

# Eyespot peek-a-boo: false eyes improve the survival of caterpillars in leaf rolls

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## Abstract

Deimatic displays typically involve body parts that can conceal or reveal visual signals, potentially reducing detectability at a distance while startling predators up close. Some species may achieve this “conceal-then-reveal” effect using modified aspects of their environment (environmental deimatism hypothesis). The larvae of spicebush swallowtail butterflies (*Papilio troilus*) possess large eyespots, and rest in leaf rolls during the day. I tested the hypothesis that leaf rolls reduce eyespot conspicuousness while maintaining eyespot effectiveness by comparing avian predation on 659 artificial larvae: eyespotted and non-eyespotted, presented in leaf rolls or on open leaves. Leaf rolls reduced predation regardless of color pattern. Eyespots also reduced predation, but only for artificial larvae in leaf rolls. On open leaves, eyespots neither increased nor decreased predation. These results suggest that eyespots and leaf rolls can combine to create a deimatic display – and that this strategy likely evolved to enhance existing antipredator effects of leaf rolls.

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ABSTRACT

Deimatic displays typically involve body parts that can conceal or reveal visual signals, potentially reducing detectability at a distance while startling predators up close. Some species may achieve this “conceal-then-reveal” effect using modified aspects of their environment (environmental deimatism hypothesis). The larvae of spicebush swallowtail butterflies (*Papilio troilus*) possess large eyespots, and rest in leaf rolls during the day. I tested the hypothesis that leaf rolls reduce eyespot conspicuousness while maintaining eyespot effectiveness by comparing avian predation on 659 artificial larvae: eyespotted and non-eyespotted, presented in leaf rolls or on open leaves. Leaf rolls reduced predation regardless of color pattern. Eyespots also reduced predation, but only for artificial larvae in leaf rolls. On open leaves, eyespots neither increased nor decreased predation. These results suggest that eyespots and leaf rolls can combine to create a deimatic display – and that this strategy likely evolved to enhance existing antipredator effects of leaf rolls.

## INTRODUCTION

Conspicuous color patterns have well-known advantages in social, sexual, and antipredator contexts (Prudic *et al.* 2007; Stuart-Fox & Moussalli 2008; Aronsson & Gamberale-Stille 2009; Caro & Allen 2017). However, strong signals may also attract the attention of unwanted receivers, e.g., predators (Endler 1983; Justin Marshall 2000; Halfwerk *et al.* 2014; de Lira *et al.* 2018). Even in aposematic species, high detectability can result in high mortality through the attraction of naïve, specialist, or otherwise undeterred predators (Ruxton *et al.* 2009; Mappes *et al.* 2014; Fabricant & Herberstein 2015; Umbers *et al.* 2015). The inbuilt tradeoffs of detectability are cited as potential drivers for the evolution of intermediate aposematic signals (Ruxton *et al.* 2009), distance-dependent crypsis (Barnett *et al.* 2017, 2018), and even seasonal trends in proportions of aposematic versus cryptic species (Mappes *et al.* 2014). Beyond color traits alone, deimatic displays may allow organisms to resolve detectability tradeoffs behaviorally (Umbers *et al.* 2015, 2017; Umbers & Mappes 2015; Badiane *et al.* 2018).

Deimatic displays are broadly defined by Umbers and Mappes (2016) as “momentary, transient, conspicuous” signals that induce “a startle response” or overload “the senses of an attacking predator, such that the predator pauses, slows or stops the attack.” Deimatism generally occurs late in the predation sequence – i.e., when the initial defense(s) have failed – and may or may not involve honest advertisements of toxicity and (Umbers *et al.* 2015, 2019). These displays often blur lines between traditional categories of antipredator signalling, combining distinct types of visual defenses (aposematism, crypsis, masquerade, and/or mimicry) and sometimes additional modalities (e.g., acoustic, chemical) in a single complex display (Dookie *et al.* 2017, Badiane *et al.* 2018, Vidal-García *et al.* 2020, Whiting *et al.* 2022, Drinkwater *et al.* 2022). Deimatic displays thus offer sensory ecologists a way to study (1) how behavior intersects with morphology to shape the perception of visual signals, (2) what conditions select for the evolution of multicomponent and/or multimodal signals, and (3) how evolutionary tradeoffs can produce and maintain signal diversity (Rowe 1999, Cuthill *et al.* 2017, Stevens and Ruxton 2018, Postema *et al.* 2022). Despite a surge of theoretical interest in the past 10 years, the ecology and evolution of deimatic displays remains poorly understood relative to other forms of visual defenses (e.g., aposematism, crypsis; Umbers *et al.* 2015, 2017, Skelhorn *et al.* 2016b, Umbers and Mappes 2016). In particular, experimental evidence for the fitness consequences of deimatic displays is lacking – and, even more so, how these displays function under natural conditions (but see Umbers *et al.* 2019).

