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The effectiveness of eyespots and masquerade in protecting artificial prey across ontogenetic and seasonal shifts

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Abstract

When constraints on antipredator coloration shift over the course of development, it can be advantageous for animals to adopt different color strategies for each life stage. Many caterpillars in the genus *Papilio* exhibit unique ontogenetic color sequences: for example, early instars that masquerade as bird feces, with later instars possessing eyespots. I hypothesize that larvae abandon feces masquerade in favor of eyespots due to ontogenetic changes in signaler size. This ontogenetic pattern also occurs within broader seasonal shifts in background color and predator activity. I conducted predation experiments with artificial prey to determine how potential signaling constraints (specifically size and season) shape predation risk, and consequently the expression of ontogenetic color change in *Papilio* larvae. Seasonally, both predation and background greenness declined significantly from July to September, though there was little evidence that these patterns impacted the effectiveness of either color strategy. Caterpillar size and color strongly affected the attack rate of avian predators: attacks increased with prey size regardless of color, and eyespotted prey were attacked more than masquerading prey overall. These results may reflect a key size-mediated tradeoff between conspicuousness and intimidation in eyespotted prey, and raise questions about how interwoven aspects of behavior and signal environment might maintain the prevalence of large, eyespotted larvae in nature.

Keywords: ontogenetic color change, eyespots, masquerade, predator-prey dynamics, season

Animal coloration is diverse in both form and function (Caro et al. 2016; Caro and Allen 2017; Cuthill et al. 2017). This diversity reflects the complex evolutionary landscape experienced by visual signals: color patterns are shaped by proximate constraints on the signals themselves (e.g., aspects of signal production, propagation, and perception) as well as multiple, sometimes competing selection pressures on ultimate function (Guilford and Dawkins 1991; Hebets and Papaj 2005; Hebets et al. 2016; Patricelli and Hebets 2016). Additionally, these constraints and selection pressures may be temporally dynamic. Organisms move, grow, and experience changing life history needs as they age (Werner and Gilliam 1984; Valkonen et al. 2014; Nakazawa 2015); they encounter different abundances, compositions, and experience levels of predators over time (Endler and Mappes 2004; Nokelainen et al. 2014; Mappes et al. 2014); and the visual conditions of their habitats may shift on daily, weekly, or seasonal bases (Mills et al. 2013; Zimova et al. 2018). One way that animals contend with temporal changes in themselves, their receivers, and their environments is to adopt distinct color patterns at different life stages ("ontogenetic color change"; Booth 1990). For example, many insects are cryptic at more vulnerable, immature stages, but aposematically colored as larger and/ or more toxic adults (Boege et al. 2019). Ontogenetic color change is especially common in insects (Booth 1992; Grant

2007; Valkonen et al. 2014; Gaitonde et al. 2018), but also occurs in other invertebrates (Todd et al., 2009), amphibians (Bulbert et al. 2018), reptiles (Hawlena et al. 2006; Wilson et al. 2007), birds (Iverson and Karubian 2017), and mammals (Caro 2005). Though this strategy is taxonomically widespread, adaptive hypotheses for this phenomenon are rarely tested experimentally (but see Wilson et al. 2007; Valkonen et al. 2014; Bulbert et al. 2018).

