Integrating multiple pressures on antipredator color traits: a conceptual framework, with experimental evidence from swallowtail caterpillars (Papilionidae)

Postema, Elizabeth Grace

2023

Integrating multiple pressures on antipredator color traits: a conceptual framework, with experimental evidence from swallowtail caterpillars (Papilionidae)

By

ELIZABETH GRACE POSTEMA
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal Behavior

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Louie Yang, Chair

Andy Sih

Rick Karban

Committee in Charge

2023

ACKNOWLEDGEMENTS

-

I dedicate this dissertation, with much love, to my parents. Their unwavering support – including turning our family home into an impromptu field station – made this all possible.

-

Many hands, hearts, and minds helped my dissertation come to fruition. Foremost of these is my advisor Louie Yang, who I met at *exactly the right time* – just after deciding I "definitely did not want to go to graduate school in California." Thank you for teaching me how to fail (early and often), how to swing for the fences, and how to build an enriching life beyond research. I also thank my many other academic mentors, both formal and informal. Special thanks to my dissertation committee members, Andy Sih and Rick Karban, for their experience, insight, and encouragement. Additional thanks to Diane Ullman, for encouraging me to always look for art-science connections. Finally, many thanks to my Qualifying Exam committee, Gail Patricelli, Kate Laskowski, and Tom Hahn – even over Zoom, their excellent questions and comments substantially improved my dissertation.

My amazing graduate experience was built on the excellent undergraduate mentorship I received at Denison University. Thank you to Andy McCall and Dave Smith for immediately recognizing and nurturing my love of ecology; to Tom Schultz, for inspiring my obsession with insects, color, and artificial caterpillars; and to Sylvia Brown, for teaching me how to write.

The support of friends, family, colleagues, and loved ones has always been an essential part of my work. Making hundreds of clay models takes a lot of manpower – so thank you to Mia Lippey, Gwen Erdosh, Kirsten Sheehy, Lohit Garikpati, and Hee-Jin Chung for risking carpal tunnel syndrome for the sake of science. Mia also supplied much of the artwork used in this dissertation, including the beautiful figures in my first chapter. Thank you to my siblings, Tess, Jake, and Ben, for always cheering me on. Endless love to my wonderful friend and housemate Myles Collinson – my last years of graduate school were fueled by drinking Aperol spritzes, screaming along to the Tarzan soundtrack, and binge-watching Below Deck together. Similarly, thanks to my friend Carly Hawkins for always being ready to "Possum Out" with me at a moment's notice.

To my dearest Pavel, I owe much more to you than I could possibly fit on a single page (or even several). Without your love and patience, the last few years would have been far more difficult – and far less fun. Thank you for always noticing the hawks, rescuing spiders on my behalf, and modeling what it really means to be a serious scholar.

As a field ecologist, I was lucky enough to do my dissertation work in many beautiful places, from Davis, California to my hometown of Ann Arbor, Michigan. Thank you to the Putah Creek Riparian Reserve, Quail Ridge Reserve, Nichol's Arboretum (of University of Michigan), as well as Bird Hills Nature Reserve for the privilege and pleasure of doing research on their land. Finally, I acknowledge the many institutions and funding sources that made my work possible: UC Davis, UC Davis Graduate Studies, the Department of Entomology and Nematology, and the Animal Behavior Graduate Group; the Phi Beta Kappa Northern California Association; the Jastro-Shields Award; the Coss Wildlife Research Award; the NRS Maurer-Timm Grant; and the Gordon Research Conference.

ABSTRACT

Color is a visible fingerprint of deep ecological relationships. Color traits are shaped by intraspecific, interspecific, abiotic, and/or physiological drivers across ontogenetic and evolutionary timescales. My dissertation is concerned with how organisms integrate multiple, often conflicting selection pressures on color, given these many functions. I specifically study how selection on color traits varies across development. How do individuals avoid predation despite temporal shifts in morphology, environmental conditions, and/or behavior? Further, are there general patterns in how organisms balance these constraints over time? To answer these questions, I combine theoretical work with field experiments using artificial caterpillars.

In the first chapter of my dissertation, I review common sources of conflicting selection pressures on color strategies, and present a mechanistic framework for how organisms integrate these functions. I determine that color patterns can be intermediate, simultaneous, and/or plastic with respect to multiple pressures. These multivariate strategies encompass a wide range of defensive color phenotypes (from crypsis to aposematism) and provide avenues for future theoretical, meta-analytical, and empirical research on animal color.

In my second and third chapters, I experimentally test how specific factors shape color plasticity in swallowtail caterpillars. Using artificial swallowtail-like prey exposed to natural predators in two separate field experiments, I find that size constrains the effectiveness of both feces masquerade and eyespots; background color does not significantly influence the effectiveness of either color pattern; and environmental modification (e.g., constructing leaf shelters) not only protects prey in general, but actively enhances the “startle” effect of eyespots. Overall, in these species, ontogenetic shifts in larval color are shaped by size and behavior.

The study of animal coloration has a long history. My conceptual and experimental work builds on this history by (1) framing color patterns as functional strategies that extend beyond single-function phenotypes, (2) experimentally testing assumed constraints and functions of color, and (3) providing empirical evidence for the role of behavior in defensive color traits.

TABLE OF CONTENTS

Supplementary Information

Chapter I. Color under pressure: how multiple factors shape defensive coloration

Elizabeth G. Postema, Mia K. Lippey, and Tiernan Armstrong-Ingram

Published in Behavioral Ecology Vol. 34(1)

ABSTRACT

Behavioral ecologists have long studied the role of coloration as a defense against natural enemies. Recent reviews of defensive coloration have emphasized that these visual signals are rarely selected by single predatory receivers. Complex interactions between signaler, receiver, and environmental pressures produce a striking array of color strategies—many of which must serve multiple, sometimes conflicting, functions. In this review, we describe six common conflicts in selection pressures that produce multifunctional color patterns, and three key strategies of multifunctionality. Six general scenarios that produce conflicting selection pressures on defensive coloration are: (1) multiple antagonists, (2) conspecific communication, (3) hunting while being hunted, (4) variation in transmission environment, (5) ontogenetic changes, and (6) abiotic/physiological factors. Organisms resolve these apparent conflicts via (1) intermediate, (2) simultaneous, and/or (3) plastic color strategies. These strategies apply across the full spectrum of color defenses, from aposematism to crypsis, and reflect how complexity in sets of selection pressures can produce and maintain the diversity of animal color patterns we see in nature. Finally, we discuss how best to approach studies of multifunctionality in animal color, with specific examples of unresolved questions in the field.

INTRODUCTION

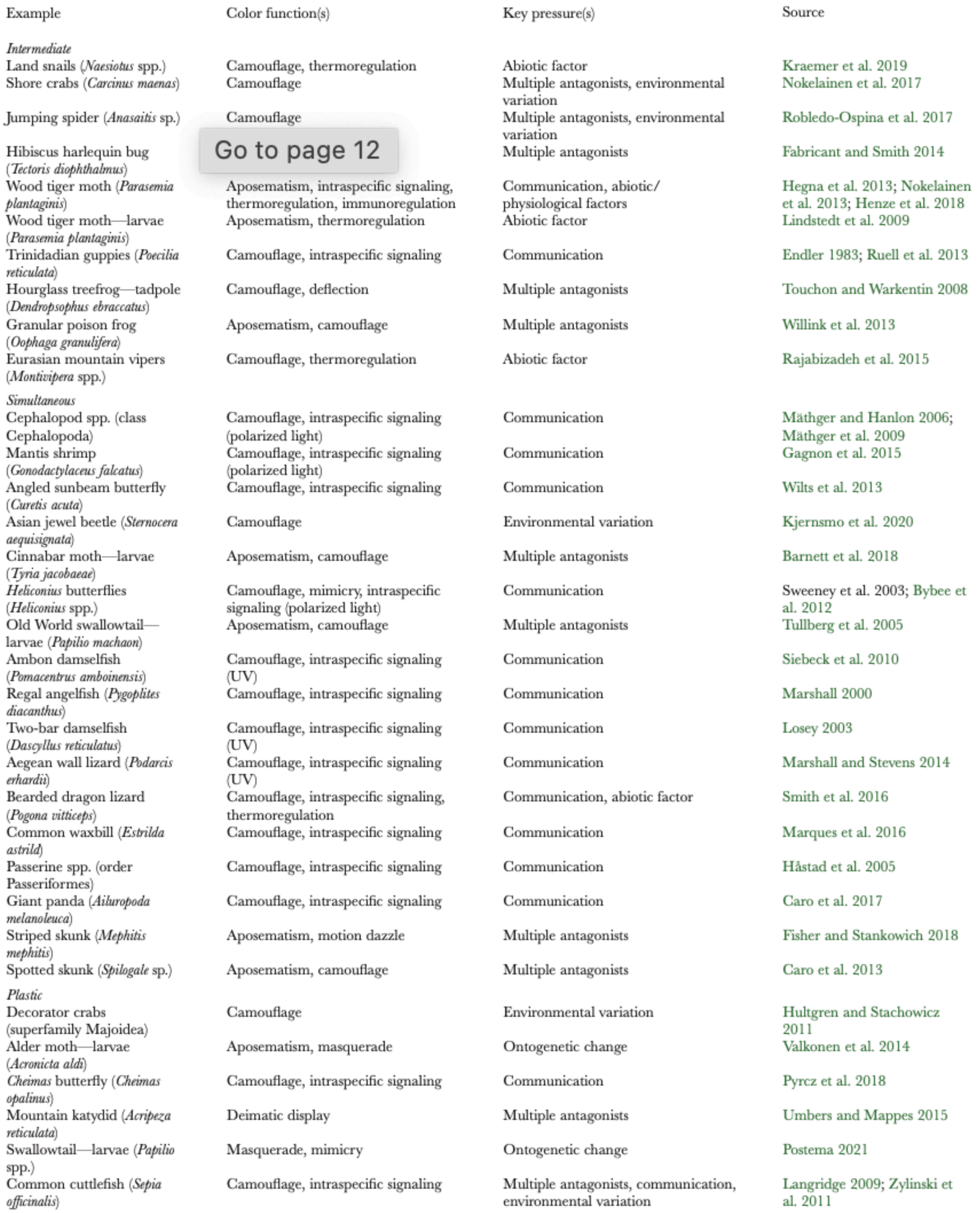
Researchers are drawn to the study of color from a kaleidoscope of perspectives (Goethe 1840; Gage 1993, 1999; Cuthill et al. 2017; Endler and Mappes 2017; Maund 2019). For biologists, color offers a uniquely visible lens into the inner workings of ecology and evolution. From Gregor Mendel's careful observations of flower, pod, and seed colors in hybrid pea plants to Henry Kettlewell's classic demonstration of industrial melanism in peppered moths, color has been central to some of the most iconic experiments in biology (Kettlewell 1955; Abbott and Fairbanks 2016). The color strategies that animals employ to thwart natural enemies, communicate with conspecifics, and contend with variable abiotic/physiological factors (among other functions) are as diverse and striking as the animals themselves (Caro et al. 2017; Cuthill et al. 2017; Endler and Mappes 2017).

Categories of coloration are generally neither exhaustive nor mutually exclusive, but standard lists of antipredator color include aposematism, camouflage, mimicry, masquerade (often considered a type of "cue mimicry"), motion dazzle, deflection, and/or deimatism (Caro and Allen 2017; Jamie 2017). Further subcategories have been delineated within each of these groups, such as the distinction between background-matching and disruptive color within camouflage, or between aggressive, Batesian, Müllerian and rewarding types of mimicry (Merilaita and Lind 2005; Dalziell and Welbergen 2016; Jamie 2017). Additionally, many species possess color patterns that fulfill multiple categories (Table 1.1), or that do not fit neatly into any one of the proposed categories. Even color strategies with seemingly opposite functions, such as aposematism and camouflage, can coexist on the same individual (Caro et al. 2013; Marshall and Stevens 2014; Barnett et al. 2018). It is likely that any given coloration will consist of multiple categories that overlap in synergistic ways – particularly when we consider the color pattern in its natural context.

Color traits evolve under complex suites of selection pressures (Hebets and Papaj 2005; Caro et al. 2016; Cuthill et al. 2017). Organisms often need to simultaneously avoid threats from multiple predators and parasites; overcome the challenges of capturing a variety of prey; cope with changes in habitat structure, light, and temperature; and navigate a complicated web of relationships with conspecifics, including competitive rivals, social partners, and/or potential mates (Endler 1993b; Caro et al. 2016; Hebets et al. 2016). The phenotypes we observe are a product of balancing these various needs across shortand long-term environmental changes. Given this complexity, it is not surprising that visual signals have been the subject of considerable research, and that recent literature has called attention to the role of multiple interacting selection pressures in shaping these signals. Cuthill et al. (2017) observed that "...researchers usually try to identify single key functions of external appearances, but individual color patterns can experience multiple, often opposing, selection pressures." The existence and influence of multiple selection pressures on color has been particularly well-documented in cryptic (see Stevens and Merilaita 2009; Duarte et al. 2017) and aposematic systems (see Speed and Ruxton 2007; Ruxton et al. 2009; Rojas et al. 2018).

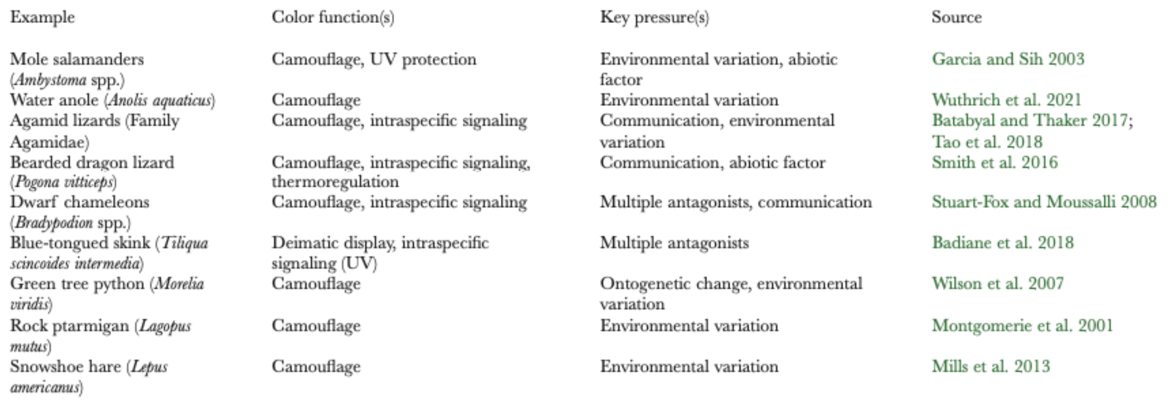
In this review we seek to describe, in more detail, the nature of these conflicts in selection pressures – both when and where they arise, and how they shape the expression of defensive color strategies. Previous reviews of complexity in defensive coloration have mostly focused on one category of defense, such as camouflage (Stevens and Ruxton 2018) or mimicry (Dalziell and Welbergen 2016), or emphasize a limited number of possible pressures, such as change over time (Caro et al. 2016) or predator behavior (Endler and Mappes 2017). Others that address animal signalling more broadly are oriented toward intraspecific communication (i.e., sexual or social contexts; Hebets et al. 2016; Patricelli and Hebets 2016) or signals that span multiple modalities (Rowe and Guilford 1999; Ratcliffe and Nydam 2008; Rowe and Halpin 2013). We aim to provide a more general framework for understanding how multiple interacting selection pressures can produce and maintain diversity in defensive visual signals. Our primary goals for this review are threefold: (I) to summarize common scenarios where "multiple, often opposing, selection pressures" shape defensive color patterns (Cuthill et al. 2017), (II) to delineate the color strategies animals exhibit in the face of those multiple pressures, and (III) to propose useful avenues for the future study of multifunctionality in animal coloration, informed by the current state of the field. We intend for this review to both organize and build upon an enormous amount of conceptual, observational, and empirical research that has been done on this topic.

Table 1.1. Sourced examples of intermediate, simultaneous, and plastic color strategies. For each example, we describe the proposed function(s) of the visual signal and the proposed key pressure(s) involved in shaping it.