The caterpillars of swallowtail butterflies (family Papilionidae) exhibit a wide variety of defensive color strategies (Gaitonde *et al.* 2018) and complementary defensive behaviors (Hossie & Sherratt 2012, 2013, 2014; Hossie *et al.* 2013, 2015). Many species possess eyespots in the final instars, presumably to deter predators by mimicking the eyes of more threatening animals (Wagner 2005; Hossie & Sherratt 2013; De Bona *et al.* 2015; Skelhorn *et al.* 2016a). Spicebush swallowtail (*Papilio troilus*) eyespots are large compared to other North American species in the same genus, such as *P. eurymedes*, *glaucus*, or *rutulus* (Wagner 2005). Like other eyespotted *Papilio* larvae, spicebush swallowtails inflate their thoraxes in response to agitation (Hossie & Sherratt 2013). This reflex increases the size of the eyespots, and may heighten the larva’s resemblance to a snake (Hossie & Sherratt 2014). However, even at rest, *P. troilus* eyespots may still be conspicuous to predators unless the larvae are otherwise concealed (Fig. 1a). Unlike most *Papilio* species, *P. troilus* larvae

construct shelters from the leaves of their host plant. They rest in these leaf rolls during the day (Fig. 1d), emerging at night to feed (Wagner 2005). Larvae in leaf rolls point their heads up towards the leaf petiole, generally making their eyespots partially visible at the small opening at the top of the roll (Wagner 2005; see supplemental table 1).

I hypothesize that the combination of eyespots and leaf rolls in *P. troilus* larvae creates the same antipredator effect as a deimatic display. I refer to this potential defensive strategy as “environmental deimatism”: the use of environmental materials to create a sudden, startling visual signal that stops or slows predation. Similar to other deimatic displays, *P. troilus* eyespots are concealed at rest, but may become suddenly apparent once a predator opens or looks into the leaf roll (Fig. 1a,d). This effect would fulfill the definition of a “momentary, transient signal” that startles a predator enough to slow or halt its attack (Umbers & Mappes 2016). Additionally, leaf rolls may offset potential detectability costs of large, conspicuous eyespots (Hossie et al. 2013, Postema 2022). While the startling “conceal-then-reveal” effect of eyespots within leaf rolls has been presumed for both *P. troilus* larvae and other lepidopteran species (Wagner 2005; Janzen et al. 2010), this assumption remains untested. In other arthropod systems, leaf rolls have been shown to play a role in predator defense (Murakami 1999; Tvardikova & Novotny 2012) among other functions (Kobayashi et al. 2015; Romero et al. 2022), but their influence on the perception of prey color patterns is not well-known. I expect that environmental deimatism may be a relatively widespread (but not well-characterized) defensive strategy among shelter-building species – e.g., spiders that spring out suddenly from leaf rolls (Postema *personal observations*), or other leaf-sheltering caterpillars with conspicuous markings (Janzen et al. 2010).

To test the environmental deimatism hypothesis, I conducted an artificial prey experiment using a combination of eyespotted and non-eyespotted clay caterpillars presented on either open or rolled host plant leaves (Fig. 1b-e). I predicted an overall protective effect of leaf rolls, as well as interactions between prey presentation (roll vs. open leaf) and color (eyespots vs. no eyespots). Specifically, I expected eyespots to decrease predation on leaf-rolled prey. For prey on open leaves, I predicted that eyespots would either increase or have no effect on predation – depending on how well eyespots deter predators without additional defensive components (Hossie and Sherratt 2013, Postema 2022). Overall, my primary aims for this experiment were (1) to conduct a test of the environmental deimatism hypothesis in the field, and (2) to quantify the potential costs of conspicuous color signals that lack additional behavioral components.

