

Morphology and Antipredator Behavior in Larval *Orgyia leucostigma* (Lepidoptera: Lymantriidae)

Authors: Castellanos, Ignacio, and Zuria, Iriana

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MORPHOLOGY AND ANTIPREDATOR BEHAVIOR IN LARVAL *ORGYIA LEUCOSTIGMA*
(LEPIDOPTERA: LYMANTRIIDAE)

Additional key words: caterpillar, costs, defense, tussock moth

Predation pressure usually leads to selection for prey adaptations that reduce susceptibility to predation. These often include behavioral and morphological defenses (Endler 1986, Lima 1998). Behavioral defenses can involve reduced activity levels, fleeing, hiding, and confrontation (Gross 1993, Greeney et al. 2012). Morphological defenses such as spines, hairs, and thick sclerotized cuticles are often post-contact defenses that typically reduce predation risk by making prey more difficult to handle or to kill, thereby increasing their probability of escaping if attacked or captured (Gross 1993, Greeney et al. 2012).

Studies on antipredator defenses in terrestrial insect prey have often focused on either behavior or morphology separately, even if both traits are frequently related (DeWitt et al. 1999, Johansson & Mikolajewski 2008). For example, the effectiveness of prey morphological defenses such as mimesis and crypsis can depend on behavior (Castellanos & Barbosa 2006, Iannou & Krause 2009). Similarly, antipredator behavior may depend on modified morphology such as the hypertrophied abdominal setae used by some species of caterpillars to detect the presence of predators (Rota & Wagner 2008).

There is evidence that prey defensive behavior and antipredator morphology such as hairs, spines, and thick sclerotized cuticles, are also related. Several studies have shown that morphologically defended prey have reduced antipredator behavior compared to morphologically undefended prey (Peckarsky 1996, Mikolajewski & Johansson 2004, Boyero et al. 2012, Vogelweith et al. 2014), suggesting that morphologically undefended prey depend strongly on behavioral antipredator defense in order to compensate for their relative vulnerability (DeWitt et al. 1999, Stankovich & Blumstein 2005). Most of the evidence that supports the contention that there is a negative relationship between antipredator behavior and antipredator morphology in insects comes from studies comparing different aquatic species that vary in their morphology (Mikolajewski & Johansson 2004, Vogelweith et al. 2014). Relatively few studies have compared these relationships in individuals of the same species (but see Stoks 1999). In this study, we experimentally manipulated individual prey morphology to determine if predation risk assessment behavior by prey is altered as a consequence of changes

in antipredator morphology. We conducted our experiments with larvae of the white-marked tussock moth *Orgyia leucostigma* (J. E. Smith) (Lepidoptera: Lymantriidae), which are covered with conspicuous defensive hairs (Payne 1917, Castellanos et al. 2011b), and display antipredator behavior responses that include walking away and dropping from the host plant leaf (Castellanos et al. 2011a, b).

The defensive hairs of larvae of *O. leucostigma* were removed in order to test if individuals without hairs would be more vulnerable to predation and would alter their behavioral response to predation risk due to *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). The behavioral responses were compared to those of individuals with hairs. Caterpillar antipredator behaviors depend on their relative vulnerability to predators, as well as, over an evolutionary time scale, the costs of the behavior (Stamp 1986, Castellanos & Barbosa 2006, McClure & Despland 2011). Thus, we hypothesized that caterpillars without hairs would be more vulnerable to predation than individuals with hairs and that morphologically defended *O. leucostigma* would escape by dropping from host plant less often than morphologically undefended individuals.

Orgyia leucostigma are external polyphagous, solitary foliage feeders of a large number of species in various tree genera (Payne 1917). *Orgyia leucostigma* larvae used in the experiments originated from a laboratory colony established from field collections at Patuxent Wildlife Refuge Research Center (PWRRC) (39° 02' 30" N latitude, 76° 47' 30" W longitude), Maryland, USA. Larvae eclosing from egg masses were reared individually in 237-ml plastic containers, and fed *Acer negundo* L. (Aceraceae) (box elder) foliage.

The stink bug *P. maculiventris* is a generalist predator that feeds primarily on larval Lepidoptera and Coleoptera (McPherson 1982), and is a common member of the insect community in PWRRC. It actively searches for prey while walking on the foliage of plants, and is able to perceive prey within a few millimeters, or after physically contacting the prey (Evans 1982). Upon encountering prey, *P. maculiventris* extend their proboscis and slowly attempt to insert it into the nearest prey tissues (Evans 1982, I. Castellanos personal observation). *Podisus maculiventris* individuals used in

the experiments originated from adults collected in early spring at PWRRC and were fed with lymantriid, noctuid, geometrid and tenebrionid larvae, as well as water and green beans (Mallampalli et al. 2002). Prior to the experiment, hunger level was standardized for each predator by starving individuals for 24 hours.