Swallowtail butterflies in the genus *Papilio* express a diversity of ontogenetic color sequences, including species whose larvae masquerade as bird feces at early instars, whereas later instars possess eyespots and green countershading (Wagner 2005; Shapiro and Manolis 2007; Gaitonde et al. 2018; Figure 1A). Other swallowtail species are aposematically patterned at later instars (e.g., *Papilio zelicaon, P. polyxenes, P. machaon*), and a small number of citrus-feeding species (e.g., *P. cresphontes, P. thoas*) retain feces coloration throughout larval development. In the evolutionary history of the swallowtail family (Papilionidae), the ontogenetic switch from feces masquerade to eyespots is the ancestral state (Gaitonde et al. 2018). Late-instar aposematic striping evolved primarily in species with toxic hostplants, presumably to warn predators of sequestered chemical defenses while remaining cryptic at a distance (Tullberg et al. 2005). Although the individual color strategies of swallowtail larvae are relatively
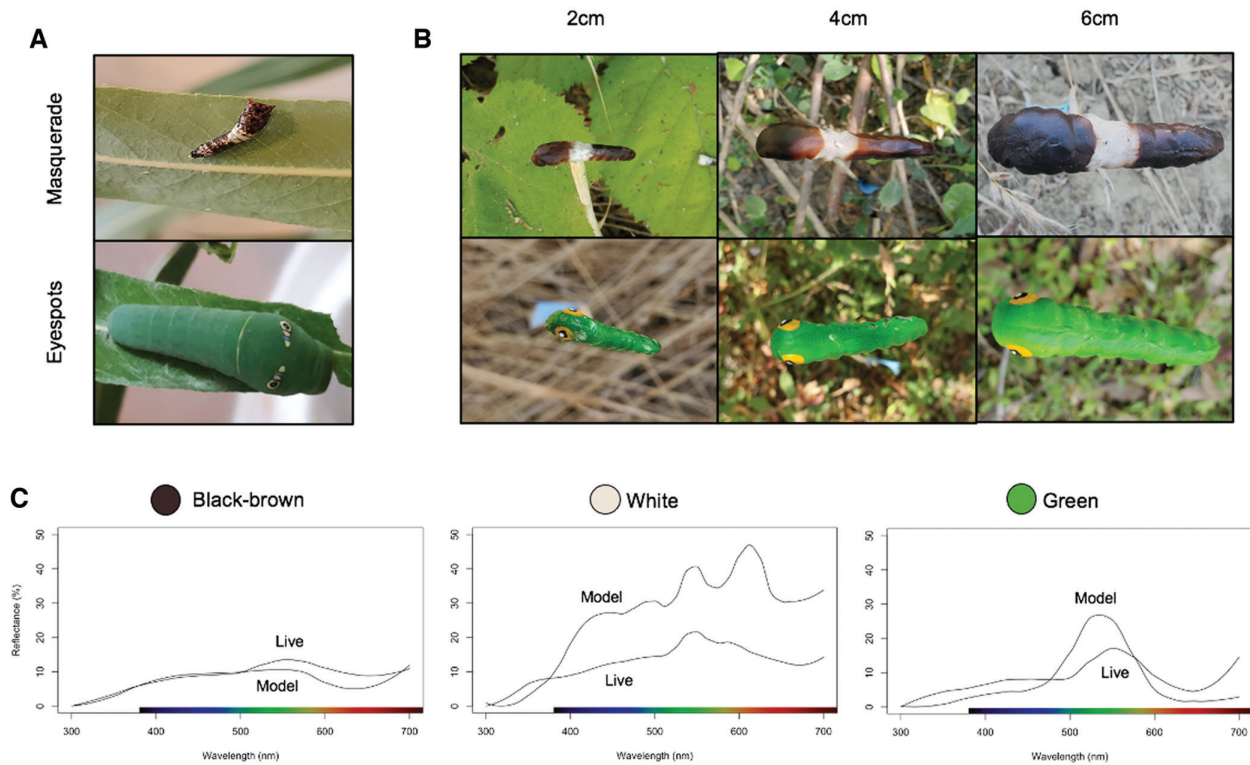
Figure 1. (A) Live larvae of the western tiger swallowtail (*Papilio rutulus*). Top: an early-instar larva masquerading as bird feces. Bottom: a 5th instar larva with prominent eyespots. (B) Three sizes of plasticine swallowtail larvae (2, 4, and 6 cm) painted to resemble either the younger feces-masquerading morph or the older eyespotted morph. (C) Reflectance measurements of larval color elements, taken from live and artificial larvae of both morphs: black-brown (feces masquerade), white (feces masquerade), and green (eyespots). Curves represent the average percentage of reflectance of 300- to 700-nm wavelengths across 3 measurements.

well-characterized from mechanistic, ecological, and evolutionary perspectives, it remains unclear why the majority of swallowtail larvae switch away from feces masquerade as they age (Futahashi and Fujiwara 2008; Hossie and Sherratt 2012, 2013; Gaitonde et al. 2018).

In other insect species, mimetic strategies are most effective when the mimic and its model are similar in size and behavior (Suzuki and Sakurai 2015). For example, avian predators are more likely to attack twig-mimicking caterpillars when those larvae are larger or smaller than model twigs (Skelhorn et al. 2010b). Feces masquerade in swallowtails, similarly, may become less convincing as larvae exceed the size of common bird feces (Valkonen et al. 2014). Additionally, as bird feces are immobile, feces masquerade may be less effective in older larvae that are more active (Valkonen et al. 2014). Conversely, eyespots are generally associated with larger lepidopteran larvae; this is likely because larger eyespots are more intimidating to predators (Hossie et al. 2015) or because they bear greater resemblance to putative snake models (Hossie and Sherratt 2014). It is also unlikely that eyespots are rendered ineffective by movement in the way feces masquerade might be, as model eyes are found on active, mobile animals (Stevens 2005; Skelhorn et al. 2016b). The intimidation effect of eyespots may even be enhanced by movements that suddenly reveal the eyespots when threatened ("deimatic displays"; Umbers et al. 2015, 2017), and/or specific snake-like defensive behaviors that increase model fidelity (Hossie and Sherratt 2013, 2014). Together, these patterns suggest a plausible adaptive mechanism for the switch from masquerade to eyespots: a decrease in effectiveness of masquerade against

predators as larvae become larger and more active, combined with an increase in the effectiveness of eyespots along the same ontogenetic axis.