## MATERIALS AND METHODS

### *Site Description and Host Plant Selection*

I conducted field predation trials at two sites in Ann Arbor, MI, approximately 4km apart (Bird Hills Nature Area, “Bird Hills”: 42°18’09.1”N 83°45’37.9”W; Nichols Arboretum, “Arboretum”: 42°16’48.9”N 83°43’20.5”W; see supplemental figure 1). Both sites were composed of mixed coniferous-deciduous forest, and contained host plants of *P. troilus* caterpillars such as sassafras (*Sassafras albidum*) and tulip tree (*Liriodendron tulipifera*). I selected individual plants haphazardly across subsites, with at least 5m between each plant. All plants were checked for *P. troilus* larvae before the start of each trial and previously occupied plants (n = 17; see supplemental figure 1) were not used. Potential predators of *P. troilus* were present at the sites throughout the experiment (see supplemental table 2), as well as live *P. troilus* larvae and adults. I ran two predation trials at these sites: Trial 1 (July 3<sup>rd</sup> to July 12<sup>th</sup>) and Trial 2 (July 20<sup>th</sup> to July 30<sup>th</sup>).

### *Surveys of Naturally Occurring Leaf Rolls*

To characterize the overall community of leaf-sheltering organisms at my study site, I conducted surveys of naturally occurring leaf rolls at Bird Hills from August 23<sup>rd</sup>-27<sup>th</sup>, 2022. Surveys were conducted along five 50m linear transects that overlapped with areas previously used for the predation experiment. I pre-established each transect on a digital map of the study site, and used GPS points from the map to start and end the physical transects on-site. I surveyed all plants of a specific height range (over 0.5m and under 2m tall) within one meter of the transect for rolled or folded leaf structures; plants were marked as having either 0, 1, 2, or >3 leaf rolls. Leaf rolls varied in structure but were counted in the survey as long as they were (a) at least partially enclosed, and (b) clearly constructed by an animal. Leaves that were curled due to

desiccation or disease were not considered leaf rolls, as I was most interested in the contents of intentionally constructed rolls that resembled structures made by *P. troilus* larvae.

I recorded the contents of up to 3 leaf rolls per plant by carefully opening each structure by hand. Organisms were visually identified to broad taxonomic groups (e.g., spiders, springtails, beetles). Rolls with either non-living debris or nothing inside were recorded as “empty.” As some organisms immediately jumped or dropped out of the rolls, I also recorded the escape behavior of all observed organisms: jumping, dropping, or no response. In total, I surveyed 464 leaf rolls across 457 individual plants. Of these plants, ~10% were also host plants of *P. troilus*, including spicebush (*Lindera benzoin*), sassafras, and tulip tree.

#### *Host Plant and Habitat Measurements*

Background color, texture, complexity, and lighting can impact the perception of visual signals (Endler 1993). To account for the influence of background on artificial prey detection and/or perception, I measured two main habitat characteristics: canopy openness and plant size. I anticipated that more open canopies would increase avian attack rates (Blake & Hoppes 1986; Richards & Coley 2008), possibly by providing better lighting conditions for prey detection. In terms of plant size, I expected that individual prey items would be easier to find on smaller plants, also increasing avian attacks. Plant height measurements were taken (in cm) from ground-level at the base of the stem to the tip of the uppermost leaf. Plants used in the experiment were an average of 124.9cm tall (sd: 60.1cm). I measured canopy openness by taking upward digital photos with a 180° hemispheric lens at plant height directly above each plant, with the camera held level with the ground. I then processed these photos using ImageJ (version 1.53) to calculate the proportion of open sky relative to vegetative cover in each image.