Example	Color function(s)	Key pressure(s)	Source
Intermediate			
Land snails (*Naesiotus* spp.)	Camouflage, thermoregulation	Abiotic factor	Kraemer et al. 2019
Shore crabs (*Carcinus maenas*)	Camouflage	Multiple antagonists, environmental variation	Nokelainen et al. 2017
Jumping spider (*Anasaitis* sp.)	Camouflage	Multiple antagonists, environmental variation	Robledo-Ospina et al. 2017
Hibiscus harlequin bug (*Tectoris diophthalmus*)	[illegible]	Multiple antagonists	Fabricant and Smith 2014
Wood tiger moth (*Parasemia plantaginis*)	Aposematism, intraspecific signaling, thermoregulation, immunoregulation	Communication, abiotic/ physiological factors	Hegna et al. 2013; Nokelainen et al. 2013; Henze et al. 2018
Wood tiger moth—larvae (*Parasemia plantaginis*)	Aposematism, thermoregulation	Abiotic factor	Lindstedt et al. 2009
Trinidadian guppies (*Poecilia reticulata*)	Camouflage, intraspecific signaling	Communication	Endler 1983; Ruell et al. 2013
Hourglass treefrog—tadpole (*Dendropsophus ebraccatus*)	Camouflage, deflection	Multiple antagonists	Touchon and Warkentin 2008
Granular poison frog (*Oophaga granulifera*)	Aposematism, camouflage	Multiple antagonists	Willink et al. 2013
Eurasian mountain vipers (*Montivipera* spp.)	Camouflage, thermoregulation	Abiotic factor	Rajabizadeh et al. 2015
Simultaneous			
Cephalopod spp. (class Cephalopoda)	Camouflage, intraspecific signaling (polarized light)	Communication	Mäthger and Hanlon 2006; Mäthger et al. 2009
Mantis shrimp (*Gonodactylaceus falcatus*)	Camouflage, intraspecific signaling (polarized light)	Communication	Gagnon et al. 2015
Angled sunbeam butterfly (*Curetis acuta*)	Camouflage, intraspecific signaling	Communication	Wilts et al. 2013
Asian jewel beetle (*Sternocera aequisignata*)	Camouflage	Environmental variation	Kjernsmo et al. 2020
Cinnabar moth—larvae (*Tyria jacobaeae*)	Aposematism, camouflage	Multiple antagonists	Barnett et al. 2018
Heliconius butterflies (*Heliconius* spp.)	Camouflage, mimicry, intraspecific signaling (polarized light)	Communication	Sweeney et al. 2003; Bybee et al. 2012
Old World swallowtail—larvae (*Papilio machaon*)	Aposematism, camouflage	Multiple antagonists	Tullberg et al. 2005
Ambon damselfish (*Pomacentrus amboinensis*)	Camouflage, intraspecific signaling (UV)	Communication	Siebeck et al. 2010
Regal angelfish (*Pygoplites diacanthus*)	Camouflage, intraspecific signaling	Communication	Marshall 2000
Two-bar damselfish (*Dascyllus reticulatus*)	Camouflage, intraspecific signaling (UV)	Communication	Losey 2003
Aegean wall lizard (*Podarcis erhardii*)	Camouflage, intraspecific signaling (UV)	Communication	Marshall and Stevens 2014
Bearded dragon lizard (*Pogona vitticeps*)	Camouflage, intraspecific signaling, thermoregulation	Communication, abiotic factor	Smith et al. 2016
Common waxbill (*Estrilda astrild*)	Camouflage, intraspecific signaling	Communication	Marques et al. 2016
Passerine spp. (order Passeriformes)	Camouflage, intraspecific signaling	Communication	Håstad et al. 2005
Giant panda (*Ailuropoda melanoleuca*)	Camouflage, intraspecific signaling	Communication	Caro et al. 2017
Striped skunk (*Mephitis mephitis*)	Aposematism, motion dazzle	Multiple antagonists	Fisher and Stankowich 2018
Spotted skunk (*Spilogale* sp.)	Aposematism, camouflage	Multiple antagonists	Caro et al. 2013
Plastic			
Decorator crabs (superfamily Majoidea)	Camouflage	Environmental variation	Hultgren and Stachowicz 2011
Alder moth—larvae (*Acronicta aldi*)	Aposematism, masquerade	Ontogenetic change	Valkonen et al. 2014
Cheimas butterfly (*Cheimas opalinus*)	Camouflage, intraspecific signaling	Communication	Pyrcz et al. 2018
Mountain katydid (*Acripeza reticulata*)	Deimatic display	Multiple antagonists	Umbers and Mappes 2015
Swallowtail—larvae (*Papilio* spp.)	Masquerade, mimicry	Ontogenetic change	Postema 2021
Common cuttlefish (*Sepia officinalis*)	Camouflage, intraspecific signaling	Multiple antagonists, communication, environmental variation	Langridge 2009; Zylinski et al. 2011

Table 1.1. Continued



Example	Color function(s)	Key pressure(s)	Source
Mole salamanders (*Ambystoma* spp.)	Camouflage, UV protection	Environmental variation, abiotic factor	Garcia and Sih 2003
Water anole (*Anolis aquaticus*)	Camouflage	Environmental variation	Wuthrich et al. 2021
Agamid lizards (Family Agamidae)	Camouflage, intraspecific signaling	Communication, environmental variation	Batabyal and Thaker 2017; Tao et al. 2018
Bearded dragon lizard (*Pogona vitticeps*)	Camouflage, intraspecific signaling, thermoregulation	Communication, abiotic factor	Smith et al. 2016
Dwarf chameleons (*Bradypodion* spp.)	Camouflage, intraspecific signaling	Multiple antagonists, communication	Stuart-Fox and Moussalli 2008
Blue-tongued skink (*Tiliqua scincoides intermedia*)	Deimatic display, intraspecific signaling (UV)	Multiple antagonists	Badiane et al. 2018
Green tree python (*Morelia viridis*)	Camouflage	Ontogenetic change, environmental variation	Wilson et al. 2007
Rock ptarmigan (*Lagopus mutus*)	Camouflage	Environmental variation	Montgomerie et al. 2001
Snowshoe hare (*Lepus americanus*)	Camouflage	Environmental variation	Mills et al. 2013

MULTIPLE PRESSURES ON COLORATION

Defensive coloration generally involves the transmission of a signal to potential receivers – or, in the case of crypsis, the non-transmission of cues (Hebets et al. 2016; Merilaita et al. 2017). As such, color is directly influenced by the animal's capacity for signal production, the phylogenetic, ontogenetic, and physiological constraints on that capacity, the nature of its receiver(s), and the environment the signal is transmitted through (Endler 1993a; Endler 1993b; Hebets and Papaj 2005; Hebets et al. 2016). While these constraints may not necessarily be in conflict, conflicts in the function of coloration are relatively common, and often produce multipurpose defensive strategies (Table 1.1). In the following sections, we summarize six common scenarios where we expect conflicts in selection pressures to produce multifunctional coloration: (i) multiple antagonists, (ii) conspecific communication, (iii) hunting while being hunted, (iv) variation in transmission environment, (v) ontogenetic changes, and (vi) abiotic and physiological factors.

Multiple Antagonists

A common conflict arises when an animal uses coloration to defend against multiple natural enemies. The most effective deterrence strategy may differ between antagonists, as a result of differences in visual capacities (Fabricant and Smith 2014), experience levels (Mappes et al. 2014) and/or methods of attack (Touchon and Warkentin 2008; Fisher and Stankowich 2018). Reviews of animal coloration (and signalling more generally) consistently emphasize the importance of receiver psychology in shaping the evolution of signals (Guilford and Dawkins 1991; Rowe 1999; Endler and Mappes 2004; Stevens and Merilaita 2009; Rowe 2013). Color perception varies widely between taxa, from mono- to tetrachromacy and beyond (e.g., the detection of polarized light; Sontag 1971; Kamermans and Hawryshyn 2011; Honkavaara et al. 2002; Gagnon et al. 2015; Cuthill et al., 2017). Though differences in perception are sometimes undetectable to the human eye, developments in visual modelling software in tandem with use of spectrometry and/or digital image processing have enabled researchers to test how prey species appear to a variety of predatory receivers (Stoddard 2012; Caro et al. 2017; Stoddard and Osorio 2019; Nokelainen et al. 2022). For example, in modeling how a twig-masquerading orb-weaver (*Wixia abdominalis*) appears to its dichromatic hymenopteran and tetrachromatic avian predators, Xavier et al. (2018) show that this species is able to remain inconspicuous in the eyes of both.

Among predators with similar visual physiologies, variation in predator experience alone can produce conflicting selection pressures on color. For example, avian nestlings that have yet to learn caterpillar warning signals may kill and eat conspicuous (but toxic) prey, while more experienced adults forage for cryptic (nontoxic) prey instead. As a result, Mappes et al. (2014)

suggest that the direction of selection can shift on a seasonal axis: during times of year where naïve predators are common, crypsis may be favored as a strategy over aposematism, and vice-versa. This experience-driven conflict in the direction of selection has also been proposed as an explanation for the fine striping in some caterpillar species: the stripes blend together cryptically at a distance, perhaps avoiding detection by most predators (particularly naïve predators), but appear aposematic at close ranges to deter predators once detected (Tullberg et al. 2005; Barnett et al. 2017).

Lastly, differences in antagonist attack method may select for distinct color strategies. Tadpoles of a neotropical treefrog (*Dendropsophus ebraccatus*), for example, face opposing selection pressures on color from both aquatic insect predators and fish (Touchon and Warkentin 2008). The former are ambush hunters, whereas the latter use a chase-down strategy. While ambush hunters can be misdirected from vital organs with a bright red tail spot, this pattern is less effective in deflecting attacks during chases. In nature, the range of *D. ebraccatus* tail color – red, colorless, and in-between – reflects the gradient of selection pressures exerted by these two predator types (Robertson et al. 2009). Similar cases of predator-driven polymorphisms have been documented in shore crabs (*Carcinus maenas*), granular poison frogs (*Oophaga granulifera*), and some species of jumping spider (*Anasaitis* sp.) (Willink et al. 2013; Nokelainen et al. 2017; Robledo-Ospina et al. 2017). As these and other examples demonstrate, multiple antagonists can impose conflicting selection pressures on a number of different levels, within and across predator taxa.

The bulk of defensive coloration literature focuses on predators as the main antagonistic agents of selection. However, parasites, parasitoids, and pathogens may also shape defensive signals, either when these antagonists detect their hosts visually, or when the host's color patterns derive from pigments that are also immunologically involved. In general, there is far less information on parasites as a selection pressure on defensive visual signals, perhaps because many of these taxa rely on non-visual cues to find hosts (Turlings et al. 1993), or those that do use visual cues rely more on shape, size, and/or movement than color (Morehead and Feener 2000; Stireman 2002). Still, parasites have been shown to play a major role in the evolution of certain color patterns (e.g. striping in zebras likely developed to deter biting flies; Caro et al., 2019). Additionally, certain color pigments (such as melanin in insects, or carotenoids in songbirds) are involved in defending organisms against pathogens or microbial natural enemies (Smilanich et al. 2009; Sugumaran and Barek 2016; Weaver et al. 2017). How organisms integrate the immune function(s) of these pigments with their role in defensive color strategies is not well-studied (but see Nokelainen et al. 2013).

To better understand the relationship between immune pigments and defensive coloration, it may be helpful to look at similar cases in reproductive contexts. Several hypotheses concerned with immunological functioning have been proposed to explain the maintenance of "honest" signalling in sexual ornaments, as summarized by Weaver et al. (2017). For example, if certain pigments are limited (e.g., by diet), resource tradeoffs between immune health and robust sexual signalling could keep the display an honest indication of the signaler's health or fitness ("resource tradeoff hypothesis"). Alternatively, the "shared pathway" hypothesis proposes that sexual color displays and immune functioning may be physiologically linked in such a way that the presence of one necessitates the presence of the other (Hill 2011). These hypotheses (among others) are readily transferable to predator-prey signalling contexts: e.g., are there resource allocation tradeoffs between the use of pigments for immune health versus defensive color patterns (and/or additional functions of color), given that pigments may be costly to synthesize

(Talloen 2004)? Physiologically, is the expression of immune-related pigments linked to the expression of certain color patterns (Mikkola and Rantala 2010), or are these pathways typically decoupled? Factorial experiments to test these questions - perhaps via the manipulation of pigment availability, combined with the presence or absence of an immune challenge and/or visual predator - would be useful in clarifying this relationship.

Conspecific Communication

Animals must often communicate different information to antagonists and conspecifics. The differences between how *natural* and *sexual* selection act on the evolution of visual signals are a well-documented case of this conflict. Opposing directions of natural and sexual selection have been proposed as a hypothesis for why many animals (particularly birds) alternate plastically between "basic" and bright, showy breeding coloration throughout the year; while conspicuous color may be good for attracting mates, it may also inadvertently attract the attention of visual predators (Gluckman 2014; Pascual et al. 2014). A similar pattern is often expressed ontogenetically, where more conspicuous colors only develop when the individual is mature enough to breed (Booth 1990).

Communication conflicts can extend beyond sexual signalling into broader social contexts. For example, the Ambon damselfish (*Pomacentrus amboinensis*) belongs to hierarchical social networks that require conspicuous signals for accurate individual recognition. However, like many reef fish, this species must also avoid detection by predators. To effectively achieve both conspecific communication and camouflage against predators, the color pattern is high-contrast and individually recognizable to UV-sensitive conspecifics while remaining cryptic to UV-insensitive predators (Siebeck et al. 2010). Other forms of covert communication include physically revealing and concealing ornaments (e.g., retractable dewlaps in anoles; Nicholson et al. 2007) and pattern partitioning (e.g., lizards with cryptic patterns on areas most visible to aerial predators, and social/sexual signals on areas most visible to ground-level conspecifics; Marshall and Stevens 2014; Smith et al. 2016). Pattern partitioning may also be enhanced by active modification of pigments, as in cuttlefish (Brown et al. 2012; Zylinski et al. 2011). Of the multiple pressures we describe in this review, conspecific communication is one of the more commonly studied (Table 1.1), and its ability to produce multifunctional color patterns has been particularly well-demonstrated in cichlid and poecilid fishes, as well as adult wood tiger moths (Marshall 2000; Losey 2003; Siebeck et al. 2010; Henze et al. 2018).

Hunting While Being Hunted

Some species may experience selection on visual signals from both the "prey" and the "predator" perspective. Studies of color defense often focus on prey species, so research on the unique signalling demands of these species is minimal. Whether conflicts in these scenarios are rare (e.g., the need to hunt and avoid being hunted are synergistic with respect to visual signals; Cheney 2010) or simply understudied is unclear. However, research by Yeh et al. (2015) provides evidence that selection by predators and prey can indeed interact to shape the expression of visual signals. The oval St. Andrew's cross spider (*Argiope aemula*), like other diurnal orb weavers, decorate their webs with zig-zagging silken structures; these decorations ("stabilimenta") attract prey, particularly when their geometry is consistent throughout the day. The conspicuousness of these structures also attracts predators, though to a lesser degree when the stabilimenta's shape is frequently changed. In natural populations, variation in design consistency likely reflects the competing needs of prey attraction and predator avoidance (Yeh et al. 2015).

Variation in Transmission Environment

The perception of color, at its core, hinges on interactions between the intensity/angle of light, the reflectance(s) of the colored object, and the background that the object appears against (Endler 1993a; Endler 1993b). Biological "backgrounds" are rarely simple, homogenous, or static; for example, the color palette of a temperate forest changes drastically from spring to fall, and from day to night (Kelber and Roth 2006; Xie et al. 2018). This indicates that a single color strategy may not be effective in all the environments an organism encounters (Caro et al., 2016). Beyond background color, other aspects of habitat such as leaf shape (Gaitonde et al. 2018), texture (Kang et al., 2012), visual complexity (Merilaita 2003), pattern heterogeneity (Kang et al., 2016; Michalis et al., 2017), and angle of light (Kjernsmo et al. 2020), may also constrain defensive color patterns in ways that select for multifunctional strategies.

In cryptic species where background-matching is particularly important, common solutions for dynamic environments include color change and/or behavioral modifications (Caro et al., 2016; Shapiro, 1976; Stevens and Ruxton, 2018). Several species can quickly and flexibly camouflage against a variety of backgrounds (Hanlon 2007; Zylinski et al 2011l; Akkyanak 2017); other species actively select habitats they camouflage most effectively against (Camacho 2020), with some even orienting their bodies to better match specific patterns (Kang et al. 2012; Webster et al. 2008), or self-decorating with camouflaging materials (Montgomerie et al. 2001; Hultgren and Stachowicz 2011). Cryptic species that neither change color nor optimize for a single microhabitat may compromise between background types in a heterogenous habitat via "intermediate" color patterns (Merilaita et al. 1999). While the intermediate pattern may be less effective than the optimal cryptic pattern for each background type, these patterns can persist by matching moderately well across multiple backgrounds. This prediction is supported both theoretically (Merilaita et al. 1999) and empirically (Merilaita et al. 2001). Perhaps predictability, given its core mechanism, the effects of transmission environment on crypsis are relatively well-documented. Less is known about how variation in transmission environment influences aposematism, mimicry, masquerade, and other non-cryptic strategies (but see Prudic et al. 2007; Postema 2021).

Ontogenetic Changes

Signalers themselves are often as dynamic as the environments they inhabit. Over the course of development, organisms may shift along physiological and/or behavioral axes that constrain the effectiveness of certain color signals (Booth 1990). For example, several species of feces-mimicking caterpillars (*Acronicta alni, Papilio* spp.) become larger and more active as they approach pupation. These changes render the masquerade less convincing to predators, as both size- and behavior-matching is key to the efficacy of mimetic strategies (Skelhorn et al. 2010; Skelhorn et al. 2016; Wang et al. 2017). It is likely that these ontogenetic constraints contribute to *A. alni* and *Papilio* larvae changing color strategy dramatically at later instars: both species shift to patterns that may be more effective on larger, more active individuals, such as aposematism or false eyespots (Smith et al. 2014; Valkonen et al. 2014; Postema 2021; Hossie et al. 2015). Ontogenetic changes in vulnerability, habitat selection, reproductive status, and diet (discussed further in section vi) may also contribute to shifts in color pattern across development. Selection pressures imposed by ontogeny seem to disproportionality result in color change strategies, perhaps because developmental changes can be easily paired, physiologically, with color expression (Booth 1990; Table 1.1).