In multivoltine swallowtail species (such as *P. rutulus*, which produces up to 3 generations from early spring to late fall), these individual-level ontogenetic shifts are nested within broader seasonal shifts. Aspects of both the visual environment and an organism's predators may be seasonally dynamic. Seasonal changes in predator experience (i.e., the abundance of young, naïve predators vs. older, experienced predators) have been shown to select for distinct color strategies: when naïve predators are abundant, cryptic strategies are more successful than aposematic strategies, and vice versa (Mappes et al. 2014). Prey may also be exposed to changing abundances of certain predators on a seasonal basis, with some taxa (e.g., birds) being more dependent on visual hunting strategies than others (Guilford and Dawkins 1991; Mason et al. 2018). For example, many avian species forage more heavily on insects during the spring and summer months as they breed, nest, and provision new offspring (Yoshikawa and Osada 2015). Lastly, as the perception of animal coloration depends on signaling environment (including color, lighting, and heterogeneity), seasonal shifts in substrate have the potential to make certain colorations more or less salient to predatory receivers (Endler 1993; Merilaita 2003). Seasonal effects on color have been primarily studied in cryptic species that change color to match seasonally variable landscapes (Mills et al. 2013; Caro et al. 2016; Zimova et al. 2018), as well as in aposematic species to a lesser extent (Mappes et al. 2014); whether seasonal changes influence the effectiveness of

other protective color patterns, such as mimicry and masquerade, are not well-known.

I conducted artificial prey experiments to answer 2 main questions about ontogenetic color change in swallowtail larvae, given this range of dynamic signaling constraints. (1) How do changes in body size and color traits affect predation risk? Based on the hypothesis that color may change over the course of development due to the size-dependent nature of antipredator color signals, I predicted that eyespots would most effectively deter predators in large (late-instar) prey, whereas feces masquerade would be most effective in small (early-instar) prey. (2) Does the effectiveness of feces masquerade and/or eyespots vary across seasons? Color-specific patterns of predation may be seasonally variable if feces masquerade and/or eyespots depend on background-matching or predator experience to function effectively. Alternatively, exposure to dynamic seasonal conditions might select for color patterns that function independently of background color/predator experience, leading to similar seasonal patterns of predation across color strategies.

Materials and methods

Study locations

For field predation experiments, I established 5 200-m long linear transects (at least 150 m apart) in and around Davis, California, USA (Transects A and B: Russell Ranch, 38°32′17.9″ N 121°52'07.3″W; Transects C and D: Putah Creek Riparian Reserve, 38°31′24.8″N 121°47'01.4″W; Transect E: riparian land near the Center for Aquatic Biology and Aquaculture, 38°31′43.0″N 121°46′55.6″W; Supplemental Materials, Figure S1). I conducted 2 predation trials with artificial prey between July 13–19 and September 11–16 2019. Larvae of both *P. rutulus* and *P. eurymedon* are present at various stages of development during these months (Shapiro and Manolis 2007). Transects A–D are similar in habitat type, composed of heterogeneous patches of dense riparian vegetation, and oak savannah along stretches of Putah creek. Transect E lays along a shallow drainage ditch and is far drier and grassier. I observed adult swallowtails of various species (*P. rutulus, P. eurymedon, P. zelicaon)* at all 5 locations throughout the experiment. I also observed an abundance of potential predators: particularly small- to medium-sized insectivorous/omnivorous birds (species list in Supplementary Materials, Table S1), groups of wild turkeys (*Meleagris gallopavo*), ground squirrels (*Otospermophilus beecheyi*), and western fence lizards (*Sceloporus occidentalis*; Postema EG, personal observations).

Artificial prey construction

I constructed artificial prey with the combined coloration features of 2 local *Papilio* species, the western tiger *P. rutulus* and pale *P. eurymedon* swallowtail in order to provide a generalized prey model. The larvae of these species both exhibit an ontogenetic switch from feces masquerade to eyespots after the 3rd molt. These species are also nontoxic, increase dramatically in size as they develop, and, in the case of *P. rutulus*, produce 2 to 3 generations from February to November (Shapiro and Manolis 2007). Although the patterns of the artificial prey were generalized, the body shape was derived specifically from an artist's 3D rendering of a swallowtail larva. I created 3D-printed molds for 3 artificial prey lengths: 2, 4, and 6 cm. I then created "blank"

artificial prey by pressing white, nonairdrying plasticine clay (Van Aken Plastalina® Modeling Clay) into the molds, trimming any excess clay, and smoothing out the seams. To create feces masquerade and eyespot color morphs for each size class, I used a combination of alcohol-based airbrush inks for the base pattern and acrylic paints for fine details (Figure 1B). For the eyespotted prey, I created a countershaded effect by first applying a light green basecoat and then painting a coat of darker green on the dorsal side; research by Hossie and Sherratt (2012) emphasizes the importance of countershading to effective background matching in these species. Using this method, I created 960 artificial caterpillars: 480 per trial, 60 per size–color combination. Finally, to standardize prey presentation, I affixed the models to thin 25-cm-long wooden stakes by pressing the tip of the stake into the middle of each model's ventral side.