#### *Artificial Prey Construction*

I constructed artificial *P. troilus* larvae by pressing white modeling clay (Van Aken Plastalina?) into 3D-printed molds (see supplemental file “3D mold.zip”). The resulting unpainted prey are 4cm long, approximately the size of a 4<sup>th</sup> or 5<sup>th</sup> instar larva. Larvae are eyespotted at this point in development (Fig. 1a). To attach prey to host plants, I inserted a short loop of 26-gauge flexible craft wire into each clay caterpillar, leaving the two ends of the loop exposed from the ventral side. I applied three layers of acrylic airbrush paint (CREATEX tan, yellow-green, and dark green) to create the appearance of green countershading, which is an important aspect of visual defense for many swallowtail species (Rowland *et al.* 2007). Using yellow and black acrylic paint, I hand-painted eyespots on half of the prey (“eyespotted”, Fig. 1b), while leaving the rest blank (Fig. 1c). I preserved the paint with one coat of Krylon? matte finish spray. Finally, I measured the reflectances of both real and artificial *P. troilus* caterpillars using an Ocean Optics Flame Miniature (FLAME-S-UV-VIS-ES) spectrometer with Ocean Optics PX-2 Pulsed Xenon light source, calibrated with a 99% Labsphere reflectance standard (see supplemental figure 2).

#### *Predation Experiment*

For the predation trials, I deployed four different treatments of artificial larvae in a 2 x 2 factorial design: eyespotted in leaf rolls, eyespotted on open leaves, non-eyespotted in leaf rolls, and non-eyespotted on open leaves. I affixed prey to individual host plants, interspersed by both treatment and host plant species (*S. albidumor* *L. tulipifera* ). I generated unique treatment assignments for each trial. During deployment, I selected the artificial prey’s location on the plant haphazardly and measured its height (in cm) from the ground. Prey were placed 11.0-281.0 cm high on plants (mean: 83.5, SD: 44.1cm), comparable to the heights of live *P. troilus* prey I observed in the field (25-164cm, mean: 83.8, SD: 40.5cm; supplemental table 1).

For the open leaf treatment group, I attached artificial prey to the adaxial side of fully expanded host plant leaves by poking the loose wire-ends of each prey through the leaf, then twisting them tightly around the midrib. For the prey in leaf rolls, I attached them to the leaf in the same way, then folded the leaf over the artificial prey and secured it shut with a strip of Scotch? double-sided tape (Fig. 1e). I positioned all prey with the “head” pointed up towards the leaf petiole, which reflects this species’ typical resting position (Fig. 1a, 1f). During Trial 2, I also included a fifth treatment group of eyespotted prey in leaf rolls, oriented

down away from the petiole, to test the effect of eyespot orientation on predator perception. However, as I was unable to confidently determine whether predators perceived this treatment group as eyespotted or not, I excluded data on these prey from the final analysis. Before the start of the trial, I took a photo of each artificial caterpillar in place.

I collected artificial prey after approximately 5 days of exposure (mean: 121 hours, SD: 8 hours). At the end of each trial, I visually inspected prey for evidence of predation, using the pre-trial photographs as a baseline for non-attacked prey. Avian and mammalian attacks are clearly distinguishable by the shape of the bite-marks in the clay (see supplemental figure 3). I recorded any missing prey items (that could not be found after carefully scouring a 1m<sup>2</sup> area around the original location) as attacked by an unknown predator. I photographed all recoverable prey with visible attack marks. In a few cases (n = 9), either the artificial caterpillar or the entire leaf roll fell from the plant with no sign of predator damage; these prey were excluded from analysis. I also excluded one artificial caterpillar in which a live *P. troilus* caterpillar had crawled into a leaf roll, and three artificial prey where the plant could not be found (and thus the clay caterpillar could not be recovered). In total I deployed 809 artificial caterpillars. Of those, data from 659 artificial caterpillars are included in the analysis, excluding prey attacked by non-avian predators. Because birds are common visual predators of insects (Nyffeler *et al.* 2018), avian predation patterns are the most informative (relative to mammalian or unknown predators) for the goals of this study (Hossie and Sherratt 2012, 2013). Additionally, as expected, mammalian and unknown predators showed little variation in predation between experimental treatments (see supplemental figure 4). As it is difficult to detect evidence of arthropod attacks using clay caterpillars, these types of predators were not considered in this experiment.