Abiotic and Physiological Factors

Certain abiotic and physiological factors can impose selection on animal coloration. In terms of abiotic factors, melanin-based color patterns offer both enhanced heat retention and protection from UV rays, though these functions are often balanced against the need to camouflage from predators (Garcia and Sih 2003; Lindstedt et al. 2009; Rajabizadeh et al. 2015). Conversely, lighter colors can prevent overheating via increased light reflectance (Brenner and Hearing 2008; Kraemer et al. 2019). For ectotherms that are more dependent on environmental conditions for thermoregulation, abiotic selection pressures on coloration may be particularly strong (Trullas et al. 2007). Among wood tiger moths (*Parasemia plantaginis*), for example, the black-and-orange caterpillars face a hard tradeoff between aposematism and thermoregulation. Larvae with orange and black patches of equal size exhibit a stronger aposematic signal, while those with larger black patches grow faster and larger in cold environments due to enhanced heat absorption (Lindstedt et al. 2009). These conflicting selection pressures maintain a range of larval color polymorphisms that vary in the size and number of black patches across geographic thermal clines. In some species, orientation behavior may play a key role in mediating interactions between crypsis, thermoregulation, and UV protection; models by Penacchio et al. (2015) show that certain orientations can achieve all three functions compatibly, though some may optimize thermoregulation and UV protection to the detriment of crypsis**.**

Temperature may also interact with humidity to influence coloration. According to Gloger's rule, there is a strong correlation between dark coloration and warm/humid climates, as well as between reddish coloration and warm/dry climates. This pattern is relatively consistent across endotherms, with some exceptions**;** for example, Marcondes et al. (2021) observe that birds in the family Furnariidae tend to have both darker and more rufous plumage in cooler, wetter habitats. Whether this pattern is directly influenced by color-dependent effects of heat and moisture, or through some combination of variables confounded with hot, humid environments (e.g. parasite abundance, vegetation density, light level) is unclear, though the latter appears more likely (Delhey 2019; Marcondes et al. 2021).

The role of certain pigments in tissue toughness is an important but often overlooked physiological factor that may shape animal coloration. In birds, melanin can help resist abrasion to feathers (Bonser 1995), while in insects, melanin is essential to the process of exoskeleton hardening (sclerotization) (Anderson 2010). To our knowledge, there are few (if any) empirical studies that address how organisms balance these functions with the need to avoid predation. Of course, the use of pigments in tissue strengthening may not result in strict resource-allocation tradeoffs with defensive coloration. In some cases, these functions may even be synergistic. For example, weevils in the genus *Pachyrhynchus* are both highly sclerotized and notably dark (melanized) in coloration, with contrasting iridescent patches that advertise their toughness (Wang et al. 2021).

Finally, how organisms produce or acquire pigments can shape the expression of defensive color patterns (Fabricant et al. 2013). In some systems, pigmentation is directly influenced by the compounds and minerals present in the organism’s food source (McGraw 2006; Isaksson 2009). In others – particularly in aposematic organisms – variation in diet can indirectly produce variation in defensive coloration. For example, the nymphs of spotted lanternflies (*Lycorma delicatula*) change dramatically from black to aposematic red over development; this is associated with a narrowing of the diet to more toxic host plants, from which they can sequester defensive chemicals (and thus “back up” the aposematic signal; Song et al. 2018).

Interacting Constraints

In nature, a single species may experience many interacting selection pressures that change over time (Booth 1990) and/or space (Caro et al. 2016). As described in the section above, *P. plantaginis* larvae experience a color-mediated tradeoff between aposematism and thermoregulation. The apparent dyad of selection pressures becomes at least a triad when *P. plantaginis* larvae develop into adult moths: adults are aposematic, thermoregulate via dark pigmentation, and also use hindwing coloration to communicate with sexual partners (Hegna et al. 2013; Henze et al. 2018). Each pressure offers an opposing direction of selection. Yellow hindwings best advertise toxicity, highly melanized wings are most efficient at heat absorption in cold conditions, and white hindwings are most attractive to females. As a result, across geographic clines in temperature and predator community composition, we see an incredible diversity of adult moth phenotypes in natural populations. Beyond this triad of selection pressures, *P. plantaginis* color patterns are likely shaped by additional factors: e.g., frequency-dependent selection, and possibly the involvement of melanin in immune functioning (Nokelainen et al. 2013; Gordon et al. 2015).

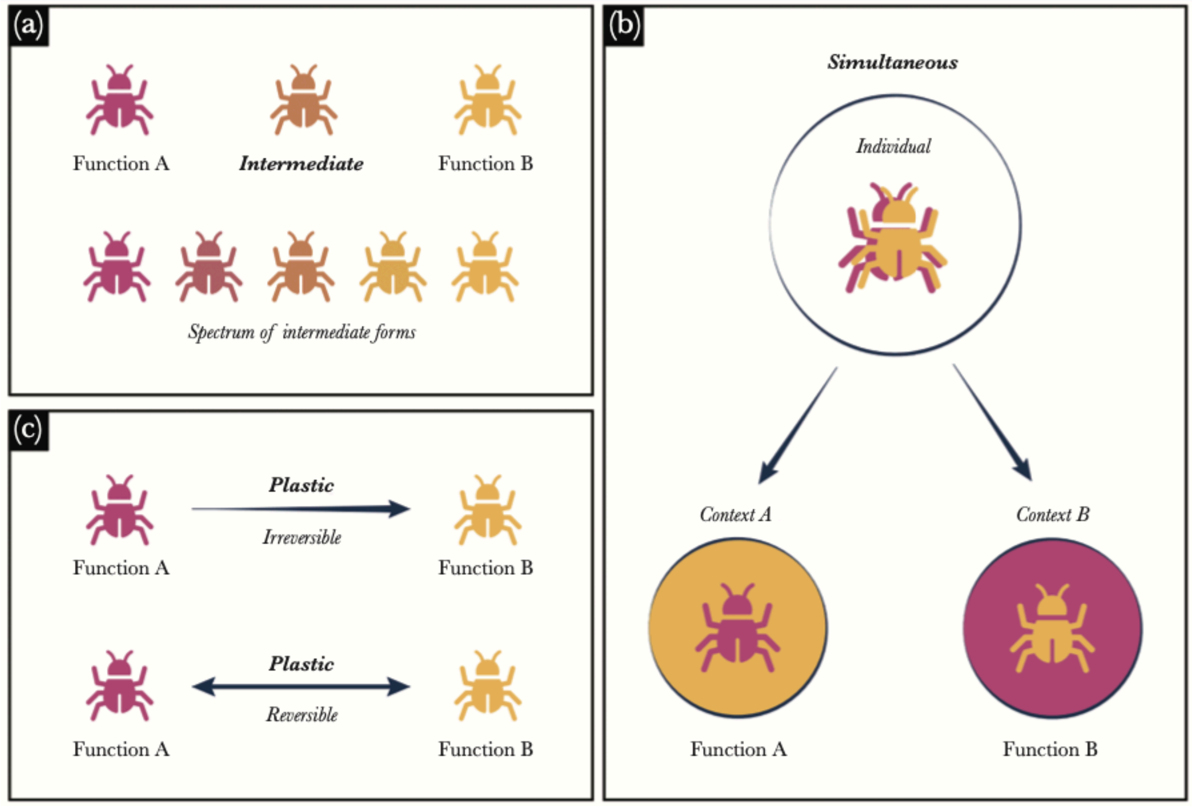


Fig. 1.1. Representations of how (A) intermediate, (B) simultaneous, and (C) plastic color strategies can serve multiple functions, using a hypothetical animal signaler with two possible functions of color. Note that for (B), the two layers represent two color patterns coexisting on the same individual, and that context refers to a combination of receiver and/or environmental characteristics that affect visual signals.

MULTIFUNCTIONAL COLOR STRATEGIES

Achieving multifunctionality in defensive coloration can involve either an immutable color pattern, or a color pattern that changes over time. We refer to various color change strategies as **plastic.** Among non-changing color defenses, we delineate two main types: **intermediate** and **simultaneous** strategies. These differ primarily the degree of expected polymorphism, as well as the exact role that transmission environment and receiver psychology play. Importantly, we observe a variety of defensive signals (from crypsis to aposematism) and possible pressures (signaler, receiver, or environmental) across all three strategies.

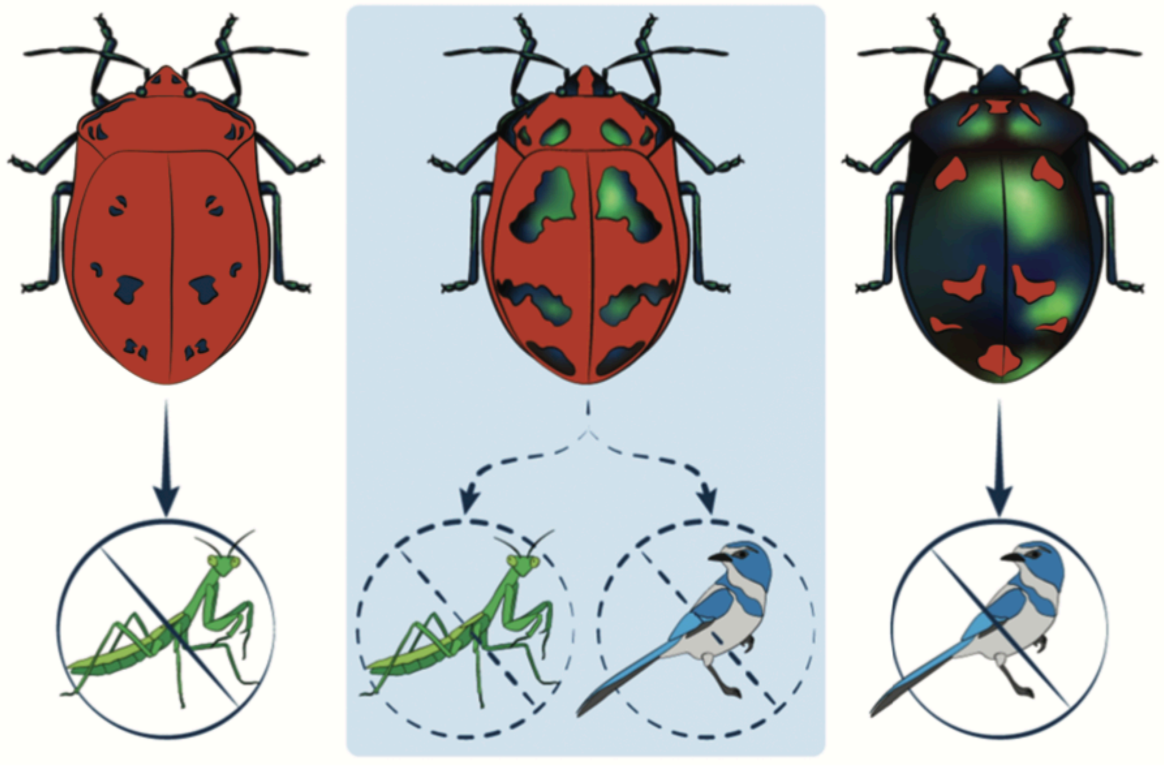


Fig. 1.2. Intermediate color strategies, as demonstrated by the hibiscus harlequin bug (Tectocoris diophthalmus), typically occur along polymorphic spectra. The matte orange morph (left) is cryptic to monochromatic insect predators. The iridescent green morph (right) deters tetrachromatic avian predators. The intermediate morph's combination of matte orange and iridescent green patches (middle) can defend against both predator types, though it is less effective against each.

(i) **Intermediate** color strategies satisfy conflicting pressures with a single coloration that represents some point between two or more optimal color strategies. This strategy has been well-studied in the context of how organisms effectively camouflage against heterogenous habitats, using the same term (Merilaita et al. 1999; Merilaita et al. 2001). Evidence from both modeled scenarios and live predation trials suggests that, in some cases, multiple background types can select for intermediate levels of crypsis – i.e., patterns that compromise between two background types, rather than optimally matching one or the other (Merilaita et al. 2001; Houston 2007). Selection for intermediate patterns may also depend on aspects of predator behavior, such as predator travel time between background types (Houston et al. 2007).

Intermediates also exist in non-cryptic systems, and often occur along population-wide spectra (Fig. 1.1; Table 1.1). This is true of *Tectocoris diophthalmus*, a colorful stinkbug with both avian and insect predators (Fabricant and Herberstein 2015). *T. diophthalmus* vary in color considerably, ranging from matte orange to iridescent green (Fabricant et al. 2013). While iridescence is an effective aposematic deterrent to avian predators (more so than solid orange), this coloration also makes *T. diophthalmus* more visible to insect predators that are undeterred by advertisements of toxicity (Fabricant et al., 2014; Fabricant and Herberstein, 2015). Mantises, monochromatic predators that use luminance contrast rather than color to hunt (Sontag 1971), are less likely to attack solid orange individuals relative to iridescent individuals. These conflicting selection pressures result in intermediate *T. diophthalmus* morphs with varying sizes of iridescent and orange patches (Fabricant and Herberstein 2015; Fig. 1.2). Geographic gradients in both temperature and rainfall likely play a role in shaping this species' coloration as well (Fabricant et al. 2018).

Both *T. diophthalmus* and other species (such as *D. ebraccatus* tadpoles) demonstrate that intermediates can exist along a wide spectrum of color morphs (Touchon and Warkentin 2008). In these scenarios, the full scale of color polymorphism across populations likely depends, to some degree, on the local proportions of different predator types (among other factors; Fabricant et al. 2018). For example, we would expect to see intermediates in areas where both predator types are relatively abundant and active (birds and mantids for *T. diophthalmus,* fish and aquatic insects for *D. ebbraccutus*; Fabricant and Herberstein 2015). This is true of male three-spined sticklebacks (*Gasterosteus aculeatus*) and Trinidadian guppies (*Poecilia reticulata*), whose sexual ornaments vary in conspicuousness depending on the local level of predation (Endler 1983; Dick et al. 2018; Gygax et al. 2018).

(ii) We consider seemingly singular color patterns that serve multiple functions, depending on combinations of receiver traits and/or aspects of habitat structure, **simultaneous.** While the propagation, reception, and effectiveness of *any* given color pattern is influenced by the nature of its environment and receivers (Guilford and Dawkins 1991; Endler 1993b; Caro et al. 2016; Patricelli and Hebets 2016), simultaneous color strategies describe a more specific phenomenon: when the key *function* of the visual signal changes as a result of variation in visual properties of the transmission environment and/or the characteristics of its receiver(s) (Fig. 1.1). For example, some reef fish possess blue and yellow patterns that are either cryptic or conspicuous depending on a combination of receiver distance and background color/texture. The regal angelfish (*Pygoplites diacanthus*) appears highly conspicuous, but in fact matches the spectral reflectance of average reef color with remarkable accuracy when the pattern's blue and yellow components blend at a distance. This species can thus signal to conspecifics at close distances or against open-water backgrounds, while remaining cryptic to predators from a distance or against variegated coral backgrounds (Cook et al. 2012).

Simultaneous color defenses are common in species that need to communicate with conspecifics without alerting visual predators, and often involve color patterns beyond the human-visible spectrum (Table 1.1). For example, the mottled exoskeleton of the mantis shrimp *Gonodactylaceus falcatus* blends in well with the sea floor, but its drab appearance hides a secret channel of communication: patterns of circularly polarized light (Gagnon et al. 2015; Fig. 1.3). Polarized light is not commonly visible to vertebrates, likely making these signals undetectable to most fish predators (Kamermans and Hawryshyn 2011). *G. falcatus* are able to discriminate circularly polarized light, however, and appear to use these patterns to covertly signal burrow ownership to conspecifics. Secret communication channels outside of the visual light spectrum have also been characterized in many lizards, *Heliconius* butterflies, and several species of damselfish (Losey 2003; Siebeck et al. 2010; Bybee et al. 2012; Marshall and Stevens 2014).

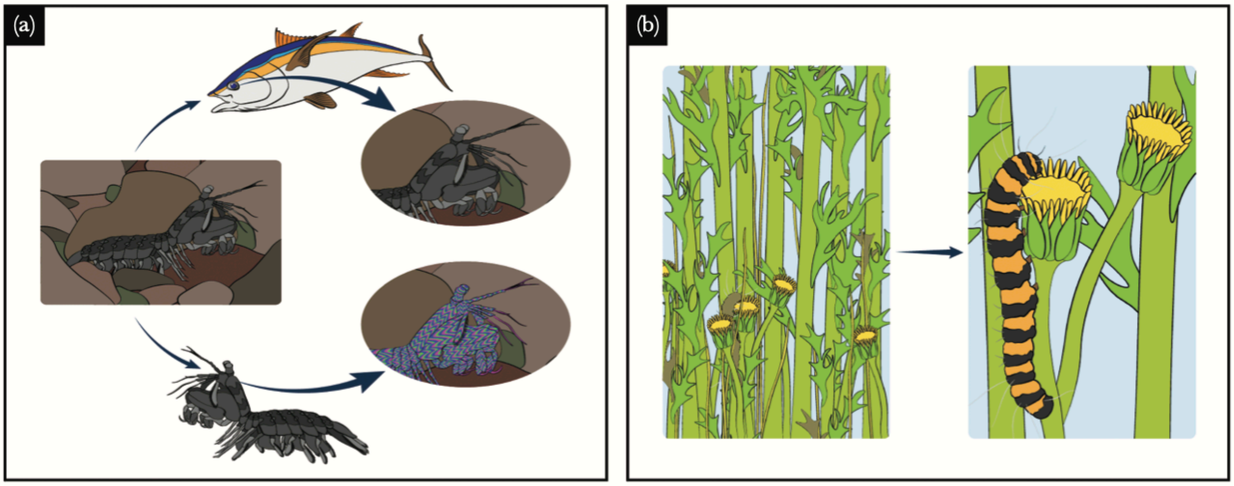


Fig. 1.3. (A) Gonodactylaceus falcatus exhibits simultaneous coloration via circularly polarized light patterns. When a predatory fish (top) views this species, G. falcatus appears well

camouflaged against its rocky habitat. Meanwhile, conspecifics (bottom) can perceive circularly polarized light, potentially allowing for covert communication (Gagnon et al. 2015). (B) Several striped caterpillar species, such as the cinnabar moth (Tyria jacobaeae), are simultaneously camouflaged at a distance (left) and aposematic up-close (right).