Experimental procedure. The dorsal, lateral, anterior, and posterior hairs of 29 fifth-instar *O. leucostigma* were cut (hair removal treatment) using curved dissecting scissors, leaving approximately 0.10 cm long hair shafts. An additional 30 larvae were used with "intact" hairs. Intact caterpillars had dorsal, lateral, anterior, and posterior hairs with lengths of 0.82 ± 0.04 , 0.64 ± 0.03 , 0.84 ± 0.04 , and 0.90 ± 0.06 cm, respectively (Mean \pm SE, $n = 10$); hair length was measured under a microscope while caterpillars remained motionless on a box elder leaf. In order to control for the possible effect of the procedure of hair cutting per se, we cut approximately 0.10 cm of the tips of the hairs of 22 fifth-instar individuals (clipping control) and compared their antipredator behavioral responses to those of intact caterpillars. Treated and clipping control larvae were allowed to recover for a period of five hours before being exposed to a predator.

Caterpillars belonging to the three treatments were individually placed on the leaf of an *A. negundo* branch inserted into a vial with water and positioned 0.5 m above a laboratory bench, and allowed to acclimate for a period of thirty minutes. After the acclimation period, a single adult *P. maculiventris* was placed on a vertical stem in contact with the experimental leaf. After the predator began walking on the experimental leaf, the stem was removed. The defensive behaviors (i.e., walking away from the predator, dropping from the leaf, or confrontation) of caterpillars that survived the attacks, as well as those that tried to escape but failed and were predated upon, were recorded. Confrontation behavior consisted of the prey moving its head from side to side or attempting to bite or biting the predator until the predator left the leaf. Head movement commonly occurred for several minutes, which discouraged continual stalking by predators. We also observed the prey body parts (hairs or cuticle) with which the predator's proboscis came in contact. A caterpillar was recorded as a survivor if the predator or the caterpillar left the leaf, with the caterpillar left unharmed. All trials were conducted with different *P. maculiventris* adults and *O. leucostigma* larvae, in the laboratory at an ambient room temperature of $25 \pm 2^\circ\text{C}$. The frequencies of different behavioral responses of caterpillars, as well as their survival, were compared using chi-square tests of independence or Fisher's exact tests when the assumptions of the chi-square test were

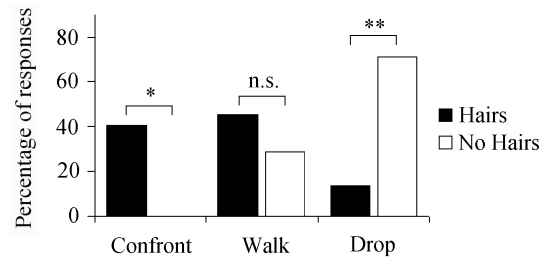


FIG. 1. The behavioral responses of surviving fifth-instar *Orgyia leucostigma* with hairs (hairs) and with their hairs removed (no hairs) that were exposed to *Podisus maculiventris* on an *Acer negundo* leaf (* $P < 0.05$, ** $P < 0.01$, and n.s. is not significant).

not met (Agresti 2007). The family-wise error rate for multiple comparisons was controlled using a Bonferroni correction (Sokal & Rohlf 1995).

Results. Upon encountering caterpillars both with and without hairs, *P. maculiventris* approached individuals with their same stereotypic behavior, extending their proboscis and slowly attempting to insert it into the nearest part of the prey (Evans 1982, I. Castellanos personal observation). The behavioral responses of *O. leucostigma* larvae to predators occurred when their hairs or cuticle were contacted. Caterpillars with intact hairs that responded by confronting or walking did so when predators contacted their hairs. However, caterpillars with intact hairs that responded by dropping did so when the predator's proboscis contacted a caterpillar's cuticle, which occurred when *P. maculiventris* was able to reach the ventral (unprotected) cuticle of larvae, typically when they were at the edge of a leaf and the predator was on the opposite side of the leaf. Caterpillars without hairs responded by walking or dropping when the stink bug's proboscis contacted their cuticle. Predators interacted with caterpillars in all trials except in three occasions, one belonging to the hair removal treatment and two to the intact caterpillar treatment; these trials were discarded from the analyses.