Field experiment

To test the effects of size, color, and season on the survival of artificial caterpillars, I conducted field predation trials with 6 categories of artificial prey: (1) 6 cm with eyespots; (2) 6 cm feces masquerade; (3) 4 cm with eyespots; (4) 4 cm feces masquerade; (5) 2 cm with eyespots; and (6) 2 cm feces masquerade (Figure 1B). I set out the artificial prey along 5 200 m transects in each trial (Supplemental Materials, Figure S1). I deployed 96 artificial caterpillars per transect, ~5 m apart and randomly interspersed, with equal numbers of each color–size treatment: 16 each of the 6 color–size combinations per transect. I took photographs of each prey item immediately after deployment (prior to predator exposure) to distinguish new predator damage from marks made during construction, transport, or set-up. Using those pictures, I was also able to visually estimate background greenness (from 0%, bare ground or dry plant material, to 100%, full vegetation cover; Supplemental Materials, Figure S2). I collected the prey models after approximately 48 h (mean = 48.6 h, SD = 1.8 h).

During collection, I checked for evidence of predation and scored these marks as avian, mammal, or unknown based on the shape of the indentation (Supplemental Materials, Figure S3). If models were moved from their original position, I searched an area of ~5 m to locate evidence of each missing model. If I did not find the model after searching, I recorded it as preyed on by an unknown predator. Some models had melted in the sun (N = 19) and were excluded from the analysis. Additionally, models that were damaged or lost in transit (N = 22) were not included. In total, 919 artificial caterpillars of the 960 were included in the analysis.

Color measurement

I collected spectral data from both live *P. rutulus* and artificial prey using an Ocean Optics JAZ spectrometer and deuterium–tungsten halogen light source. The spectrometer was calibrated to an Ocean Optics WS-1 diffuse reflectance standard and recorded wavelengths from 300 to 700 nm. I took measurements on 3 color areas of interest: the green of eyespotted larvae, the black of feces masquerade larvae, and the white of feces masquerade larvae. Three measurements were taken for each color. From these data, I generated mean reflectance curves to compare color values (green, black, and white) between live and artificial caterpillars (Figure 1C).
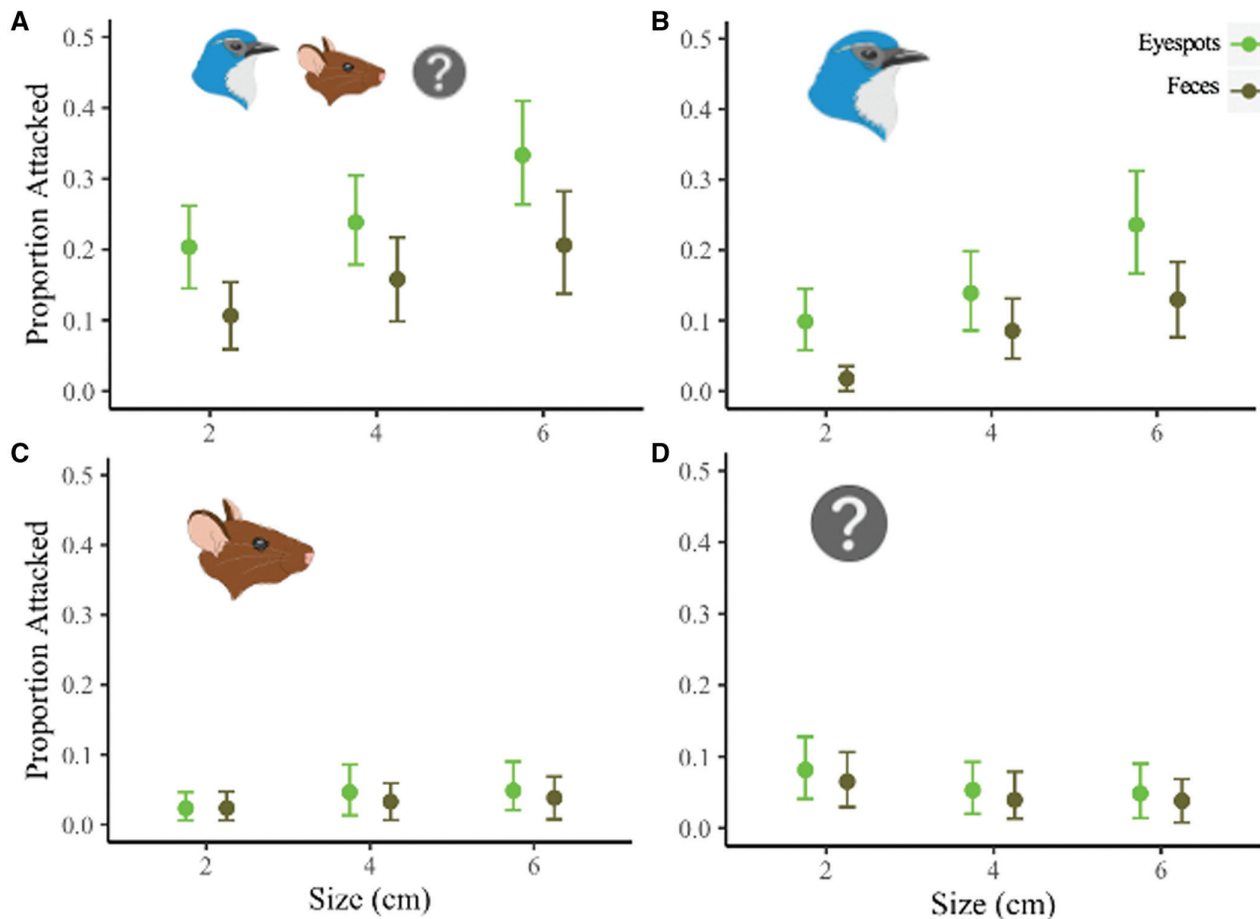
Figure 2. Proportion (mean ± *SE*) of each artificial prey type attacked by predators (out of *n* = 916 models), faceted by predator identity: (A) all types of predation included; (B) proportion attacked by avian predators; (C) proportion attacked by mammalian predators; and (D) proportion attacked by unknown predators.