Color strategies can also be simultaneous solely as a function of predator position. Studies of both cinnabar moth (*Tyria jacobaeae*) and Old World swallowtail (*Papilio machaon*) caterpillars suggest that their "aposematic" striping appears cryptic from far away (Barnett et al. 2018; Tullberg et al. 2005; Fig. 1.3). The distance-dependent nature of this signal seems to satisfy a key tradeoff faced by aposematic animals: the need to have a strong enough warning signal to deter generalist predators, without increasing encounter rates with specialist predators (or naïve predators that have not learned to associate the signal with toxicity; Ruxton et al. 2009; Mappes et al. 2014). In contrast to intermediate strategies, we do not expect systems with simultaneous color patterns to commonly be polymorphic; these strategies rely on visual illusions, differences in receiver physiologies, and/or specific background-selecting behaviors to allow for the co-occurrence of remarkably different functions on a single individual.

(iii) Color defenses can be **plastic,** morphologically and/or behaviorally, over a wide range of timescales (Umbers et al. 2014; Duarte et al. 2017). This type of multifunctional color is unique in that the organism adopts different color strategies sequentially, rather than relying on a static color pattern that fulfills multiple needs simultaneously (Fig. 1.1, 1.4). Plasticity can refer solely to color change over time, e.g. seasonal polyphenism (Mills et al. 2013; Zimova et al. 2018) or ontogenetic shifts (Booth 1990), but often explicitly includes a behavioral component (particularly in colors that change quickly) (Hanlon et al. 2008; Zylinski et al. 2011; Umbers et al. 2015). Plasticity can occur over seasons, such as arctic hares that shift between summer and winter morphs (Mills et al. 2013); over the course of days, as in color-changing crab spiders (genus *Misumena*; Anderson and Dodson 2015); or within seconds, from deimatic mountain katydids (Umbers and Mappes 2015) to the impressively mutable mimic octopus (Hanlon 2007; Hanlon et al. 2010). Spatiotemporal variation in predation and habitat likely plays an especially important role in mediating the timescale over which antipredator color plasticity occurs (Caro et al. 2016). Experimental work with mole salamanders (*Ambystoma barbouri* and *A. texanum*) supports this hypothesis; each species exhibits a different degree of behavioral and/or physical background-matching depending on the level of predation risk, refuge availability, and UV stress (Garcia and Sih 2003).

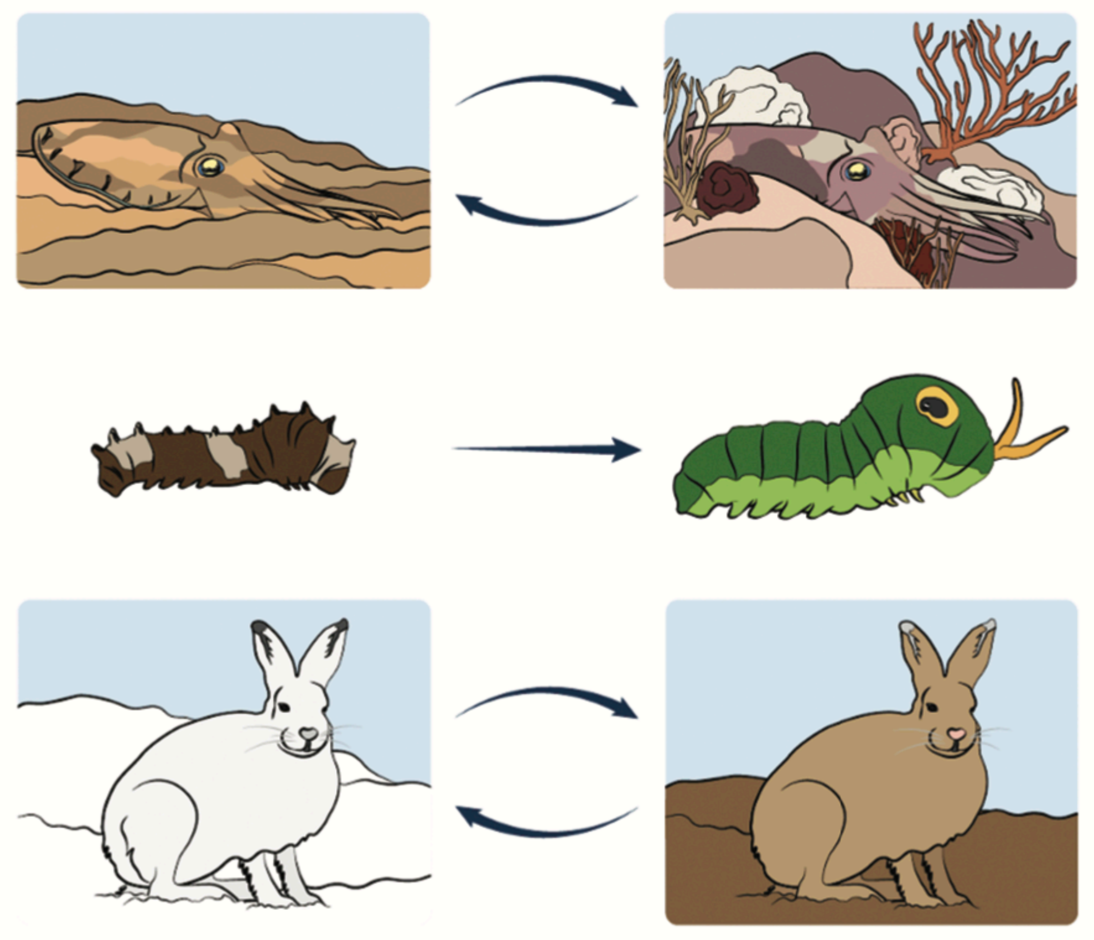


Fig. 1.4. Plastic color defenses occur in a variety of taxa, across a range of timescales, and operate via several different mechanisms. From top to bottom: the common cuttlefish (*Sepia officinalis*) can change both the color and texture of their skin within seconds, perfectly matching a variety of backgrounds; spicebush swallowtail larvae (*Papilio troilus*) move through a predictable sequence of color defense strategies over a course of weeks as they develop; the pelage of arctic hares (*Lepus arcticus*) changes with seasonal shifts in landscape color. Double arrows indicate reversible color change, whereas the single arrow represents a permanent switch.

Color plasticity appears to be an effective strategy for dealing with the challenges of multiple selection pressures, given its inherent flexibility; however, this flexibility may come at a cost (Caro et al. 2016). Time lags in plasticity can increase the likelihood of phenotypic mismatches, if the cue for color change no longer produces the "correct" color (such as in arctic species faced with unusually early snowmelts) (Mills et al. 2013). Other constraints may be energetic or phylogenetic in nature. The mimic octopus, for example, depends on specialized chromatophores for its split-second, reversible color change, and its ability to mimic the specific behaviors and morphologies of various marine life may rely on a high degree of cognitive processing (Hanlon et al. 2010). Regardless of taxa or mechanism, plastic color strategies are some of the clearest examples of how complex constraints on coloration can result in selection for multiple functions, as those functions become visually distinct in time (Stuart-Fox and Moussalli 2008).

FUTURE DIRECTIONS IN MULTIFUNCTIONAL COLOR

The diverse color strategies we see in nature are a fingerprint of the complex signaling pressures that animals must often balance. As mammals with specific visual sensitivities, we overlook these complexities at first glance (Jacobs, 2009). However, with both technological (Stoddard 2012; Stoddard and Osorio 2019) and conceptual (Hebets et al. 2016; Cuthill et al. 2017; Endler and Mappes 2017) advances in the study of animal color, the future of visual signalling research is rich with opportunities. In the sections below, we aim to guide this effort by discussing the following key questions: (i) What factors best explain variation in multifunctional color strategies? (ii) How do physiological processes and behavior shape defensive color? and (iii) What are the most effective ways to study multifunctionality in animal color?

What factors best explain variation in multifunctional color strategies?

To begin to understand variation in multifunctional color strategies, researchers could determine when, where, and how multifunctional color types (intermediate, simultaneous, and plastic) most commonly appear in nature. Based on the subset of examples included in Table 1.1, we can suggest some potential patterns of interest. For example, balancing social/sexual communication needs with predator defense seems to disproportionately select for simultaneous strategies; both ontogenetic changes and environmental variation are often associated with plastic strategies; and the pressures that correlate with intermediate strategies appear more varied, with a slight bias toward multiple antagonists (Table 1.1). Of course, the strength of these predictions is limited by the small scope of these examples relative to the large volume of studies on animal color. As such, these patterns should serve only as starting points for further study. A well-designed meta-analytical approach would be more appropriate to determine the nature and effect sizes of these patterns (Stewart 2010).

Specifically, we ask: are certain combinations of selection pressures more likely to produce specific types of defensive color (crypsis, aposematism, etc.) and/or specific multifunctional strategies (intermediate, simultaneous, plastic)? We urge future researchers to explore this question by analyzing a set of defensive coloration experiments using clear, consistent classifications of relevant pressures, defensive color types, and multifunctional strategies. Additional covariates of interest might be geographic location, focal species taxonomic information, predator and other receiver taxonomic information, and specific color(s) and/or pigments involved (if known).

Perhaps as a subsection of the analysis proposed above, researchers could quantify which types of defensive color patterns are more or less represented in animal color research. To this end, determining an estimate of how frequently different types of defensive color appear in the literature would be a reasonable goal. While this aim is beyond the scope of the current review, a notable pattern again arises from Table 1.1. Out of the examples included in this review, the majority focus on either crypsis or aposematism; only 6 out of the 42 systems represented (14%) involve non-cryptic or non-aposematic defenses. Multiple selection pressures have been reviewed fairly explicitly in the context of both crypsis (Merilaita et al. 1999; Stevens and Ruxton 2018) and aposematism (Speed and Ruxton 2007; Ruxton et al. 2009; Rojas et al. 2018). Seemingly less is known on this topic for species that employ mimicry, masquerade, deflection, motion dazzle, and other defensive color patterns (but see Sweeney et al. 2003; Bybee et al. 2011; Fisher and Stankowich 2018; Whiting et al. 2018; Postema 2021). Presumably these defenses are equally subject to the challenges of integrating multiple functions, and are certainly worthy of study.

How do physiological processes and behavior shape defensive color?

Major breakthroughs in the study of animal coloration often involve considerable crosstalk between physiologists, behaviorists, and ecologists. Our current understanding of the importance of receiver psychology, for example, was propelled by the work of neurologists, visual physiologists, and biophysical modelers in tandem with experts in behavioral ecology (Rowe 1999; Endler and Mappes 2004; Stevens and Merilaita 2009; Rowe 2013). As discussed in previous sections, our knowledge of how the use of pigments in physiological processes (e.g. immune functioning, integument strengthening) interacts with the use of pigments in external patterning remains limited – though some hypotheses from sexual signalling contexts may be helpful in elucidating these interactions (Hill 2011). We recommend that future researchers aim to link the fine-scale metabolic processes involved in the acquisition, production, and allocation of pigments with the expression and success of defensive color patterns.

We also encourage researchers to include relevant behaviors in experimental tests of color multifunctionality whenever possible. The efficacy of a given defensive color pattern usually hinges on specific behaviors (Stevens and Ruxton 2018); examples include microhabitat selection, adjusting orientation, matching wind- or current-induced motion in vegetation, self-decorating with environmental debris, behavioral mimicry in mimetic species, and suddenly revealing bright patterns or body parts when threatened (Stachowicz and Hay 2000; Hanlon 2007; Kitamura and Imafuku 2010**;** Bian et al. 2016; Wang et al. 2017). Furthermore, behavioral flexibility often allows organisms to successfully integrate multiple selection pressures (Garcia and Sih 2003; Umbers and Mappes 2015). Despite much observational support for the role of behavior in defensive coloration, experimental work that includes behavior (and particularly *motion*) is less common (but see Hämäläinen et al. 2015). In systems where appropriate behaviors are difficult to include in experimental settings, video playbacks and/or robotics may prove useful as alternatives to live animals (Pruden and Uetz 2004; Romano et al. 2019).

What are the most effective ways to study multifunctionality in animal color?

One goal of this review is to recognize that the factors that shape animal color patterns are often complex, variable, difficult to detect, and/or unintuitive. Given these inherent challenges, how can researchers of animal color best proceed? While there is no universal answer to this question and the study of coloration will necessitate a variety of methods, one basic approach we propose is to start small. Experiments that aim to address all relevant pressures at once will likely be infeasible, if not impossible. A more successful approach seems to be the accumulation of smaller studies in a single system, dealing with only two or three selection pressures at a time. This strategy has been well-implemented in the wood tiger moth system (*P. plantaginis)*, and has gradually revealed the intricate network of selection pressures that shapes this species' color patterns as larvae and as adults **(**Lindstedt et al. 2009; Hegna et al. 2013; Nokelainen et al. 2013; Henze et al. 2018)**.**

On a more basic methodological level, we reiterate what other reviews have emphasized: that studies of animal visual signals benefit from the careful documentation and testing of relevant pressures, as opposed to assuming functions based solely on observation (Hegna et al. 2013; Nokelainen et al. 2013; Henze et al. 2018). Whenever possible, spectral data should be acquired and modeled from the perspectives of relevant predatory and/or conspecific receivers, as this can reveal hidden axes of multifunctionality (Nokelainen et al. 2022). Specific functions of a color strategy should be either empirically supported or noted as an assumed (but untested) function, as effectively demonstrated by Guerra-Grenier (2019) in a review of insect egg coloration. When considering the potential for aposematism in each species' eggs, the author lists the exact state of knowledge (hypothesized, hypothesized and indirectly supported, or hypothesized and directly supported) with supporting references. This degree of transparency – in terms of assumed versus empirically supported functions – should be standard in future studies of animal coloration.

CONCLUSION

Multiple interacting selection pressures can produce and maintain diversity in defensive color signals, both within (Hegna et al. 2013; Henze et al. 2018) and across species (Allen et al. 2013; Gaitonde et al. 2018). Conflicting constraints on color are common (Table 1.1) and often necessitate multiple functions - whether those functions are expressed in an intermediate combination (Fig. 1.2), become apparent across different conditions (Fig. 1.3), or change sequentially over time (Fig. 1.4). By reframing defensive visual signals as intermediate, simultaneous, or plastic *strategies,* we encourage the explicit consideration of how color is shaped by complex suites of selection pressures. Research that seeks only to "identify single key functions of external appearances" may paint an incomplete picture of how defensive color patterns evolve in nature, and in some cases may fail to explain diversity and/or apparent suboptimality in these signals (Cuthill et al. 2017). For example, the existence of "weak" aposematic signals can be understood in light of variation in predator physiologies, experience levels, and/or hunting methods (Endler and Mappes 2004; Mappes et al. 2014).

From protection against natural enemies (Wilson et al. 2001; Caro and Allen 2017) and environmental factors (Garcia and Sih 2003; Clusella Trullas et al. 2007) to communication with sexual and social partners (Hebets et al. 2016), color plays several key roles in natural systems. Given this multiplicity of function, compounded with signaler, receiver, and environmental constraints on signalling that may shift over space and time, we expect diversity in defensive coloration as a rule rather than an exception.

REFERENCES

Abbott S, Fairbanks DJ. 2016. Experiments on plant hybrids by Gregor Mendel. Genetics. 204(2):407–422. doi:10.1534/genetics.116.195198.

Akkaynak D, Siemann LA, Barbosa A, Mäthger LM. 2017. Changeable camouflage: how well can flounder resemble the colour and spatial scale of substrates in their natural habitats? R Soc Open Sci. 4:160824.

Allen WL, Baddeley R, Scott-Samuel NE, Cuthill IC. 2013. The evolution and function of pattern diversity in snakes. Behav Ecol. 24(5):1237–1250. doi:10.1093/beheco/art058.

Anderson SO. 2010. Insect cuticular sclerotization: a review. Insect Biochem Mol Biol. 40:166-178.

Anderson AG, Dodson GN. 2015. Colour change ability and its effect on prey capture success in female *Misumenoides formosipes* crab spiders: Colour change and foraging in a crab spider. Ecol Ent. 40(2):106–113. doi:10.1111/een.12167.

Barnett JB, Cuthill IC, Scott-Samuel NE. 2018. Distance-dependent aposematism and camouflage in the cinnabar moth caterpillar (*Tyria jacobaeae*, Erebidae). R Soc Open Sci. 5(2). doi:10.1098/rsos.171396.