### Statistical Analysis

To analyze these data, I used binomial generalized linear models with a with a complementary log-log link function in R (ver. 1.1.463). For all models, I set avian predation as the binomial response variable (0 = not attacked, 1 = attacked) and included days exposed as an offset term. For the primary model, I included the following independent variables: trial, location, leaf roll treatment (rolled, open), color treatment (eyespotted, non-eyespotted), canopy openness (a proportion, from 0 = sky fully obscured to 1 = sky not obscured by anything), and plant height (in cm). I also tested for an interaction between the roll treatment and color treatment. To determine the statistical significance of each independent variable across the model, I compared the full model to models lacking the term of interest using likelihood ratio tests (package `lmtree`). To determine the simple effects of eyespots and leaf rolls, without the interaction effect, I constructed 4 additional models using data from (1) only eyespotted prey, (2) only non-eyespotted prey, (3) only leaf-rolled prey, and (4) only open-leaf prey. For models (1) and (2), I compared full models to models without the leaf roll treatment; for models (3) and (4), I compared full models to models without the color treatment. These comparisons were also made using likelihood ratio tests.

## RESULTS

The overall avian predation rate was 13.4% of artificial caterpillars over a 5-day period. Predation did not vary significantly by trial ( $\chi^2 = 0.79$ ,  $df = 1$ ,  $p = 0.38$ ), location ( $\chi^2 = 0.55$ ,  $df = 1$ ,  $p = 0.46$ ), canopy openness ( $\chi^2 < 0.01$ ,  $df = 1$ ,  $p = 0.95$ ), or plant size ( $\chi^2 = 0.71$ ,  $df = 1$ ,  $p = 0.40$ ). Leaf rolls significantly reduced predation relative to prey on open leaves (12.9% reduction,  $\chi^2 = 24.43$ ,  $df = 1$ ,  $p < 0.001$ ). This was true of both eyespotted (17.3% reduction,  $\chi^2 = 25.77$ ,  $df = 1$ ,  $p < 0.001$ ) and non-eyespotted (8.4% reduction,  $\chi^2 = 4.54$ ,  $df = 1$ ,  $p < 0.05$ ) prey. Eyespots alone had no significant effect on predation ( $\chi^2 = 0.91$ ,  $df = 1$ ,  $p = 0.34$ ), though this was only true for prey on open leaves ( $\chi^2 = 0.13$ ,  $df = 1$ ,  $p = 0.72$ ). There was a significant interaction between leaf rolls and eyespots ( $\chi^2 = 5.96$ ,  $df = 1$ ,  $p < 0.05$ ): in leaf rolls, eyespots reduced the probability of predation (7.1% reduction,  $\chi^2 = 6.98$ ,  $df = 1$ ,  $p < 0.01$ ).

## DISCUSSION

In this study, leaf rolls not only protected prey in general, but also increased the effectiveness of eyespots at deterring visual predators (Fig. 2). This result supports the environmental deimatism hypothesis, i.e., the active use of objects in the environment to create a deimatic display. Similar to the mounds built by

bowerbirds for sexual communication (Endler *et al.* 2010), leaf rolls are not just passive “backgrounds,” but an integral part of the visual signal’s success. While environmental modification has been shown to effectively complement (or serve as) visual camouflage in several systems (Canfield 2009; Hultgren & Stachowicz 2011), these behaviors are not well-documented in other types of visual defense (e.g. aposematism, mimicry, masquerade, deimatism). Environmental deimatism may be a common strategy among other leaf-sheltering organisms, such as eyespotted hesperiid caterpillars (Janzen 2010) or spiders that abruptly jump out of rolls when disturbed (*Postema pers. observations*, Fig. 3). Beyond leaf rolls, environmental deimatism may exist as a more general strategy for shelter-using or -building species.

The overall protective effect of leaf rolls supports past experimental work on these structures’ role in predator defense (Murakami 1999; Tvardikova & Novotny 2012). However, the mechanism of protection is not entirely clear. One possibility is that leaf rolls physically hide the organism from detection. However, folded leaves are relatively noticeable against non-folded foliage; in some systems, leaf rolls even seem to act as a target for visually-oriented predators (Kobayashi *et al.* 2020). Naturally occurring leaf rolls in the study area were common, and often occupied by living organisms: over half (56%) of surveyed plants had at least one leaf roll, and over a third (34%) of rolls were occupied. Given their frequency and apparent profitability, it seems advantageous for avian predators to learn to search for prey in leaf rolls. However, the organisms inhabiting leaf rolls were not necessarily desirable prey items (Fig. 3). Surveyed rolls most commonly contained small, fast-moving spiders (49% of occupied rolls). Spiders often jumped from the roll immediately when disturbed, potentially making prey startling, hard to catch, or both. Other common prey items included very small organisms (e.g. springtails) and small weevils that often dropped to the ground when disturbed. Larger, less mobile, and more profitable prey – e.g. caterpillars – were rare (found in only ~4% of occupied rolls). The main defensive function of the leaf roll, then, may be to conceal prey *identity*. The added ambiguity and handling time of leaf rolls may make them relatively low-value foraging microhabitats, though this likely depends on the predator community’s degree of specialization, and perhaps temporal shifts in leaf roll abundance/occupancy. Predator uncertainty could further enhance the effectiveness of unexpected or startling visual signals as well.