Analyses

To analyze these data, I used binomial generalized linear mixed models with a cloglog-link function in R (package lme4). The first model pooled data across predator types, with the independent variable set as a binary measure of predation: "attacked" as 1, "not attacked" as 0. I set transect as a random effect with 5 levels, and size (2, 4, or 6 cm), color (eyespot, feces masquerade), season (July, September), percent background greenness (from 0% to 100%), and relevant 2-way interactions (size*color, color*background greenness) as fixed effects. I also included exposure time, in days, as an offset term. Finally, I constructed separate but otherwise identical models for each predator attack type (avian, mammal, and unknown) to investigate differences in predation patterns between taxa. I used likelihood ratio tests with and without the variable of interest to determine significance, and compared the fit of my models using second-order Akaike information criterion (package AICcmodavg).

Results

Predation and predator identity

Over the course of both trials, predators attacked an average of 20.5% of the artificial caterpillars. Of these attacks, 55.8% were carried out by avian predators, though models were also attacked by unknown predators (27.2%), and occasionally by small mammals (17.0% of attacks). Taxa-specific attack rates (average number attacked/total prey deployed) were as follows: 11.5% for avian, 3.5% for mammalian, and 5.6% for unknown predators.

Effects of size and color

Pooled across predator type, predation increased significantly with size ($\chi^2 = 11.16$, $df = 1$, $P < 0.001$; Figure 2A). Additionally, predation was influenced by color; eyespotted prey were attacked significantly more than feces masquerade prey ($\chi^2 = 17.51$, $df = 1$, $P < 0.001$; Figure 2A). When avian predation was analyzed separately, there was a similar pattern of increased predation on larger models ($\chi^2 = 22.87$, $df = 1$, $P < 0.001$; Figure 2B) and eyespotted models ($\chi^2 = 15.33$, $df = 1$, $P < 0.001$; Figure 2B). In both pooled and avian-specific analyses, the influence of color on predation was not size-dependent (pooled: $\chi^2 = 0.09$, $df = 1$, $P = 0.77$; avian: $\chi^2 = 1.81$, $df = 1$, $P = 0.18$). In mammal-specific analyses neither size ($\chi^2 = 1.21$, $df = 1$, $P = 0.27$) nor color ($\chi^2 = 0.62$, $df = 1$, $P = 0.43$) influenced predation. For unknown predators, smaller prey were marginally more likely to be attacked ($\chi^2 = 3.72$, $df = 1$, $P = 0.05$), whereas color had no effect on predation ($\chi^2 = 0.98$, $df = 1$, $P = 0.32$)