Barnett JB, Redfern AS, Bhattacharyya-Dickson R, Clifton O, Courty T, Ho T, Hopes A, McPhee T, Merrison K, Owen R, et al. 2017. Stripes for warning and stripes for hiding: spatial frequency and detection distance. Behav Ecol. 28(2):373–381. doi:10.1093/beheco/arw168.

Bian X, Elgar MA, Peters RA. 2016. The swaying behavior of *Extatosoma tiaratum:* motion camouflage in a stick insect? Behav Ecol. 27:83-92.

Blount JD, Metcalfe NB, Birkhead TR, Surai PF. 2003. Carotenoid modulation of immune function and sexual attractiveness in Zebra Finches. Science. 300(5616):125–127. doi:10.1126/science.1082142.

Bonser RHC, 1995. Melanin and the abrasion resistance of feathers. Condor. 97:590-591.

Booth CL. 1990. Evolutionary significance of ontogenetic colour change in animals. Biol Jour Lin Soc. 40(2):125–163. doi:10.1111/j.1095-8312.1990.tb01973.x.

Brenner M, Hearing VJ. 2008. The protective role of melanin against UV damage in human skin. Photochem Photobiol. 84(3):539–549. doi:10.1111/j.1751-1097.2007.00226.x.

Brown C, Garwood MP., Williamson Jane E. 2012. It pays to cheat: tactical deception in a cephalopod social signalling system. Biol Lett. 8(5):729–732. doi:10.1098/rsbl.2012.0435.

Bybee SM, Yuan F, Ramstetter MD, Llorente-Bousquets J, Reed RD, Osorio D, Briscoe AD. 2012. UV photoreceptors and UV-yellow wing pigments in Heliconius butterflies allow a color signal to serve both mimicry and intraspecific communication. Am Nat. 179(1):38–51. doi:10.1086/663192.20160344

Camacho C, Sanabria-Fernández A, Baños-Villalba A, Edelaar P. 2020. Experimental evidence that matching habitat choice drives local adaptation in a wild population. Proc R Soc B: Biol Sci. 287:20200721.http://dx.doi.org/10.1098/rspb.2020.0721

Caro T, Stankowich T, Kiffner C, Hunter J. 2013. Are spotted skunks conspicuous or cryptic? Ethol Ecol Evol. 25(2):144–160. doi:10.1080/03949370.2012.744359.

Caro T, Sherratt TN, Stevens M. 2016. The ecology of multiple colour defences. Evol Ecol. 30(5):797–809. doi:10.1007/s10682-016-9854-3.

Caro T, Allen WL. 2017. Interspecific visual signalling in animals and plants: a functional classification. Philos Trans R Soc Lond B Biol Sci. 372(1724).

doi:10.1098/rstb.2016.0344.

Caro T, Stoddard MC, Stuart-Fox D. 2017. Animal coloration: production, perception, function and application. Phil Trans R Soc B: Biol Sci. 372(1724):20170047. doi:10.1098/rstb.2017.0047.

Caro T, Argueta Y, Briolat ES, Bruggink J, Kasprowsky M, Lake J, Mitchell MJ, Richardson S, How M. 2019. Benefits of zebra stripes: Behaviour of tabanid flies around zebras and horses. PLOS ONE. 14(2):e0210831.doi:10.1371/journal.pone.0210831.

Cheney KL. 2010. Multiple selective pressures apply to a coral reef fish mimic: a case of Batesian–aggressive mimicry. Proc R Soc B: Biol Sci. 277(1689):1849–1855. doi:10.1098/rspb.2009.2218.

Clusella Trullas S, van Wyk JH, Spotila JR. 2007. Thermal melanism in ectotherms. Journal of Therm Biol. 32(5):235–245. doi:10.1016/j.jtherbio.2007.01.013.

Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G, Hauber ME, Hill GE, Jablonski NG, Jiggins CD, Kelber A, et al. 2017. The biology of color. Science. 357(6350):eaan0221. doi:10.1126/science.aan0221.

Dalziell AH, Welbergen JA. 2016. Mimicry for all modalities. Ecol Lett. 19(6):609–619. doi:10.1111/ele.12602.

Delhey K. 2019. A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. Bio Rev. 94:1294-1316.

Dick C, Arendt J, Reznick DN, Hayashi CY. 2018. The developmental and genetic trajectory of coloration in the guppy (*Poecilia reticulata*). Evol Dev. 20(6):207–218. doi:10.1111/ede.12268.

Duarte RC, Flores AAV, Stevens M. 2017. Camouflage through colour change: mechanisms, adaptive value and ecological significance. Phil Trans R

Soc B: Biol Sci. 372(1724):20160342. doi:10.1098/rstb.2016.0342.

Endler JA. 1983. Natural and sexual selection on color patterns in poeciliid fishes. Environ Biol Fish. 9(2):173–190. doi:10.1007/BF00690861.

Endler JA. 1993a. Some general comments on the evolution and design of animal communication systems. Phil Trans R Soc B. 340:215-225.

Endler JA. 1993b. The color of light in forests and its implications. Ecol Mono. 63(1):2–27. doi:10.2307/2937121.

Endler JA, Mappes J. 2004. Predator mixes and the conspicuousness of aposematic signals. Am Nat. 163(4):532–547. doi:10.1086/382662.

Endler JA, Mappes J. 2017. The current and future state of animal coloration research. Phil Tran R Soc B: Biol Sci. 372(1724):20160352. doi:10.1098/rstb.2016.0352.

Fabricant SA, Kemp DJ, Krajíček J, Bosáková Z, Herberstein ME. 2013. Mechanisms of color production in a highly variable shield-back stinkbug, *Tectocoris diopthalmus* (Heteroptera: Scutelleridae), and why it matters. PLOS ONE. 8(5):e64082. doi:10.1371/journal.pone.0064082.

Fabricant SA, Exnerová A, Ježová D, Štys P. 2014. Scared by shiny? The value of iridescence in aposematic signalling of the hibiscus harlequin bug. An Behav. 90:315–325. doi:10.1016/j.anbehav.2014.01.021.

Fabricant SA, Smith CL. 2014. Is the hibiscus harlequin bug aposematic? The importance of testing multiple predators. Ecol Evol. 4(2):113–120. doi:10.1002/ece3.914.

Fabricant SA, Herberstein ME. 2015. Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. Behav Ecol. 26(1):38–44. doi:10.1093/beheco/aru157.

Fabricant SA, Burdfield-Steel E, Umbers KL, Lowe L. 2018. Warning signal plasticity in hibiscus harlequin bugs. Evol Ecol 32: 10.1007/s10682-018-9946-3.

Fisher KA, Stankowich T. 2018. Antipredator strategies of striped skunks in response to cues of aerial and terrestrial predators. An Behav. 143:25–34. doi:10.1016/j.anbehav.2018.06.023.

Fitze PS, Tschirren B, Gasparini J, Richner H. 2007. Carotenoid-based plumage colors and immune function: is there a trade-off for rare carotenoids? Am Nat. 169(S1):S137–S144. doi:10.1086/510094.

Gage J. 1993. Color and culture: practice and meaning from antiquity to abstraction. 1st North American ed. Boston: Little, Brown and Company.

Gage J. 1999. Color and meaning: art, science, and symbolism. Berkeley ; Los Angeles: University of California Press.

Gagnon YL, Templin RM, How MJ, Marshall NJ. 2015. Circularly polarized light as a communication signal in mantis shrimps. Curr Biol. 25(23):3074–3078. doi:10.1016/j.cub.2015.10.047.

Gaitonde N, Joshi J, Kunte K. 2018. Evolution of ontogenic change in color defenses of swallowtail butterflies. Ecol Evol. 8(19):9751–9763. doi:10.1002/ece3.4426.

Garcia TS, Sih A. 2003. Color change and color-dependent behavior in response to predation risk in the salamander sister species *Ambystoma barbouri* and *Ambystoma texanum*. Oecologia. 137(1):131–139. doi:10.1007/s00442-003-1314-4.

Gluckman T-L. 2014. Pathways to elaboration of sexual dimorphism in bird plumage patterns. Biol J Linn Soc. 111(2):262–273. doi:10.1111/bij.12211.

Gordon SP, Kokko H, Rojas B, Nokelainen O, Mappes J. 2015. Colour polymorphism torn apart by opposing positive frequency-dependent selection, yet maintained in space. Journal of An Ecol. 84(6):1555–1564. doi:10.1111/1365-2656.12416.

Guilford T, Dawkins MS. 1991. Receiver psychology and the evolution of animal signals. An Behav. 42(1):1–14. doi:10.1016/S0003-3472(05)80600-1.

Gygax M, Rentsch AK, Rudman SM, Rennison DJ. 2018. Differential predation alters pigmentation in threespine stickleback (*Gasterosteus aculeatus*). J Evol Biol. 31(10):1589–1598. doi:10.1111/jeb.13354.

Hämäläinen L, Valkonen J, Mappes J, Rojas B. 2015. Visual illusions in predator-prey interactions: birds find moving patterned prey harder to catch. Anim Cogn. 18:1059-1068.

Hanlon R. 2007. Cephalopod dynamic camouflage. Curr Biol. 17(11):R400–R404. doi:10.1016/j.cub.2007.03.034.

Hanlon RT, Conroy L-A, Forsythe JW. 2008. Mimicry and foraging behaviour of two tropical sand-flat octopus species off North Sulawesi, Indonesia. Biol Jour Linn Soc. 93(1):23–38. doi:10.1111/j.1095-8312.2007.00948.x.

Hanlon RT, Watson AC, Barbosa A. 2010. A ‘mimic octopus’ in the Atlantic: flatfish mimicry and camouflage by *Macrotritopus defilippi*. Biol Bull. 218(1):15–24. doi:10.1086/BBLv218n1p15.

Hebets EA, Papaj DR. 2005. Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol. 57(3):197–214. doi:10.1007/s00265-004-0865-7.

Hebets EA, Barron AB, Balakrishnan CN, Hauber ME, Mason PH, Hoke KL. 2016. A systems approach to animal communication. Proc R Soc B: Biol Sci. 283(1826):20152889. doi:10.1098/rspb.2015.2889.

Hegna RH, Nokelainen O, Hegna JR, Mappes J. 2013. To quiver or to shiver: increased melanization benefits thermoregulation, but reduces warning signal efficacy in the wood tiger moth. Proc R Soc B: Biol Sci. 280(1755):20122812. doi:10.1098/rspb.2012.2812.

Henze MJ, Lind O, Mappes J, Rojas B, Kelber A. 2018. An aposematic colour-polymorphic moth seen through the eyes of conspecifics and predators - sensitivity and colour discrimination in a tiger moth. Funct Ecol. 32(7):1797–1809. doi:10.1111/1365-2435.13100.

Hill GE. 2011. Condition-dependent traits as signals of the functionality of vital cellular processes. Ecol Lett. 14:625-634.

Honkavaara J, Koivula M, Korpimaki E, Siitari H, Viitala J. 2002. Ultraviolet vision and foraging in terrestrial vertebrates. Oikos. 98(3):505–511. doi:10.1034/j.1600-0706.2002.980315.x.

Hossie TJ, Skelhorn J, Breinholt JW, Kawahara AY, Sherratt TN. 2015. Body size affects the evolution of eyespots in caterpillars. PNAS. 112:6664-6669.201415121. doi:10.1073/pnas.1415121112.

Hultgren K, Stachowicz J. 2011. Camouflage in decorator crabs: Integrating ecological, behavioural and evolutionary approaches. Pages 214-229 in M. Stevens and S. Merlaita (eds). *Animal Camouflage*. Cambridge: Cambridge University Press.

Isaksson C. 2009. The chemical pathway of carotenoids: from plants to birds. Ardea 97:125-128.

Jamie GA. 2017. Signals, cues and the nature of mimicry. Proc Biol Sci. 284(1849):20162080. doi:10.1098/rspb.2016.2080.

Goethe, JWv. 1840. Goethe's theory of colours. London: John Murray, Albemarle Street.

Kamermans M, Hawryshyn C. 2011. Teleost polarization vision: how it might work and what it might be good for. Philo Trans R Soc B: Biol Sci. 366(1565):742–756. doi:10.1098/rstb.2010.0211.

Kang C-K, Moon J-Y, Lee S-I, Jablonski PG. 2012. Camouflage through an active choice of a resting spot and body orientation in moths. Jour Evol Biol. 25(9):1695–1702. doi:10.1111/j.1420-9101.2012.02557.x.

Kang C-K, Kim YE, Jang Y. 2016. Colour and pattern change against visually heterogeneous backgrounds in the tree frog *Hyla japonica*. Sci Rep. 6(1):22601. doi:10.1038/srep22601.

Kelber A, Roth LSV. 2006. Nocturnal colour vision – not as rare as we might think. J Exp Biol. 209(5):781–788. doi:10.1242/jeb.02060.

Kettlewell HBD. 1955. Selection experiments on industrial melanism in the Lepidoptera. Heredity. 9(3):323–342. doi:10.1038/hdy.1955.36.

Kitamura T, Imafuku M. 2010. Behavioral Batesian mimicry involving intraspecific polymorphism in the butterfly *Papilio polytes.* Zool Sci. 27:217-221.

Kjernsmo K, Whitney HM, Scott-Samuel NE, Hall JR, Knowles H, Talas L, Cuthill IC. 2020. Iridescence as camouflage. Curr Biol. 30(3):551-555.e3. doi:10.1016/j.cub.2019.12.013.

Kraemer AC, Philip CW, Rankin AM, Parent CE. 2019. Trade-offs direct the evolution of coloration in Galapagos land snails. Proc R Soc B-Biol Sci. 286(1894):20182278. doi:10.1098/rspb.2018.2278.

Lindstedt C, Lindström L, Mappes J. 2009. Thermoregulation constrains effective warning signal expression. Evolution. 63(2):469–478. doi:10.1111/j.1558-5646.2008.00561.x.

Losey G. 2003. Crypsis and communication functions of UV-visible coloration in two coral reef damselfish, *Dascyllus aruanus* and *D. reticulatus*. An Behav. 66:299–307. doi:10.1006/anbe.2003.2214.

Mappes J, Kokko H, Ojala K, Lindström L. 2014. Seasonal changes in predator community switch the direction of selection for prey defences. Nat Comm. 5(1):5016. doi:10.1038/ncomms6016.

Marcondes RS, Nations JA, Seeholzer GF, Brumfield RT. 2021. Gloger's rule: climate, light environments, and color in a large family of tropical birds (Furnariidae). Am Nat. 197: https://doi.org/10.1086/713386

Marshall NJ. 2000. Communication and camouflage with the same 'bright' colours in reef fishes. Philo Trans R Soc of Lond Ser B: Biol Sci. 355(1401):1243–1248. doi:10.1098/rstb.2000.0676.

Marshall KLA, Stevens M. 2014. Wall lizards display conspicuous signals to conspecifics and reduce detection by avian predators. Behav Ecol. 25(6):1325–1337. doi:10.1093/beheco/aru126.

Maund B. 2019. Color. In: Zalta EN, editor. The Stanford encyclopedia of philosophy. Spring 2019. Metaphys Res Lab, Stanford Uni.

McGraw KJ, Ardia DR. 2003. Carotenoids, immunocompetence, and the information content of sexual colors: An experimental test. Am Nat. 162(6):704–712. doi:10.1086/378904.

Merilaita S, Tuomi J, Jormalainen V, 1999. Optimization of cryptic coloration in heterogeneous habitats. Biol J Linn Soc. 67:151-161.

Merilaita S, Lyytinen A, Mappes J, 2001. Selection for cryptic coloration in a visually heterogeneous habitat. Proc R Soc Lond B. 268:1925-1929.

Merilaita S. 2003. Visual background complexity facilitates the evolution of camouflage. Evolution 57:1248-1254.

Merilaita S, Lind J. 2005. Background-matching and disruptive coloration, and the evolution of cryptic coloration. Proc R Soc B. 272(1563):665–670. doi:10.1098/rspb.2004.3000.

Merilaita S, Scott-Samuel NE, Cuthill IC. How camouflage works. 2017. Philo Trans R Soc B: Biol Sci. 372(1724):20160341. http://dx.doi.org/10.1098/rstb.2016.0341

Michalis C, Scott-Samuel NE, Gibson DP, Cuthill IC. 2017. Optimal background matching camouflage. Proc R Soc B. 284(1858):20170709. doi:10.1098/rspb.2017.0709.

Mills LS, Zimova M, Oyler J, Running S, Abatzoglou JT, Lukacs PM. 2013. Camouflage mismatch in seasonal coat color due to decreased snow duration. PNAS. 110(18):7360–7365. doi:10.1073/pnas.1222724110.

Mikkola K, Rantala MJ. 2010. Immune defence, a possible nonvisual selective factor behind the industrial melanism of moths (Lepidoptera). Biol J Linn Soc. 99:831-838.

Montgomerie R, Lyon B, Holder K. 2001. Dirty ptarmigan: behavioral modification of conspicuous male plumage. Behav Ecol. 12:429-438.

Morehead SA, Feener DH. 2000. Visual and chemical cues used in host location and acceptance by a dipteran parasitoid. Jour In Behav. 13(4):613–625. doi:10.1023/A:1007875921705.

Nicholson KE, Harmon LJ, Losos JB. 2007. Evolution of *Anolis* lizard dewlap diversity. PLoS ONE. 2(3):e274. doi:10.1371/journal.pone.0000274.