There was no significant effect of hair clipping (clipping control) on the behavioral defenses of *O. leucostigma* caterpillars in response to *P. maculiventris* when compared with intact caterpillars (Fisher's Exact Test: $P = 0.837$). Overall, there was a significant effect of hair removal on caterpillar vulnerability to predation ($\chi^2 = 4.98$, $df = 1$, $P = 0.026$): caterpillars with their hairs removed suffered greater predation (14 out of 28, or 50%) compared to intact caterpillars (6 out of 28, or 21.4%). The removal of hairs had a significant effect on the type of defensive behavior exhibited by surviving caterpillars ($\chi^2 = 14.27$, $df = 2$, $P = 0.001$) (Fig. 1). The

percentage of intact caterpillars that survived by confronting the predators (9 out of 22, or 40.9%) was significantly greater than the percentage of caterpillars with their hairs removed that survived through that same defensive behavior (0 out of 14, or 0%) (Fisher's Exact Test: $P = 0.018$), whereas the percentage of intact caterpillars that dropped (3 out of 22, or 13.6%) was significantly smaller than the percentage of caterpillars with hairs removed that dropped (10 out of 14, or 71.4%) ($\chi^2 = 12.39$, $df = 1$, $P = 0.003$) (Fig. 1). The percentage of caterpillars that escaped predation by walking away was greater for intact individuals (10 out of 22, or 45.5%) than for individuals with their hairs removed (4 out of 14, or 28.6%), however, this difference was not statistically significant ($\chi^2 = 1.03$, $df = 1$, $P = 0.933$) (Fig. 1). Of the 6 caterpillars with intact hairs that were predated, 2 tried to escape by walking and 1 by confronting, but failed and were predated, and the predators were able to pierce the epidermis of the other 3 caterpillars without eliciting a defensive response. Of the 14 caterpillars without hairs that were predated, 3 tried to escape by walking, but failed and were predated, and the epidermis of the other 11 caterpillars was pierced by *P. maculiventris*. Once *P. maculiventris* is able to pierce the epidermis of its prey with its proboscis, it causes prey paralysis and immobilization, apparently by injecting a toxin (Berenbaum et al. 1992), and the proboscis also anchors the prey with its teeth and rasps (Cohen 1998).

Discussion. Most studies on antipredator defenses in terrestrial insect species have focused on either behavior or morphology, and given little consideration to the interplay between these two functional characteristics. Our results show that for larvae of *O. leucostigma*, morphology and behavior can act in a compensatory manner, providing evidence that both are important. That is, larvae without hairs were more susceptible to predation by invertebrate predators and dropped more often in the presence of predatory stink bugs. In contrast, the antipredator behavior exhibited by individuals with intact hairs most frequently involved confronting or walking away. Since piercing the cuticle by invertebrate predators is likely to represent a higher predation risk than contact with hairs, the caterpillars exhibit the strongest, and potentially, the most costly response (i.e., dropping from the plant), in order to compensate for a relatively greater vulnerability when predators contact the cuticle. Dislodged larvae may be exposed to adverse abiotic conditions (Roitberg & Myers 1978), must climb the original tree or reach another host tree (Castellanos et al. 2011a) and thus could be subjected to ground predation (Losey & Denno 1998), starvation (Nelson 2007), or reduced

fitness if they access host trees of inferior quality (Stamp & Bowers 1991).

Our results show that morphological antipredator defenses can influence how caterpillars perceive threats and how perceived threats can influence the magnitude of their behavioral response, and suggest that future research on antipredator defenses in terrestrial insect species should consider the combined role of behavior and morphology. It has been argued that the intensity of a prey's behavioral defenses should be inversely related to the effectiveness of its morphological defenses due to the costs associated with antipredator behavior (Peckarsky 1996, Johansson & Mikolajewski 2008). Since relatively small increments in hair length can improve protection from predators (Sugiura & Yamazaki 2014), it is plausible that small differences in caterpillar hair length within species or between closely related species might also be associated with differences in the magnitude of a defensive behavior.

An interesting area for future research would be to compare the behavioral responses of terrestrial insect individuals of the same or closely related species that vary in antipredator morphology (or chemistry) in order to determine if there are differences in investment into morphological (or chemical) versus behavioral defenses as has been shown to occur in other systems (Stankovich & Blumstein 2005, Johansson & Mikolajewski 2008, Hettzey et al. 2014). More research is needed that integrates different antipredator defenses and their relative costs in the evolution of antipredator defenses (Vencl & Srygley 2013).

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IGNACIO CASTELLANOS* AND IRIANA ZURIA, *Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Básicas e Ingeniería, Centro de Investigaciones Biológicas, 4.5 carr. Pachuca-Tulancingo s/n, Mineral de la Reforma, Hidalgo, C.P. 42184, México*, PEDRO BARBOSA *Department of Entomology, University of Maryland, College Park, Maryland, 20742, USA*, AND ASTRID CALDAS, *PO Box 37012, NHB MRC 105, Smithsonian Institution, Washington, DC 20013-7012, USA*.

*Corresponding author is Ignacio Castellanos, email address: ignacioe.castellanos@gmail.com

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