Effects of season

Background greenness decreased significantly across the season (from 18.7% to 9.5%; $F_{1,913} = 36.6$, $P < 0.001$; Figure 3A).
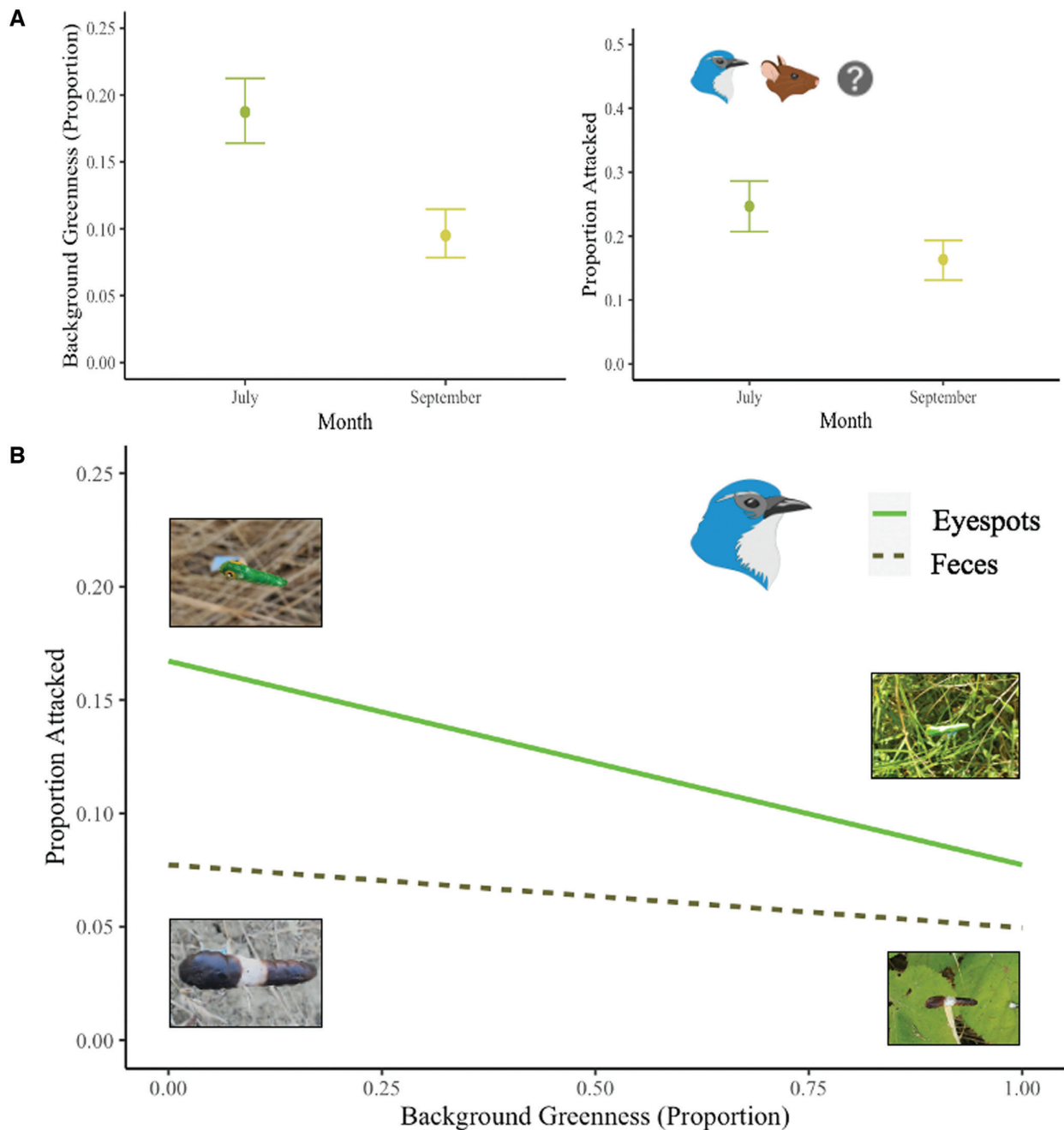
Figure 3. (A) The effects of season (months: July and September) on average background greenness (left) and proportion of each artificial prey attacked by predators overall (right). Greenness is a visual estimate from 0.0 = 0% green to 1.0 = 100% green. (B) The average proportion of model larvae attacked by avian predators across different background greenness levels. The green line represents attacks on eyespotted prey, whereas the brown dotted line represents attacks on feces-masquerading prey. There was no significant relationship between predation and background greenness, regardless of color morph.

Overall predation was significantly higher ($\chi^2 = 14.09$, $df = 1$, $P < 0.001$) in the July trial (24.7% attacked) compared with the September trial (16.3% attacked). From mid-summer to early fall, the proportions of avian and mammal attacks did not change significantly (avian: $\chi^2 = 2.99$, $df = 1$, $P = 0.08$; mammal: $\chi^2 = 0.61$, $df = 1$, $P = 0.43$), whereas unknown predator attacks decreased significantly ($\chi^2 = 20.41$, $df = 1$, $P < 0.001$).