Nokelainen O, Lindstedt C, Mappes J. 2013. Environment-mediated morph-linked immune and life-history responses in the aposematic wood tiger moth. J An Ecol. 82(3):653–662. doi:10.1111/1365-2656.12037.

Nokelainen O, Hubbard N, Lown AE, Wood LE, Stevens M. 2017. Through predators' eyes: phenotype-environment associations in shore crab coloration at different spatial scales. Biol J Linn Soc. 122(4):738–751.

Nokelainen O, Galarza JA, Kirvesoja J, Suisto K, Mappes J. 2022. Genetic colour variation visible for predators and conspecifics is concealed from humans in a polymorphic moth. J Evol Biol. 35:467-478.

Pascual J, Senar JC, Domènech J. 2014. Plumage brightness, vigilance, escape potential, and predation risk in male and female Eurasian Siskins (*Spinus spinus*). Auk. 131(1):61–72. doi:10.1642/AUK-13-220.1.

Patricelli GL, Hebets EA. 2016. New dimensions in animal communication: the case for complexity. Curr Op Behav Sci. 12:80–89. doi:10.1016/j.cobeha.2016.09.011.

Penacchio O, Ruxton GD, Lovell PG, Cuthill IC, Harris JM, 2015. Orientation to the sun by animals and its interaction with crypsis. Funct Ecol. 29:1165–1177.

Postema EG. 2021. The effectiveness of eyespots and masquerade in protecting artificial prey across ontogenetic and seasonal shifts. Curr Zool.(zoab082). doi:10.1093/cz/zoab082.

Pruden AK, Uetz GW. 2004. Assessment of potential predation costs of male decoration and courtship display in wolf spiders using video digitization playback. J Insect Behav. 17:67-80.

Rajabizadeh M, Adriaens D, Kaboli M, Sarafraz J, Ahmadi M. 2015. Dorsal colour pattern variation in Eurasian mountain vipers (genus *Montivipera*): A trade-off between thermoregulation and crypsis. Zool Anz - Comp Zool. 257:1–9. doi:10.1016/j.jcz.2015.03.006.

Ratcliffe JM, Nydam ML. 2008. Multimodal warning signals for a multiple predator world. Nature. 455(7209):96–99. doi:10.1038/nature07087.

Robertson JM, Duryea MC, Zamudio KR. 2009. Discordant patterns of evolutionary differentiation in two Neotropical treefrogs. Molec Ecol. 18(7):1375–1395. doi:10.1111/j.1365-294X.2009.04126.x.

Robledo-Ospina LE, Escobar-Sarria F, Troscianko J, Rao D. 2017. Two ways to hide: predator and prey perspectives of disruptive coloration and background matching in jumping spiders. Biol J Linn Soc. 122(4):752–764.

Rojas B, Burdfield-Steel E, De Pasqual C, Gordon S, Hernández L, Mappes J, Nokelainen O, Rönkä K, Lindstedt C. 2018. Multimodal aposematic signals and their emerging role in mate attraction. Front Ecol Evol. 6:93. doi:10.3389/fevo.2018.00093.

Romano D, Donati E, Benelli G, Stefanini C. 2019. A review on animal-robot interaction: from bio-hybrid organisms to mixed societies. Biol Cybernetics. 113:201-225.

Rowe C. 1999. Receiver psychology and the evolution of multicomponent signals. An Behav. 58(5):921–931. doi:10.1006/anbe.1999.1242.

Rowe C, Guilford T. 1999. The evolution of multimodal warning displays. Evol Ecol. 13(7):655–671. doi:10.1023/A:1011021630244.

Rowe C. 2013. Receiver psychology: a receiver's perspective. An Behav. 85(3):517–523. doi:10.1016/j.anbehav.2013.01.004.

Rowe C, Halpin C. 2013. Why are warning displays multimodal? Behav Ecol Sociobiol. 67(9):1425–1439. doi:10.1007/s00265-013-1515-8.

Ruxton GD, Speed MP, Broom M. 2009. Identifying the ecological conditions that select for intermediate levels of aposematic signalling. Evol Ecol. 23(4):491–501. doi:10.1007/s10682-008-9247-3.

Shapiro AM. 1976. Seasonal polyphenism. In: Hecht MK, Steere WC, Wallace B, editors. Evol Biol. Boston, MA: Springer US. p. 259–333.

Siebeck UE, Parker AN, Sprenger D, Mäthger LM, Wallis G. 2010. A species of reef fish that uses ultraviolet patterns for covert face recognition. Curr Biol. 20(5):407–410. doi:10.1016/j.cub.2009.12.047.

Skelhorn J, Rowland HM, Speed MP, De Wert L, Quinn L, Delf J, Ruxton GD. 2010. Size-dependent misclassification of masquerading prey. Behav Ecol. 21(6):1344–1348. doi:10.1093/beheco/arq159.

Skelhorn J, Holmes GG, Hossie TJ, Sherratt TN. 2016. Multicomponent deceptive signals reduce the speed at which predators learn that prey are profitable. Behav Ecol. 27(1):141–147. doi:10.1093/beheco/arv135.

Smilanich AM, Dyer LA, Gentry GL. 2009. The insect immune response and other putative defenses as effective predictors of parasitism. Ecology. 90(6):1434–1440. doi:10.1890/08-1906.1.

Smith KE, Halpin CG, Rowe C. 2014. Body size matters for aposematic prey during predator aversion learning. Behav Proc. 109:173–179. doi:10.1016/j.beproc.2014.09.026.

Song S, Kim S, Kwon SW, Lee S-I, Jablonksi PG. 2018. Defense sequestration associated with narrowing of diet and ontogenetic change to aposematic colours in the spotted lanternfly. Sci Reports. 8:16831.

Sontag C. 1971. Spectral sensitivity studies on the visual system of the praying mantis, *Tenodera sinensis*. J Gen Physiol. 57(1):93–112.

Speed MP, Ruxton GD. 2007. How bright and how nasty: explaining diversity in warning signal strength. Evolution. 61(3):623–635. doi:10.1111/j.1558-5646.2007.00054.x.

Stachowicz J, Hay ME. 2000. Geographic variation in camouflage specialization by a decorator crab. Am Nat. 156:59-71.

Stevens M, Merilaita S. 2009. Animal camouflage: current issues and new perspectives. Philo Trans Soc B. 364(1516):423–427. doi:10.1098/rstb.2008.0217.

Stevens M, Ruxton GD. 2018. The key role of behaviour in animal camouflage. Biol Rev. 0(0). doi:10.1111/brv.12438.

Stireman JO. 2002. Host location and selection cues in a generalist tachinid parasitoid. Ento Exp App. 103(1):23–34. doi:10.1046/j.1570-7458.2002.00958.x.

Stoddard MC. 2012. Mimicry and masquerade from the avian visual perspective. Curr Zool. 58(4):630–648. doi:10.1093/czoolo/58.4.630.

Stoddard MC, Osorio D. 2019. Animal coloration patterns: linking spatial vision to quantitative analysis. Am Nat. 193(2):164–186. doi:10.1086/701300.

Stuart-Fox D, Moussalli A. 2008. Camouflage, communication and thermoregulation; lessons from colour changing organisms. Phil Trans R Soc B: Biol Sci. 364:463-470.

Sugumaran M, Barek H. 2016. Critical analysis of the melanogenic pathway in insects and higher animals. Int J Mol Sci. 17(10):1753. doi:10.3390/ijms17101753.

Talloen W, Van Dyck H, Lens L. 2004. The cost of melanization: butterfly wing coloration under environmental stress. Evolution. 58:360-366.

Touchon JC, Warkentin KM. 2008. Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. Oikos. 117(4):634–640. doi:10.1111/j.0030-1299.2008.16354.x.

Tullberg BS, Merilaita S, Wiklund C. 2005. Aposematism and crypsis combined as a result of distance dependence: functional versatility of the colour pattern in the swallowtail butterfly larva. Proc R Soc B. 272(1570):1315–1321. doi:10.1098/rspb.2005.3079.

Turlings TCL, Wäckers FL, Vet LEM, Lewis WJ, Tumlinson JH. 1993. Learning of host-finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis AC, editors. In Learn: Ecol Evol Pers. Boston, MA: Springer US. p. 51–78.

Umbers KDL, Fabricant SA, Gawryszewski FM, Seago AE, Herberstein ME. 2014. Reversible colour change in Arthropoda. Biol Rev. 89:820-848.

Umbers KDL, Lehtonen J, Mappes J. 2015. Deimatic displays. Curr Biol. 25(2):R58–R59. doi:10.1016/j.cub.2014.11.011.

Umbers KDL, Mappes J. 2015. Postattack deimatic display in the mountain katydid, *Acripeza reticulata*. An Behav. 100:68–73. doi:10.1016/j.anbehav.2014.11.009.

Valkonen JK, Nokelainen O, JokimãKi M, Kuusinen E, Paloranta M, Peura M, Mappes J. 2014. From deception to frankness: Benefits of ontogenetic shift in the anti-predator strategy of alder moth *Acronicta alni* larvae. Curr Zool. 60(1):114–122. doi:10.1093/czoolo/60.1.114.

Wang L-Y, Rajabi H, Ghoroubi N, Lin C-P, Gorb SN. 2018. Biomechanical strategies underlying the robust body armour of an aposematic weevil. Front Physiol. 9:1410. doi: 10.3389/fphys.2018.01410

Wang M-Y, Vasas V, Chittka L, Yen S-H. 2017. Sheep in wolf's clothing: multicomponent traits enhance the success of mimicry in spider-mimicking moths. An Behav. 127:219– 224. doi:10.1016/j.anbehav.2017.03.020.

Weaver RJ, Koch RE, Hill GE. 2017. What maintains signal honesty in animal colour displays used in mate choice? Phil Trans R Soc B: Biol Sci. 372: 20160343. http://dx.doi.org/10.1098/rstb.2016.0343

Willink B, Brenes-Mora E, Bolaños F, Pröhl H. 2013. Not everything is black and white: color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. Evolution. 67(10):2783–2794. doi:10.1111/evo.12153.

Wilson K, Cotter SC, Reeson AF, Pell JK. 2001. Melanism and disease resistance in insects. Ecol Lett. 4(6):637–649. doi:10.1046/j.1461-0248.2001.00279.x.

Xavier GM, Brito VLG, Gonzaga MO. 2018. Colour matching in an orb-web spider: a twig-masquerading species as seen by potential predators. J Zool. 306(1):48–57. doi:10.1111/jzo.12568.

Xie Y, Civco DL, Silander JA. 2018. Species-specific spring and autumn leaf phenology captured by time-lapse digital cameras. Ecosphere. 9(1):e02089. doi:10.1002/ecs2.2089.

Yeh C-W, Blamires SJ, Liao C-P, Tso I-M. 2015. Top down and bottom up selection drives variations in frequency and form of a visual signal. Sci Rep. 5(1):9543. doi:10.1038/srep09543.

Zimova M, Hacklaender K, Good JM, Melo-Ferreira J, Alves PC, Mills LS. 2018. Function and underlying mechanisms of seasonal colour moulting in mammals and birds: what keeps them changing in a warming world? Biol Rev. 93(3):1478–1498. doi:10.1111/brv.12405.

Zylinski S, How MJ, Osorio D, Hanlon RT, Marshall NJ. 2011. To be seen or to hide: visual characteristics of body patterns for camouflage and communication in the Australian giant cuttlefish *Sepia apama*. Am Nat 177(5):681–690. doi:10.1086/659626.

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

Elizabeth G. Postema

Published in Current Zoology Vol. 68(4)

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.

INTRODUCTION

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 wide- spread, 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 2.1A). 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 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 150m 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; Figure S2.1). 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 Table S2.1), 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, non-air-drying plasticine clay (VanAken 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 2.1B). 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.

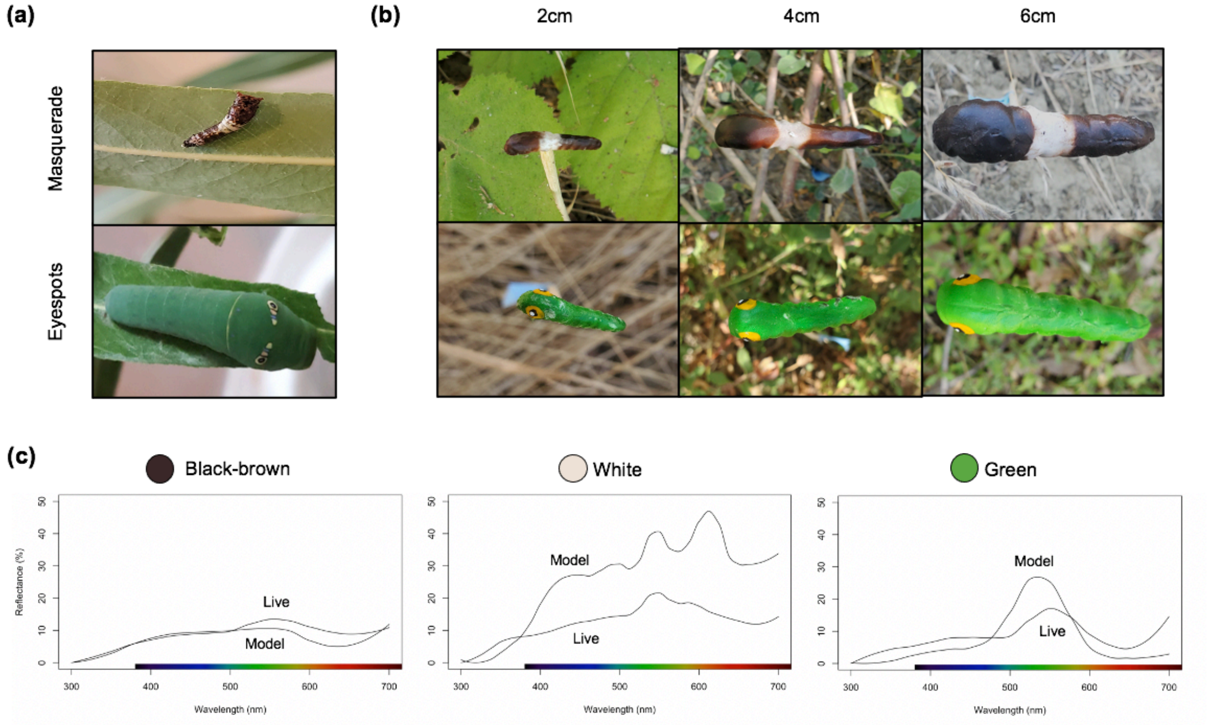


Fig. 2.1. (a) Live larvae of the western tiger swallowtail (*Papilio rutulus*). Top: an early-instar larva masquerading as bird feces. Bottom: a 5th instar larva with prominent eyespots. (b) Three sizes of plasticine swallowtail larvae (2, 4, and 6 cm) painted to resemble either the younger feces-masquerading 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.

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) 2cm feces masquerade (Figure 2.1B). I set out the artificial prey along 5 200 m transects in each trial (Figure S2.1). 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; Figure S2.2). 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 (Figure S2.3). 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 2.1C).

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.

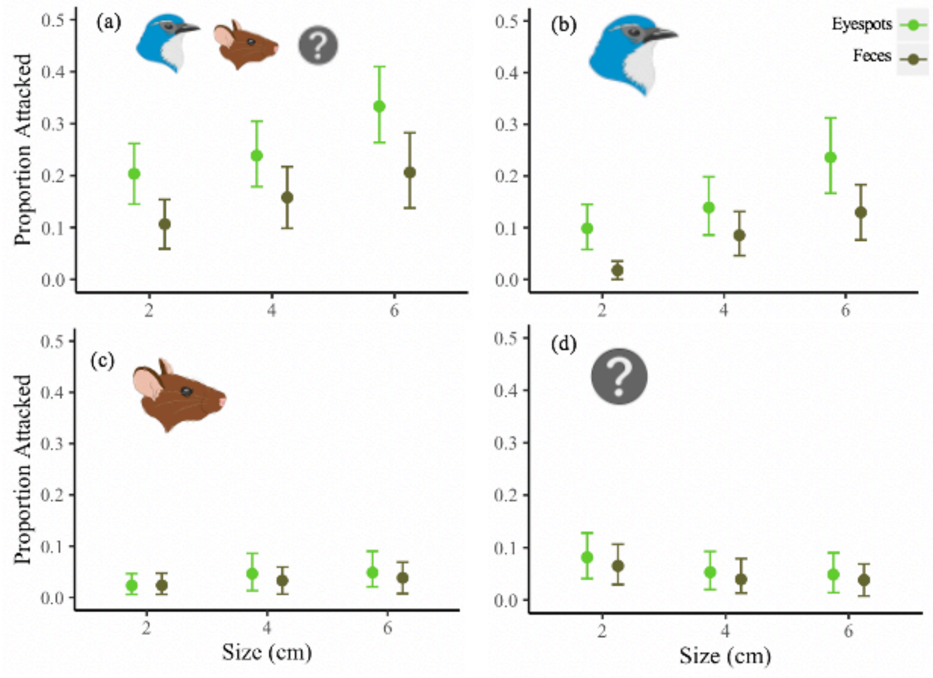


Fig. 2.2. Proportion (mean ± SE) of each artificial prey type attacked by predators (out of n = 916 models), faceted by predator identity: (A) all types of predation included; (B) proportion attacked by avian predators; (C) proportion attacked by mammalian predators; and (D) proportion attacked by unknown predators.