Eyespots did not significantly increase predation risk on open leaves relative to non-eyespotted prey on open leaves (Fig. 2). This could suggest that prey with large eyespots are not more detectable to visual predators than prey without eyespots. Given that birds are highly attuned to eye-like stimuli, this seems unlikely (De Bona *et al.* 2015). Alternatively, readily visible eyespots may be more detectable to predators, but simultaneously function to deter predators at a distance. In this scenario, the combined effects of eyespots (increased detection and predator deterrence) may be counterbalanced. This hypothesis is supported by the fact that the majority of *Papilio* species that possess eyespots rest on open leaves, as well as the general positive association between body size and presence of eyespots in lepidopteran larvae (Wagner 2005; Hossie *et al.* 2015; Gaitonde *et al.* 2018). The fear of paired, eye-like patterns appears to be relatively innate for avian predators (Merilaita *et al.* 2011). This contrasts with other conspicuous color strategies, such as aposematism, where learning is more central to the pattern’s antipredator effect (Hämäläinen *et al.* 2020). If eye mimicry does not require predators to have prior negative experience with the “model” organism, then costs of being conspicuous due to encounters with naïve predators may be minimal.

Habitat characteristics may have also played a role in the perception and effectiveness of eyespots in this study. Both field sites were densely vegetated, with an average canopy openness of 14.3% (SD: 8.3%) – i.e., ~86% of the area above each artificial caterpillar was covered by vegetation. In complex, highly vegetated, and low-light environments, it may be difficult for predators to distinguish between real and fake eyes, or it may be too risky to spend a long time investigating (Janzen *et al.* 2010). This may also help to explain why eyespots did not significantly increase predation, despite presumably higher predator detection, compared to non-eyespotted prey on open leaves (Fig. 2). While there was no direct support for the influence of background conditions (such as canopy openness and plant height) on avian predation in this study, I did not experimentally manipulate these characteristics. In other studies of visual signalling, habitat heterogeneity, vegetation density, and lighting conditions have had effects on the perception of animal color patterns (Gotceitas & Colgan 1989; Endler 1993; Coker *et al.* 2009; Seymoure *et al.* 2018). To better understand

the effect of environmental context on the perception of eyespots, it would be useful to directly observe predator responses to eyespotted and non-eyespotted prey across various habitat types.

It may be useful to consider *P. troilus* leaf rolls as an example of Dawkin’s “extended phenotype” (1999). There are clear consequences of the leaf roll on caterpillar fitness, as well as synergistic interactions between leaf rolling and color traits (Fig. 2). In this system, selection is acting on multiple interacting levels: on the structure of the roll, the expression of leaf-rolling behavior, and the organism’s color patterns (Laland 2004; Hunter 2018). This makes the evolution of environmental deimatism a question of both morphology and behavior. Umbers et al (2017) suggest two potential pathways for how deimatic displays evolve: the “defense-first” and “startle-first” hypotheses. In the former, initially cryptic prey gain constitutive defenses (e.g., toxins), which then selects for conspicuous color patterns to advertise toxicity, and finally a concealing mechanism to create the “startle” effect. In the latter, initially cryptic prey develop a sudden movement that deters predators, which is later enhanced by a conspicuous visual component (and additional chemical defenses, in some species). Given that *P. troilus* larvae are generally considered non-toxic (Wagner 2005), the “startle-first” hypothesis may be more likely. Via this pathway, we would expect larvae to have evolved the leaf-rolling behavior (a proxy for the “sudden movement”) before the development of large, conspicuous eyespots. It is less likely that leaf-rolling developed simply as a way to conceal conspicuous eyespots, as there were no obvious detectability costs of eyespots for prey on open leaves (Fig. 2). This aligns with Schaedlin and Taborsky’s (2009) observation that external structures involved in signalling often provide an initial, direct fitness benefit to the signaler, that then selects for a progressively stronger signal. A phylogenetic comparative study, tracking both color traits and deimatic behaviors across the evolutionary history of swallowtails and/or other relevant lepidopteran groups, could potentially clarify when and how the behavior-morphology pairing arose (Janzen *et al.* 2010; Vidal-García *et al.* 2020).

