approximately 0.5 mm of the tips of the hairs of 60 third instars (providing a clipping control). Of these 60 control caterpillars, 20 were exposed to each of the 3 predators and their survival was compared with that of caterpillars subjected to the hair removal treatment. A caterpillar was recorded as a survivor after the predator or the larva left the leaf.

**Statistical analyses**

The frequencies of different behavioral responses of caterpillars and of the consequences of the responses to predators, as well as differences in survival, were compared among treatments using chi-square tests of independence or Fisher’s exact tests when the assumptions of the chi-square test were not met (Agresti 1996). Hair bending velocities prior to the initiation of an antipredator behavior were compared using a nonparametric Kruskal–Wallis analysis of variance. The family-wise error rate for multiple comparisons was controlled using a Bonferroni correction (Sokal and Rohlf 1995). We report means ± standard error.

**RESULTS**

**Behavioral responses to predator attacks**

The behavioral responses of *O. leucostigma* larvae to the different predators invariably occurred as soon as their hairs were touched; contact with the body was not necessary. The caterpillars that escaped from wasps (13 out of 23) did so in response to touch by a wasp’s antennae (62% of survivors), mouthpart (23%), or leg (15%). Caterpillars that escaped from stink bugs (16 out of 21) did so in response to touch by the beak (93% of survivors) or the stink bug’s antennae (7%). All the caterpillars that escaped from spined assassin bugs (15 out of 20) did so in response to touch by an assassin bug’s foreleg.

The array of behaviors displayed by caterpillars (i.e., dropping, walking, or lack of a response) in response to predator contact with their hairs depended on the predator making the contact (Figure 2). All the caterpillars that escaped predation from wasps and spined assassin bugs did so by dropping from the leaf, whereas the response to stink bugs was more diverse (Figure 2). The percentage of caterpillars that dropped in response to stink bugs (26.6%) was significantly smaller than the percentage of caterpillars that dropped in response to wasps and spined assassin bugs (Fisher’s Exact test: $P < 0.0001$).

**Caterpillar hair displacement by predators**

The displacement velocities of hairs differed among predators ($\chi^2 = 16.59$, degree of freedom $[df] = 2$, $P = 0.0003$). The velocities at which wasps, spined assassin bugs, and stink bugs bent hairs were 86 mm/s ± 18 ($N = 6$), 152 mm/s ± 34 ($N = 9$), and 0.40 mm/s ± 0.13 ($N = 9$), respectively. Hair displacement velocities did not differ between wasps and spined assassin bugs ($\chi^2 = 0.78$, df = 1, $P = 0.4$). Wasps, which pounce on their prey, and spined assassin bugs, which strike at their prey, elicited *O. leucostigma* dropping behaviors, and both species bent hairs with significantly higher velocities than stink bugs ($\chi^2 = 10.29$, df = 1, $P = 0.004$, and $\chi^2 = 12.90$, df = 1, $P = 0.009$, respectively, with Bonferroni correction).

**Responses to controlled stimuli**

Mechanically simulated predator attacks confirmed that the behavioral responses of *O. leucostigma* caterpillars depended on the rate at which the hairs were bent ($\chi^2 = 34.65$, df = 2, $P = 0.001$). Larvae predominantly dropped (66.7%) in response to the 1-Hz stimuli (reproducing the hair bending velocity of wasps and spined assassin bugs) ($\chi^2 = 22.48$, df = 1, $P = 0.001$) and predominantly walked (78.8%) in response to the 0.01-Hz stimuli (hair bending velocity of stink bugs) ($\chi^2 = 34.09$, df = 1, $P = 0.001$) (Figure 3).

**Consequences of responses to predators**

Survival among manipulated caterpillars differed significantly among treatments (i.e., able to drop, able to walk, or deprived
Caterpillar hairs trigger defensive responses

Hair removal and caterpillar survival

Survival was more than 2 times greater for caterpillars with hairs than for caterpillars without hairs for the 3 predators. Caterpillar survival was significantly reduced when attacked by wasps, spined assassin bugs, and stink bugs (\( \chi^2 = 5.60, df = 1, P = 0.018 \), \( \chi^2 = 16.13, df = 1, P = 0.001 \), and \( \chi^2 = 4.64, df = 1, P = 0.031 \), respectively) (Figure 5). The survival for the no-hair caterpillars did not differ among predator types (Fisher’s Exact test: \( P = 0.245, N = 15 \)).

DISCUSSION

Most predator–prey studies that have investigated antipredator defenses have focused on either behavior or morphology, giving little consideration to the interplay between these 2 functional characteristics. This study shows that in a lepidopteran larva, morphology and behavior are linked and act complementarily in an adaptive way when its antipredator defense is built up.

Larval hairs were shown not only to provide a signal about the incidence of predator attack but also transmitted information about the type of predator. In particular, the differences in behavioral responses by larval *O. leucostigma* were based on differences in the velocity at which their hairs were bent. During attacks on larval prey, both wasps and spined assassin bugs generated high hair displacement velocities that triggered a dropping behavior. In contrast, stink bugs, which generated low displacement velocities, triggered mainly a walking response. Similarly, caterpillars predominantly dropped in response to mechanically reproduced high hair bending velocities and predominantly walked away in response to mechanically reproduced low hair bending velocities.