Effects of Size and Color – Pooled across predator type, predation increased significantly with size (χ^2=11.16, df =1, P < 0.001; Figure 2.2A). Additionally, predation was influenced by color;

eyespotted prey were attacked significantly more than feces masquerade prey (χ^2 =17.51, df =1, P<0.001; Figure 2.2A). When avian predation was analyzed separately, there was a similar pattern of increased predation on larger models (χ^2=22.87, df =1, P < 0.001; Figure 2.2B) and eyespotted models (χ^2 =15.33, df =1, P < 0.001; Figure 2.2B). In both pooled and avian-specific analyses, the influence of color on predation was not size-dependent (pooled: χ^2=0.09, df =1, P =0.77; avian: χ^2=1.81, df =1, P=0.18). In mammal-specific analyses neither size (χ^2=1.21, df =1, P=0.27) nor color (χ^2=0.62, df =1, P=0.43) influenced predation. For unknown predators, smaller prey were marginally more likely to be attacked (χ^2=3.72, df =1, P=0.05), whereas color had no effect on predation (χ^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 2.3A). Overall predation was significantly higher (χ^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: χ^2=2.99, df =1, P=0.08; mammal: χ^2=0.61, df =1, P=0.43), whereas unknown predator attacks decreased significantly (χ^2=20.41, df =1, P < 0.001). There was no effect of percent background greenness on predation in both pooled (χ^2=2.40, df=1, P=0.12) and predator-specific analyses (avian: χ^2=2.19, df=1, P=0.14; mammal: χ^2=1.71, df =1, P=0.19; unknown: χ^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 (χ^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 (χ^2= 0.20, df=1, P=0.66; Figure 2.3B).

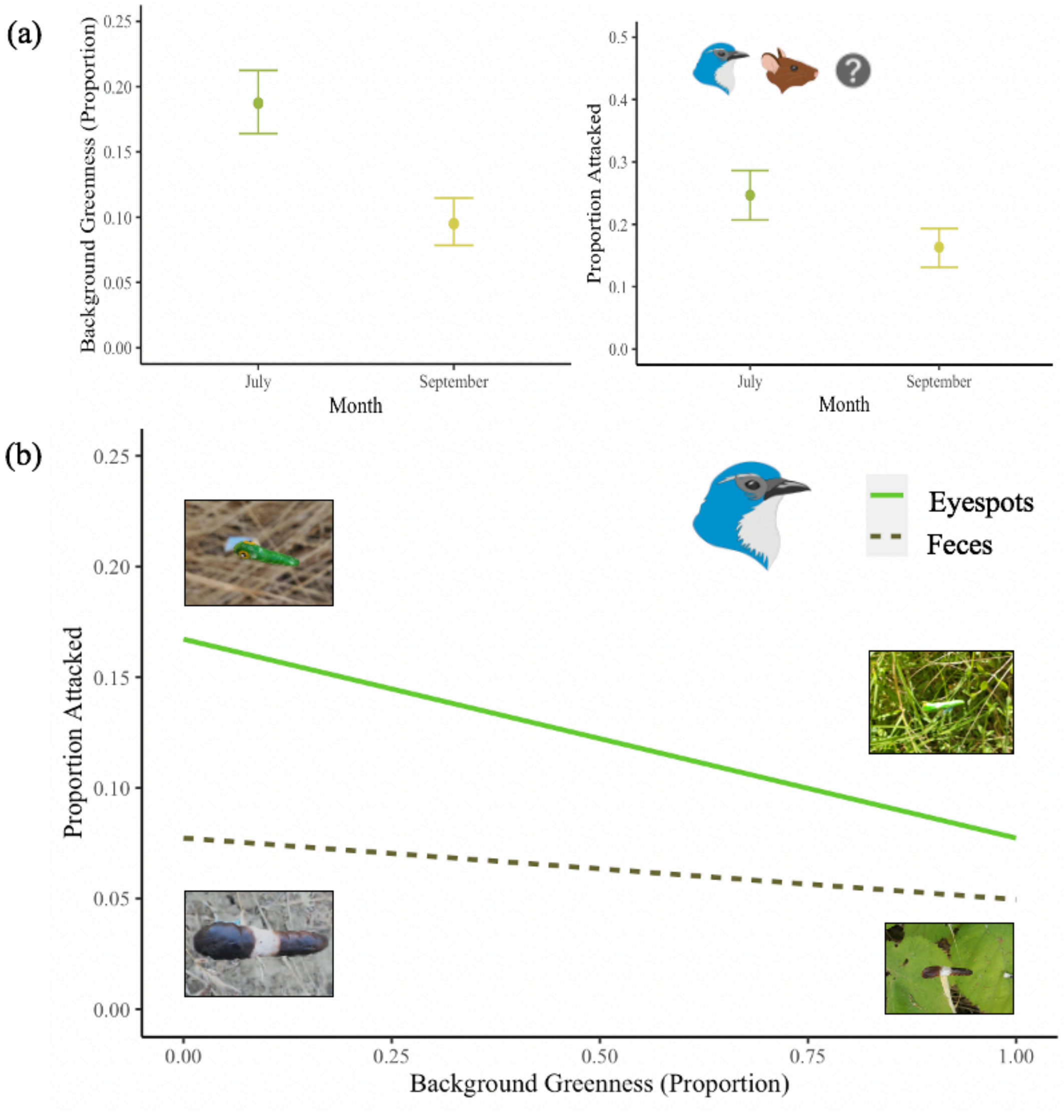


Fig. 2.3. (a) The effects of season (months: July and September) on average background greenness (left) and proportion of each artificial prey attacked by predators overall (right). Greenness is a visual estimate from 0.0 = 0% green to 1.0 = 100% green. (b) The average proportion of model larvae attacked 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.

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 2.2). Avian predation was highest on the largest size category of each color morph, and significantly higher on eyespotted prey than masquerading prey (Figure 2.2B). 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 2.2). 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 2.2C). Differences in size may have also been more detectable by avian predators than terrestrial mammals (Figure 2.2B 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 2.2C 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 2.2D). 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 2.3A). 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 these 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 2.3A). However, I found little evidence that background color influenced predator perception of larval coloration (Figure 2.3B). 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 2.3B). 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, P. 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 non-statistically significant increase in predation risk for eyespotted prey on non-green backgrounds in July (Figure S2.4). 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 2.2), 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 2.3). 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).

REFERENCES

Arenas LM, Troscianko J, Stevens M, 2014. Color contrast and stability as key elements for effective warning signals. Front Ecol and Evol 2:1–12.

Aronsson M, Gamberale-Stille G, 2009. Importance of internal pattern contrast and contrast against the background in aposematic signals. Behavl Ecol 20:1356–1362.

Boege KA, Agrawal A, Thaler JS, 2019. Ontogenetic strategies in insect herbivores and their impact on tri-trophic interactions. Curr Opin Insect Sci 32: 61–67.

Booth CL, 1990. Evolutionary significance of ontogenetic colour change in animals. Biol J Linn Soc 40:125–163.

Booth CL, 1992. Ontogenetic color change and mating cues in Largus californicus (Hemiptera: largidae). Ann of the Entomol Soc Am 85:331–334.

Bulbert MW, White TE, Saporito RA, Kraus F, 2018. Ontogenetic colour change in Oreophryne ezra (Anura: microhylidae) reflects an unusual shift from conspicuousness to crypsis but not in toxicity. Biol J Linn Soc 123:12–20.

Caro T, 2005. The adaptive significance of coloration in mammals. Bioscience 55:125–136.

Caro T, Allen WL, 2017. Interspecific visual signalling in animals and plants: a functional classification. Philos T Roy Soc B 372:20160344.

Caro T, Sherratt TN, Stevens M, 2016. The ecology of multiple colour defences. Evol Ecol 30:797–809.

Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G et al., 2017. The biology of color. Science 357:eaan0221.

Endler JA, 1993. The color of light in forests and its implications. Ecol Monogr 63:2–27.

Endler JA, Mappes J, 2004. Predator mixes and the conspicuousness of aposematic signals. Am

Nat 163:532–547.

Futahashi R, Fujiwara H, 2008. Juvenile hormone regulates butterfly larval pattern switches. Science 319:1061–1061.

Gaitonde N, Joshi J, Kunte K, 2018. Evolution of ontogenic change in color defenses of swallowtail butterflies. Ecol Evol 8:9751–9763.

Grant JB, 2007. Ontogenetic colour change and the evolution of aposematism: a case study in panic moth caterpillars. J Anim Ecol 76:439–447.

Guilford T, Dawkins MS, 1991. Receiver psychology and the evolution of animal signals. Anim Behav 42:1–14.

Hawlena D, Boochnik R, Abramsky Z, Bouskila A, 2006. Blue tail and striped body: why do lizards change their infant costume when growing up? Behav Ecol 17:889–896.

Hebets EA, Barron AB, Balakrishnan CN, Hauber ME, Mason PH et al., 2016. A systems approach to animal communication. P Roy Soc B-Biol Sci 283:20152889.

Hebets EA, Papaj DR, 2005. Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol 57:197–214.

Hossie TJ, Sherratt TN, 2012. Eyespots interact with body colour to protect caterpillar-like prey from avian predators. Anim Behav 84:167–173.

Hossie TJ, Sherratt TN, 2013. Defensive posture and eyespots deter avian predators from attacking caterpillar models. Anim Behav 86:383–389.

Hossie TJ, Sherratt TN, 2014. Does defensive posture increase mimetic fidelity of caterpillars with eyespots to their putative snake models? Curr Zool 60:76–89.

Hossie TJ, Sherratt TN, Janzen DH, Hallwachs W, 2013. An eyespot that "blinks": an open and shut case of eye mimicry in Eumorpha caterpillars (Lepidoptera: sphingidae). J Nat Hist

47:2915–2926.

Hossie TJ, Skelhorn J, Breinholt JW, Kawahara AY, Sherratt TN, 2015. Body size affects the evolution of eyespots in caterpillars. P Roy Soc B-Biol Sci 112:6664–6669.

Iverson ENK, Karubian J, 2017. The role of bare parts in avian signaling. The Auk 134:587–611.

Jacobs GH, 2009. Evolution of colour vision in mammals. Philos T Roy Soc B 364:2957–2967.

Jones MP, Pierce KE, Ward D, 2007. Avian vision: a review of form and function with special consideration to birds of prey. J Exot Pet Med 16:69–87.

Mand T, Tammaru T, Mappes J, 2007. Size dependent predation risk in cryptic and conspicuous insects. Evol Ecol 21:485.

Mappes J, Kokko H, Ojala K, Lindstro¨m L, 2014. Seasonal changes in predator community switch the direction of selection for prey defences. Nat Commun 5:5016.

Mason LD, Wardell-Johnson G, Luxton SJ, Bateman PW, 2018. Predators show seasonal predilections for model clay spiders in an urban environment. Sci Rep 8:12444.

Merilaita S, 2003. Visual background complexity facilitates the evolution of camouflage. Evolution 57:1248–1254.

Merilaita S, Lind J, 2005. Background-matching and disruptive coloration, and the evolution of cryptic coloration. Proc R Soc B Biol Sci 272:665–670.

Michalis C, Scott-Samuel NE, Gibson DP, Cuthill IC, 2017. Optimal background matching camouflage. Proc R Soc B Biol Sci 284:20170709.

Mills LS, Zimova M, Oyler J, Running S, Abatzoglou JT, Lukacs PM, 2013. Camouflage mismatch in seasonal coat color due to decreased snow duration. Proc Natl Acad Sci USA 110:7360–7365.

Nakazawa T, 2015. Ontogenetic niche shifts matter in community ecology: a review and future perspectives. Popul Ecol 57:347–354.

Nokelainen O, Valkonen J, Lindstedt C, Mappes J, 2014. Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. J Anim Ecol 83:598–605.

Paluh DJ, Hantak MM, Saporito RA, 2014. A test of aposematism in the dendrobatid poison frog Oophaga pumilio: the importance of movement in clay model experiments. J Herpetol 48:249-254.

Patricelli GL, Hebets EA, 2016. New dimensions in animal communication: the case for complexity. Curr Opin Behav Sci 12:80–89.

Prudic KL, Skemp AK, Papaj DR, 2007. Aposematic coloration, luminance contrast, and the benefits of conspicuousness. Behav Ecol 18:41–46.

Robinson SK, Holmes RT, 1982. Foraging behavior of forest Birds: the relationships among search tactics, diet, and habitat structure. Ecology 63:1918.

Shapiro AM, Manolis TD, 2007. Field Guide to Butterflies of the San Francisco Bay and Sacramento Valley Regions. Berkeley (CA): University of California Press.

Skelhorn J, Halpin CG, Rowe C, 2016a. Learning about aposematic prey. Behav Ecol 27:955–964.

Skelhorn J, Holmes GG, Hossie TJ, Sherratt TN, 2016b. Eyespots. Curr Biol 26:R52–R54.

Skelhorn J, Rowland HM, Ruxton JD, 2010a. The evolution and ecology of masquerade. Biol J Linn Soc 99:1–8.

Skelhorn J, Rowland HM, Speed MP, De Wert L, Quinn L et al., 2010b. Size-dependent misclassification of masquerading prey. Behav Ecol 21: 1344–1348.

Speed MP, 2000. Warning signals, receiver psychology and predator memory. Anim Behav

60:269–278.

Stevens M, 2005. The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. Biol Rev 80:573.

Stevens M, Ruxton GD, 2018. The key role of behaviour in animal camouflage. Biol Rev 94:116–134.

Suzuki TN, Sakurai R, 2015. Bent posture improves the protective value of bird dropping masquerading by caterpillars. Anim Behav 105:79–84.

Todd PA, Qiu W, Chong KY, 2009. Ontogenetic shifts in carapace patterning and/or colouration in intertidal and subtidal brachyuran crabs. Raffles Bull Zool 57:543–550.

Tullberg BS, Merilaita S, Wiklund C, 2005. Aposematism and crypsis combined as a result of distance dependence: functional versatility of the colour pattern in the swallowtail butterfly larva. Proc R Soc B Biol Sci 272: 1315–1321.

Umbers KDL, De Bona S, White TE, Lehtonen J, Mappes J et al., 2017. Deimatism: a neglected component of antipredator defence. Biol Letters 13: 20160936.

Umbers KDL, Lehtonen J, Mappes J, 2015. Deimatic displays. Curr Biol 25: R58–R59.

Valkonen JK, Nokelainen O, Jokim~aki M, Kuusinen E, Paloranta M et al., 2014. From deception to frankness: benefits of ontogenetic shift in the anti-predator strategy of alder moth Acronicta alni larvae. Curr Zool 60: 114–122.

Wagner DL, 2005. Caterpillars of Eastern North America: A Guide to Identification and Natural History. 1st edn. Princeton (NJ): Princeton University Press.

Werner EE, Gilliam JF, 1984. The ontogenetic niche and species interactions in size-structured populations. Annu Rev of Ecol Syst 15:393–425.

Wilson D, Heinsohn R, Endler JA, 2007. The adaptive significance of ontogenetic colour change in a tropical python. Biol Letters 3:40–43.

Yoshikawa T, Osada Y, 2015. Dietary compositions and their seasonal shifts in Japanese resident birds, estimated from the analysis of volunteer monitoring data. PLoS ONE 10:1–16.

Zimova M, Hacklaender K, Good JM, Melo-Ferreira J, Alves PC et al., 2018. Function and underlying mechanisms of seasonal colour moulting in mammals and birds: what keeps them changing in a warming world? Biol Rev 93:1478–1498.

Chapter III. Eyespot peek-a-boo: environmental deimatism in a leaf-rolling caterpillar

Elizabeth G. Postema

ABSTRACT

Animal color patterns often require synergistic behaviors to create effective defenses against visual predators. For example, deimatic displays involve body parts that can actively conceal or reveal bright color patches, potentially reducing detectability at a distance while startling predators up close. Some species may achieve this “conceal-then-reveal” effect by modifying aspects of their environment (environmental deimatism hypothesis). The larvae of spicebush swallowtail butterflies (Papilio troilus) possess large eyespots, but hide in rolled leaf structures during the day. Using artificial swallowtail larvae, I tested the hypothesis that these structures simultaneously reduce eyespot visibility and increase their effectiveness at startling visual predators. I compared wild avian predation rates on 659 artificial larvae from four treatments groups: eyespotted and non-eyespotted, presented in leaf rolls or on open leaves. Leaf rolls reduced predation regardless of color pattern, likely by concealing prey identity. Eyespots also reduced predation, but only for artificial prey in leaf rolls; on open leaves, eyespots neither increased nor decreased predation. These results support the environmental deimatism hypothesis, and suggest that this strategy may have evolved to enhance the protective effect of leaf rolls, rather than to reduce costs of prey conspicuousness.

INTRODUCTION

Bright, high-contrast 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; 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 (Umbers et al., 2015, 2019). These displays often cross 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 (Drinkwater et al., 2022). Deimatic displays thus offer ecologists a way to study (1) how behavior intersects with morphology to shape the perception of color traits, (2) what conditions select for the evolution of multicomponent and/or multimodal defenses, and (3) how evolutionary tradeoffs can produce and maintain diversity in traits (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 (Wagner, 2005), presumably to deter predators by mimicking the eyes of more threatening animals (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. 3.1a). 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. 3.1d), 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; Fig. 3.1f).

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. 3.1a,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 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, as observed by 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. 3.1b-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 & Sherratt, 2013; Postema, 2022). 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.