Given that leaf-rolling is an effective antipredator strategy for *P. troilus* larvae, and appears to work synergistically with the species’ defensive color strategy (Fig. 2), why is leaf-rolling not observed more generally across swallowtails? One possible constraint is the time and energy investment involved in constructing multiple leaf rolls over the course of larval development. After larvae lay down layers of silk, leaves may take over an hour to fully fold into a roll (supplemental video 1). These periods of high activity and potential exposure to predators are not accounted for in this study, but may temper the antipredator benefit of leaf rolls. Secondly, some host plants may not be conducive to the formation of leaf rolls. The leaves of common *P. troilus* host plants are relatively thin, wide, and flexible compared to common host plants of other eyespotted swallowtail species (e.g., *Populus* spp., *Salix* spp.; Wagner 2005). While many *Papilio* larvae form Velcro-like silk pads to rest on, the leaves of their host plants may be too stiff, thick, or narrow to easily fold into full leaf rolls. Larvae in the swallowtail family (Papilionidae) use a diverse array of host plants, and their later-instar color defenses correspond closely to evolutionary shifts in host plant usage – e.g., aposematism has mainly evolved in larvae that use narrow-leafed, toxic plants, while cryptic or mimetic strategies are associated with more dense, nontoxic plants (Gaitonde *et al.* 2018). It would be worth investigating how other aspects of host plant morphology (particularly leaf width and thickness) may have shaped the evolution of leaf-rolling, deimatism, and color traits among insects (Janzen *et al.* 2010).

The results of this study provide support for the environmental deimatism hypothesis, and, more generally, the key role of behavior in defensive visual signals (Ruxton *et al.* 2009; Cuthill *et al.* 2017; Stevens & Ruxton 2018). They also suggest that deimatic displays can arise without strong costs to conspicuousness, though this likely depends on the mechanism of predator deterrence (learned vs. reflexive avoidance). To better understand the ecology and evolution of defensive visual signals, it is essential to consider color patterns less as static characters, and more as “multivariate optima”; i.e., complex strategies that may involve selection on morphology, behavior, and/or extended phenotypes beyond the body of the organism (Dawkins 1999, Laland 2004, Cuthill *et al.* 2017, Stuart-Fox 2022, Postema *et al.* 2022).

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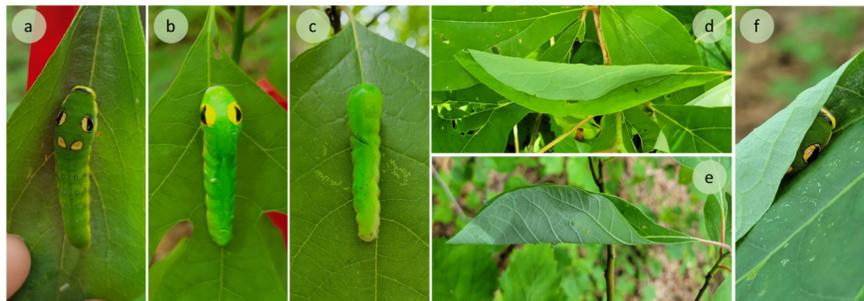
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**Figure 1.** (a) A live spicebush swallowtail (*Papilio troilus* ) larva on sassafras (*Sassafras albidum* ). Its leaf roll is held open, with strands of silk visible above the head. Eyespotted (b) and non-eyespotted (c) artificial larvae on open *S. albidum* leaves. (d) A true leaf roll with a live *P. troilus* larva inside. (e) An artificial leaf roll with an artificial larva inside. (f) A live *P. troilus* larva in a leaf roll, its eyespots partially visible up-close. Photographs by EGP.