There was no effect of percent background greenness on predation in both pooled- ($\chi^2 = 2.40$, $df = 1$, $P = 0.12$) and predator-specific analyses (avian: $\chi^2 = 2.19$, $df = 1$, $P = 0.14$; mammal: $\chi^2 = 1.71$, $df = 1$, $P = 0.19$; unknown: $\chi^2 = 0.02$, $df = 1$,

$P = 0.90$). Additionally, the relationship between predation and model color did not differ significantly by background greenness across predator types ($\chi^2 = 0.16$, $df = 1$, $P = 0.69$). Although there appears to be a slight trend of increased avian predation on eyespotted prey against less green backgrounds, this is not supported statistically ($\chi^2 = 0.20$, $df = 1$, $P = 0.66$; Figure 3B).

Discussion

Life stage-specific aspects of the signaler, such as size and color, had the greatest impact on the risk of predation by

visual predators (Figure 2). Avian predation was highest on the largest size category of each color morph, and significantly higher on eyespotted prey than masquerading prey (Figure 2B). These results beg the question of why a number of *Papilio* species adopt eyespots only at later, larger instars—or why larvae abandon masquerade at all, if large feces masqueraders experience less predation than their eyespotted counterparts. The counterintuitive nature of these results may reflect a key tradeoff faced by eyespotted species: the balance between conspicuousness and intimidation. The contrasting yellow and black eyespots of many *Papilio* species are likely conspicuous to avian predators, as these same colors are common in aposematic patterns (Stevens 2005; Prudic et al. 2007; Arenas et al. 2014). Aposematic species are known to experience tradeoffs between signal conspicuousness and efficacy: large, bright, high-contrast patterns are most effective at advertising toxicity, but also result in increased detectability to naïve or specialist predators (Mänd et al. 2007). Similarly, eyespots that are large and boldly colored may be more effective at startling predators (Hossie et al. 2015), at the cost of being detected more easily and/or habituated to more quickly (Stevens 2005). Some species resolve this tradeoff behaviorally (Umbers et al. 2017). It is common to see eyespots paired with specific movements to create "deimatic" or startle effects: for example, otherwise cryptic moths flashing eyespotted hindwings only when threatened (Stevens 2005; Umbers et al. 2015), or *Eumorpha* caterpillars physically "blinking" eyespots open and shut via muscle contractions (Hossie et al. 2013). Live swallowtail larvae, unlike artificial prey, may be able to hide or reduce the conspicuousness of eyespots when not imminently threatened by predators, maintaining a startle effect without attracting unwanted attention at a distance (Hossie et al. 2013; Umbers et al. 2015). Swallowtail larvae exhibit a variety of defensive behaviors and movements that could contribute to this effect, including thorax-puffing, assuming a reared posture, swaying, and hiding in leaf-rolls when at rest (Postema EG, personal observations; Wagner 2005; Hossie and Sherratt 2013). It would be worthwhile for future studies to tease apart the relative contributions of short-term defensive behaviors (e.g., thorax-puffing, swaying, leaf-rolling) vs. broad movement patterns (e.g., activity level, microhabitat selection) to the effectiveness of eyespots in large insect prey.

Overall, the size- and color-dependent patterns of predation I observed are consistent with the hunting strategies of avian predators (Figure 2). Insectivorous birds mainly rely on vision to find prey, and are physiologically well-equipped for color discrimination (Robinson and Holmes 1982; Jones et al. 2007). By comparison, most small mammal predators have poor color vision and are often nocturnal, and thus may be less likely to be attracted or deterred by particular color patterns (Jacobs 2009; Figure 2C). Differences in size may have also been more detectable by avian predators than terrestrial mammals (Figure 2B and C). Birds likely approached artificial prey from above, where large prey become more visible as targets, whereas small mammals likely encountered prey incidentally while moving across the landscape (Robinson and Holmes 1982; Jones et al. 2007). Predation by "unknown" predators was similar to that of mammalian predators, with no significant effect of coloration (Figure 2C and D). Small prey were slightly more likely than large prey to be attacked by unknown predators—this is likely because small prey are more easily removed and transported away from their original location (Figure 2D). These results, in agreement with previous studies using artificial swallowtails, suggest that avian predators are likely the primary receivers that shape these species' color patterns (Hossie and Sherratt 2012, 2013).