The specificity in the responses of caterpillars to stink bugs and wasps appears to be adaptive in the light of the results of
the present study. When attacked by *P. maculiventris*, caterpillars predominantly responded by walking away. This was an adequate response because survival increased compared with that of caterpillars that were deprived from the possibility of responding. On the other hand, results of the experiments with *P. fuscatus* showed that survival of larvae increased only if they could drop. This difference in the behavioral response of *O. leucostigma* to stink bugs and wasps may be determined by the cost associated with dropping. Dislodged larvae will require reaching another host tree and could be subjected to ground predation (Losey and Denno 1998; Nelson 2007; Castellanos I, Barbosa P, Caldas A, unpublished data).

Nevertheless, larvae of *O. leucostigma* dropped in response to spined assassin bugs even if walking away was shown to be sufficient to escape from this predator. Two possible explanations may account for the seemingly maladaptive dropping behavior by *O. leucostigma* in response to assassin bugs. It is well likely that this species of prey has not evolved a sensory system that allows it to distinguish among the signals produced by wasps and spined assassin bugs, both of which produce high hair displacement velocities. Alternatively, larvae may still be able to detect differences among the signals (assassin bugs generated higher displacement velocities than wasps), but the signal produced by *S. diadema* may be within the range produced by other fast hair displacement predators that can predate on caterpillars that escape by walking (see Djamai et al. 2001; Caldwell et al. 2009).

Air currents generated by the wing beats of approaching predators (Tautz and Markl 1978; Triblehorn and Yager 2006) and substrate borne vibrations produced by invertebrate predators walking on a leaf (Castellanos and Barbosa 2006) can alone cause insects to respond defensively. However, these stimuli are unlikely to account for the behaviors of *O. leucostigma* observed in this study. First, larvae invariably showed defensive behaviors only after their hairs were touched and displaced, either by predators or controlled stimuli. Indeed, tactile hairs are not displaced by airflow, they are deflected by much larger forces resulting from direct contact with a stimulating object (Barth and Dechant 2003). Second, spined assassin bugs and stink bugs forage for prey by walking and stop for several seconds (sometimes minutes) near larvae before making contact with their hairs, and most of the attacks by wasps occurred while the predators were walking, after having landed on leaves (Castellanos I, personal observation).

The behavioral responses of *O. leucostigma* caterpillars (no reaction, walking, and dropping) represent increasingly strong responses. Such graded reactions have previously been attributed to different stimulus intensities produced by predators that approach insect prey from a distance (Markl and Tautz 1975; Fullard 1979; Yager et al. 1990; Schulze and Schul 2001; Jones et al. 2002; ter Hofstede and Fullard 2008), and the results of this study actually conform to this qualitative pattern; however, our study is novel because it also provides a quantitative analysis of this pattern. Nevertheless, our design did not allow us to separate the different parameters of the signal: a stimulus causing a higher hair bending velocity was necessarily also more intense. An exciting area for further studies would be to diversify the artificial stimuli in order to determine the number of parameters of a signal a prey animal is able to distinguish.

This study shows that the hairs of larvae of *O. leucostigma* provide at least 2 advantages in the presence of invertebrate predators: in addition to providing the sensory input needed to trigger predator-specific behavioral responses, they physically hamper predator strikes. The latter aspect is illustrated by the tendency of the larvae with their hairs removed to be more vulnerable to predators. At the present, however, we do not know if the same (e.g., Markl and Tautz 1975) or different types of hairs (e.g., Paydar et al. 1999; Hiraguchi et al. 2003) are responsible for the predator-specific responses.

Most studies on behavioral responses of prey to predator stimuli have been conducted on prey individuals that respond before they are attacked or captured, that is, from a distance (Hoy et al. 1989; Lima and Dill 1990; Curio 1993; Kats and Dill 1998; Dicke and Grostal 2001). In this study, we have demonstrated that caterpillars also are able to respond differentially to stimuli produced by predators even during contact, that is, after an attack has initiated, and that the response appears adaptive. Responding effectively only after a predator has initiated an attack offers an opportunity to reduce the costs associated with antipredator behaviors (Abrams 1994; Bouskila et al. 1995). This may be a useful strategy given that even though predators may be in proximity to prey they may not always attack or capture a prey.

Although hairs may represent a substantial initial energetic investment (Bowers 1993; Montior and Bernays 1995), they may be relatively inexpensive to maintain. Furthermore, they also may be involved in other essential functions such as thermoregulation (Casey and Hegel 1981; Kukal et al. 1988) and crypsis (Sandre et al. 2007). More research is needed that integrates both morphological and behavioral antipredator defenses, particularly the functional link between antipredator morphology and behavior, their relative costs, and the consideration of multiple functions in the evolutionary ‘choice’ of an anti-predator defense.

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