**Figure 2.** Mean proportions of artificial prey in each leaf roll treatment group (rolled versus open) attacked by avian predators,  $\pm$  SE (n = 659). Yellow points represent eyespotted prey, while green points represent non-eyespotted prey. Illustrations by Mia Lippey.

**Figure 3.** (Left) Total counts of each organism type found in surveys of naturally occurring leaf rolls (n = 464). Within each organism category, counts of individuals that displayed escape behaviors in response to the leaf roll being disturbed (dropping, jumping, or no response) are represented in yellow, red, and brown, respectively. (Right) Examples of naturally occurring leaf rolls I observed in the field; leaf rolls varied in size, structure, and plant species. Photographs and illustrations by EGP.

**Figure 1.**



**Figure 2.**

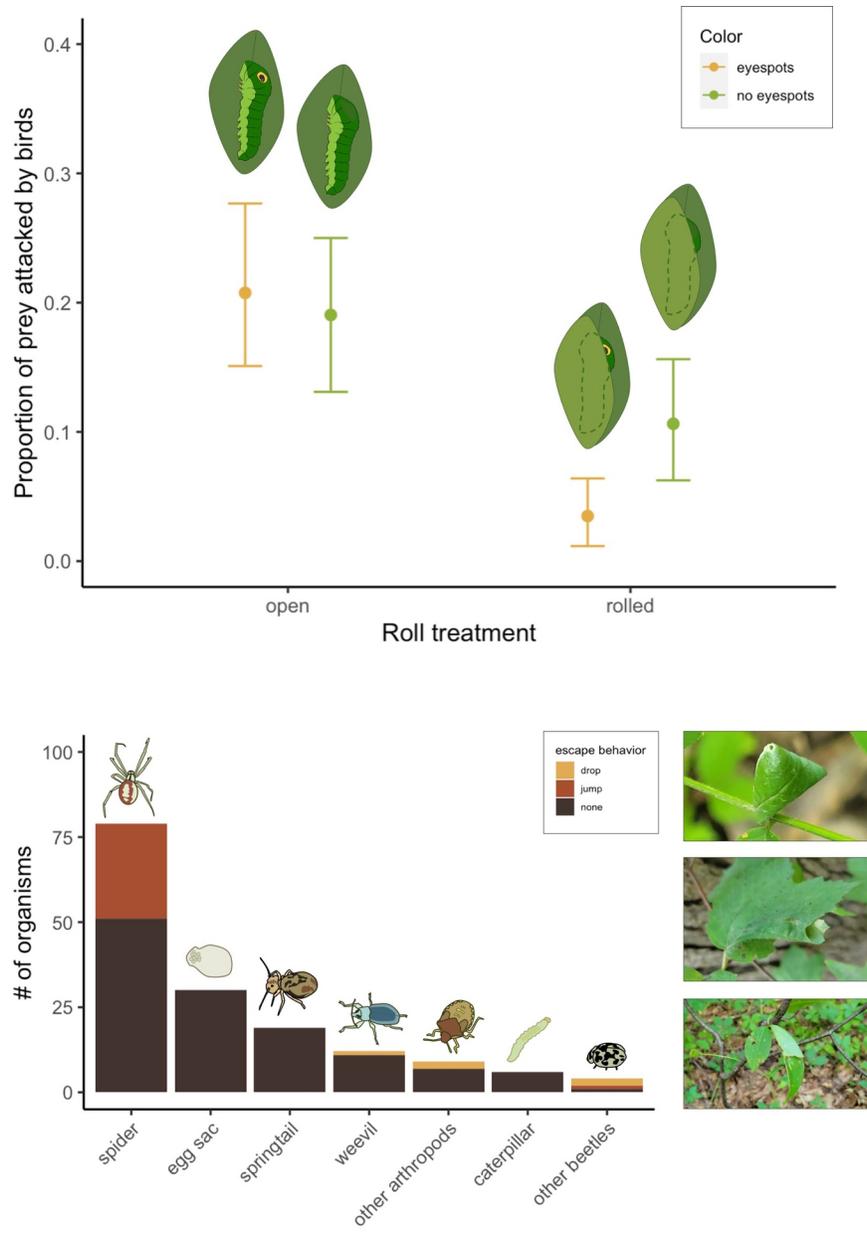


Figure 3.