In terms of seasonal effects on predation risk, predator attacks declined by almost a third from July to September (Figure 3A). This pattern is primarily driven by a significant seasonal drop-off in attacks by unknown predators; meanwhile, both mammal and avian attacks remained consistent across the experiments. There are several possible explanations for this apparent drop-off in predation pressure. First, certain predators may have simply been less active or abundant in September when compared with July. The transition from July to September in northern California marks the end of the summer growing period, and corresponds to a general decline in the activity of plants and animals. By the end of September, the majority of low-lying plants and deciduous trees are dry and/or leafless, and insects are markedly less active in the cooler, drier weather (Postema EG, personal observations). Predators may have also learned to avoid unprofitable prey. As I conducted the 2 experimental trials at roughly the same locations, ∼8 weeks apart, it is likely that many of the predators in the first trial were also present in the second. Of these overlapping predators, those who attacked models in the first trial may have remembered that the artificial prey were not edible in the same way that predators can quickly learn to avoid aposematic prey (Speed 2000; Mappes et al. 2014; Skelhorn et al. 2016a). To avoid possible effects of predators learning that artificial prey are inedible, some studies use edible pastry for their models (Hossie and Sherratt 2012, 2013). Clarifying which of the aforementioned or other explanations best account for the observed seasonal decline in "unknown" predation would require a more detailed record of both predator behavior at the individual level and predator abundance or activity.

The color of the background vegetation became significantly less green from summer to fall, in line with the seasonal drying and senescing of plants at the end of the California growing season (Figure 3A). However, I found little evidence that background color influenced predator perception of larval coloration (Figure 3B). For the feces-masquerading models this is not surprising. The protection awarded by masquerade depends primarily on misclassification, rather than a lack of detectability, though there are certainly examples of patterns that benefit from both (Skelhorn et al. 2010a). The effects of background color on eyespots are slightly harder to predict, as these are not well-documented, though one can make inferences from both aposematic and cryptic patterns. In conspicuous species, higher contrast between the animal's body color and the background tends to make the warning signal more effective, whereas the opposite is true of background-matching species (Merilaita and Lind 2005; Aronsson and Gamberale-Stille 2009; Michalis et al. 2017). Past researchers have classified the eyespotted instars of swallowtail larvae as cryptic, given their solid green bodies and association with green foliage (Gaitonde et al. 2018). However, eyespots are a functionally distinct category of color defense that may or may not include elements of crypsis in addition (Stevens 2005). Tree-feeding *Papilio* larvae present an interesting case of combined (while seemingly contrasting) color defenses, and it is most likely that their method of avoiding visual predators is a combination of context-dependent strategies (Tullberg et al. 2005; Hossie and Sherratt 2012).

In this experiment, I found little evidence of crypsis as background color had no effect on the predation of eyespotted prey (Figure 3b). However, these results were likely influenced by the method of prey presentation: artificial prey were affixed to stakes of a standard height, and placed relatively low to the ground, rather than attached directly to vegetation. The larvae of local eyespotted *Papilio* species (*P. rutulus, eurymedon*) are tree-feeding species, and generally rest on the leaves of their host plants during the day (Shapiro and Manolis 2007). In this habitat, larvae would be flush with the surface of green leaves as well as surrounded by green foliage—2 aspects of signal environment that would likely enhance the effectiveness of background matching in the green eyespotted prey, but might not significantly affect the visibility and/or signal efficacy of masquerading prey (Merilaita and Lind 2005; Prudic et al. 2007). In addition, background matching in swallowtail larvae may be further improved via behavioral mechanisms (particularly those that conceal eyespots) that artificial prey were unable to express (Stevens and Ruxton 2018). These potential methodological limitations are highlighted by a slight, but nonstatistically significant increase in predation risk for eyespotted prey on nongreen backgrounds in July (Supplementary Materials, Figure S4). Given that predation was higher in July than September, the penalty for having improperly concealed eyespots (or being unnaturally exposed) may have been correspondingly higher.

Although the relationship between color and predation risk is mediated by multiple constraints (Endler 1993; Caro et al. 2016; Cuthill et al. 2017), both the expression and the relative importance of each constraint may change over time. In a swallowtail larva's shift from masquerade to eyespots, size and color had the greatest impact on predation risk (Figure 2), whereas seasonal changes in background color did not seem to strongly influence prey perception (though this was likely impacted by prey not being affixed directly to host plant foliage; Figure 3). The switch away from masquerade is likely mediated by upper size limits on larval resemblance to bird feces, but the switch from masquerade to eyespots specifically remains a more complicated question. There are likely important larval behavior(s) that help to resolve the unexpectedly high predation on large eyespotted prey—particularly those that reduce eyespot visibility to avian predators at a distance but increase their saliency up close—but this hypothesis requires experimental testing in the swallowtail system (Stevens 2005; Umbers et al. 2017). Future studies using artificial prey should carefully consider how the absence of movement and/or behavior, in combination with aspects of signal environment, might influence how the study species' color patterns are perceived (Paluh et al. 2014; Hossie et al. 2015; Stevens and Ruxton 2018).

Acknowledgments

Conflict of Interest Statement

The author declares that they have no conflicts of interest.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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