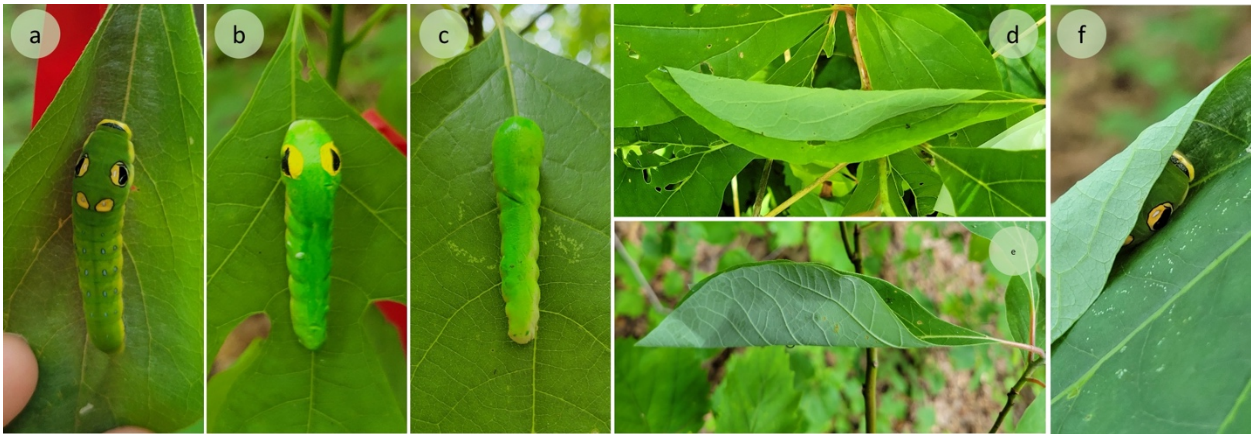


Figure 3.1. (a) A live spicebush swallowtail (Papilio troilus) larva on sassafras (Sassafras albidum). Its leaf roll is held open, with strands of silk visible above the head. Eyespotted (b) and non-eyespotted (c) artificial larvae on open S. albidum leaves. (d) A true leaf roll with a live P. troilus 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.

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; Figure S3.1).

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; Figure S3.1) were not used. Potential predators of *P. troilus* were present at the sites throughout the experiment (Table S3.1), as well as live *P. troilus* larvae (Table S3.2) and adults. I ran two predation trials at these sites, from July 3rd to 12th, and July 20th to 30th, 2021.

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 23rd-27th, 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 primarily interested in intentionally constructed rolls that resembled *P. troilus* leaf rolls.

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 perception, I measured two key 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. For plant size, I expected that individual prey items would be easier to find on smaller plants, resulting in higher avian predation. I took plant height measurements (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. The resulting unpainted prey were 4cm long, approximately the size of a 4th or 5th instar larva. Larvae have eyespots at this point in development (Fig. 3.1a). 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. 3.1b), while leaving the rest blank (Fig. 3.1c). 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 (Figure S3.2).

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. albidum* or *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; Table S3.2).

For the open leaf treatment group, I attached artificial prey to the upper side of fully expanded host plant leaves. I did this 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. 3.1e). I positioned all prey with the "head" pointed up towards the leaf petiole, which reflects this species' typical resting position (Fig. 3.1a,f). 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 (Figure S3.3). I recorded any missing prey items (that could not be found after carefully scouring a 1m^2 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, I included data from 659 artificial caterpillars in the analysis, excluding prey attacked by non-avian predators. Birds are common visual predators of insects (Nyffeler et al., 2018), making avian predation patterns 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 (Figure S3.4). 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 lmtest). 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, $\chi^2 = 6.98$, $df = 1$, $p < 0.01$).

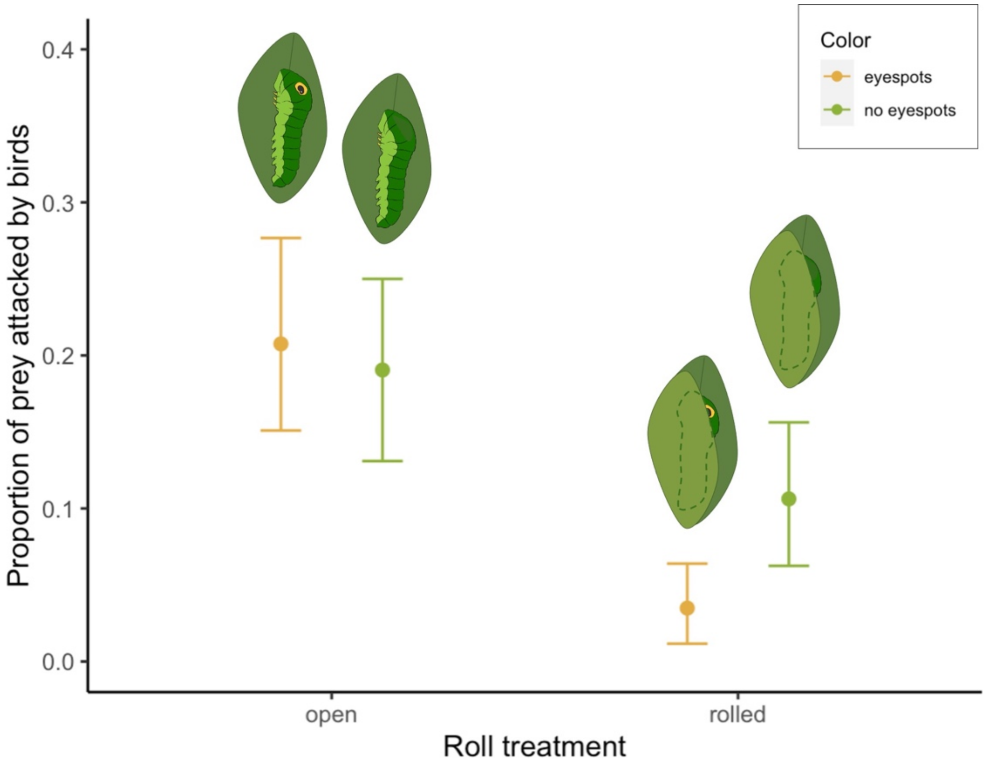


Figure 3.2. Mean proportions of artificial prey in each treatment group attacked by avian predators, ± SE (n = 659). Yellow points represent eyespotted prey, while green points represent non-eyespotted prey. Illustrations by Mia Lippey.

DISCUSSION

In this study, leaf rolls not only protected prey in general, but also increased the effectiveness of eyespots at deterring visual predators (Fig. 3.2). 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 categories of visual defense. Environmental deimatism may be a common strategy among other leaf-sheltering organisms, such as eyespotted hesperiid caterpillars (Janzen et al., 2010) or spiders that abruptly jump out of rolls when disturbed (Postema *pers. observations,* Fig. 3.3). Beyond leaf rolls, environmental deimatism may exist as a more general strategy for shelter-using or -building species.

The protective effect of leaf rolls I observed aligns with past research on these structures' role in predator defense (Murakami, 1999; Tvardikova & Novotny, 2012). However, the mechanism of protection is somewhat unclear. 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 may even 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. 3.3). 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 and occupancy. Predator uncertainty could further enhance the effectiveness of unexpected or startling visual signals.

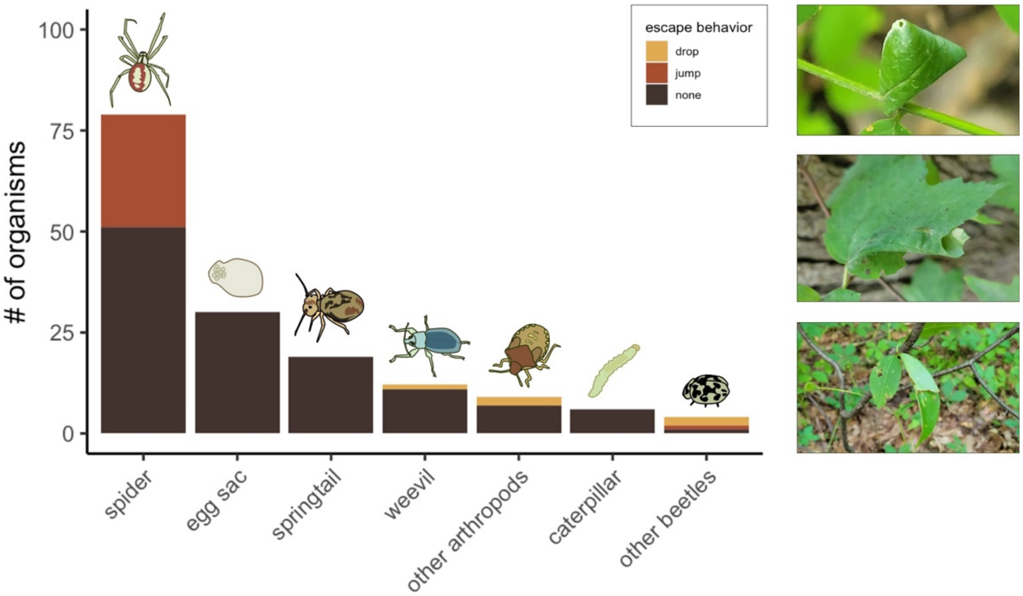


Figure 3.3. (Left) Total counts of each organism type found in surveys of naturally occurring leaf rolls (n = 464). Within each organism category, counts of individuals that displayed escape behaviors in response to the leaf roll being disturbed (dropping, jumping, or no response) are

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.

Eyespots did not significantly increase predation risk on open leaves relative to non-eyespotted prey on open leaves (Fig. 3.2). 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 (Wagner, 2005; Gaitonde et al., 2018), as well as the general positive association between body size and presence of eyespots in lepidopteran larvae (Hossie et al., 2015). 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. 3.2). 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 (1999) "extended phenotype." There are clear consequences of the leaf roll on caterpillar fitness, as well as synergistic interactions between the leaf rolling behavior and color traits (Fig. 3.2). 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. 3.2). 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. 3.2), 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 (Video S1). 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).

REFERENCES

Aronsson, M. & Gamberale-Stille, G. (2009). Importance of internal pattern contrast and contrast against the background in aposematic signals. *Behav Ecol*, 20, 1356–1362.

Badiane, A., Carazo, P., Price-Rees, S.J., Ferrando-Bernal, M. & Whiting, M.J. (2018). Why blue tongue? A potential UV-based deimatic display in a lizard. *Behav Ecol Sociobiol*, 72, 1-11.

Barnett, J.B., Cuthill, I.C. & Scott-Samuel, N.E. (2018). Distance-dependent aposematism and camouflage in the cinnabar moth caterpillar (*Tyria jacobaeae*, Erebidae). *R Soc Open Sci*, 5, 171396.

Barnett, J.B., Redfern, A.S., Bhattacharyya-Dickson, R., Clifton, O., Courty, T., Ho, T., *et al.* (2017). Stripes for warning and stripes for hiding: spatial frequency and detection distance. *Behav Ecol*, 28, 373–381.

Blake, J.G., & Hoppes, W.G. (1986). Influence of resource abundance on use of tree-fall gaps by birds in an isolated woodlot. *Auk,* 103, 328-340.

Canfield, M.R. (2009). The double cloak of invisibility: phenotypic plasticity and larval decoration in a geometrid moth, *Synchlora frondaria*, across three diet treatments. *Ecol Entomol*, 34, 412–414.

Caro, T. & Allen, W.L. (2017). Interspecific visual signalling in animals and plants: a functional classification. *Philos Trans R Soc Lond B Biol Sci*, 372, 20160344.

Coker, D.J., Pratchett, M.S. & Munday, P.L. (2009). Coral bleaching and habitat degradation increase susceptibility to predation for coral-dwelling fishes. *Behav Ecol*, 20, 1204–1210.

Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E., *et al.* (2017). The biology of color. *Science*, 357, eaan0221.

Dawkins, R. (1999). *The Extended Phenotype: The Long Reach of the Gene*. Revised edition. Oxford University Press, Oxford, UK.

De Bona, S., Valkonen, J.K., López-Sepulcre, A. & Mappes, J. (2015). Predator mimicry, not conspicuousness, explains the efficacy of butterfly eyespots. *Proc Royal Soc B*, 282, 20150202.

Dookie, A.L., Young, C.A., Lamothe, G., Schoenle, L.A. & Yack, J.E. (2017). Why do caterpillars whistle at birds? Insect defence sounds startle avian predators. *Behav Processes*, 138, 58–66.

Drinkwater, E., Allen, W.L., Endler, J.A., Hanlon, R.T., Holmes, G., Homziak, N.T., *et al.* (2022). A synthesis of deimatic behaviour. *Biol Rev*, 97, 2237-2267.

Endler, J.A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environ Biol Fish*, 9, 173–190.

Endler, J.A. (1993). The color of light in gorests and its implications. *Ecol Monogr*, 63, 2–27.

Endler, J.A., Endler, L.C. & Doerr, N.R. (2010). Great bowerbirds create theaters with forced perspective when seen by their Audience. *Curr Biol*, 20, 1679–1684.

Fabricant, S.A. & Herberstein, M.E. (2015). Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. *Behav Ecol*, 26, 38–44.

Gaitonde, N., Joshi, J. & Kunte, K. (2018). Evolution of ontogenetic change in color defenses of swallowtail butterflies. *Ecol Evol*, 8, 9751–9763.

Gotceitas, V. & Colgan, P. (1989). Predator foraging success and habitat complexity: quantitative test of the threshold hypothesis. *Oecologia*, 80, 158–166.

Halfwerk, W., Jones, P.L., Taylor, R.C., Ryan, M.J. & Page, R.A. (2014). Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual display. *Science*, 343, 413–416.

Hämäläinen, L., Mappes, J., Rowland, H.M., Teichmann, M. & Thorogood, R. (2020). Social learning within and across predator species reduces attacks on novel aposematic prey. *J Anim Ecol*, 89, 1153–1164.

Hossie, T.J. & Sherratt, T.N. (2012). Eyespots interact with body colour to protect caterpillar-like prey from avian predators. *Anim Behav*, 84, 167–173.

Hossie, T.J. & Sherratt, T.N. (2013). Defensive posture and eyespots deter avian predators from attacking caterpillar models. *Anim Behav*, 86, 383–389.

Hossie, T.J. & Sherratt, T.N. (2014). Does defensive posture increase mimetic fidelity of caterpillars with eyespots to their putative snake models? *Curr Zool*, 60, 76–89.

Hossie, T.J., Sherratt, T.N., Janzen, D.H. & Hallwachs, W. (2013). An eyespot that "blinks": an open and shut case of eye mimicry in *Eumorpha* caterpillars (Lepidoptera: Sphingidae). *J Nat Hist*, 47, 2915–2926.

Hossie, T.J., Skelhorn, J., Breinholt, J.W., Kawahara, A.Y. & Sherratt, T.N. (2015). Body size affects the evolution of eyespots in caterpillars. *PNAS*, 112, 201415121.

Hultgren, K. & Stachowicz, J. (2011). Camouflage in decorator crabs: integrating ecological, behavioural and evolutionary approaches. In: *Animal Camouflage: Mechanisms and Function*, [eds. Stevens, M., & S. Merilaita], Cambridge University Press, Cambridge, UK, pp. 212–236.

Hunter, P. (2018). The revival of the extended phenotype. *EMBO Rep*, 19, e46477.

Janzen, D.H., Hallwachs, W. & Burns, J.M. (2010). A tropical horde of counterfeit predator eyes. *PNAS*, 107, 11659–11665.

Kobayashi, C., Matsuo, K. & Kawata, M. (2020). Contradictory effects of leaf rolls in a leaf-mining weevil. *Sci Rep*, 10, 12180.

Kobayashi, C., Matsuo, K., Watanabe, K., Nagata, N., Suzuki-Ohno, Y., Kawata, M., *et al.* (2015). Arms race between leaf rollers and parasitoids: diversification of plant-manipulation behavior and its consequences. *Ecol Monogr*, 85, 253–268.

Laland, K.N. (2004). Extending the extended phenotype. *Biol Philos*, 19, 313–325.

Leslie, A.J. & Fleming, N. (1990). Role of the osmeterial gland in swallowtail larvae (Papilionidae) in defense against an avian predator. *J Lepid Soc*, 44, 245-251.

de Lira, J.J.P.R., Perez-Jvostov, F., Gotanda, K.M., Kou-Giesbrecht, S., Pease, S.K., Jackson, M., *et al.* (2018). Testing for a whole-organism trade-off between natural and sexual selection: are the male guppies preferred by females more likely to be eaten by predators? *Evol Ecol Res*, 19, 441–453.

Mappes, J., Kokko, H., Ojala, K. & Lindström, L. (2014). Seasonal changes in predator community switch the direction of selection for prey defences. *Nat Commun*, 5, 5016.

Marshall, N.J. (2000). Communication and camouflage with the same 'bright' colours in reef fishes. *Philos Trans R Soc Lond B Biol Sci*, 355, 1243–1248.

Merilaita, S., Vallin, A., Kodandaramaiah, U., Dimitrova, M., Ruuskanen, S. & Laaksonen, T. (2011). Number of eyespots and their intimidating effect on naïve predators in the peacock butterfly. *Behav Ecol*, 22, 1326–1331.

Murakami, M. (1999). Effect of avian predation on survival of leaf-rolling lepidopterous larvae. *Pop Ecol*, 41, 135–138.

Nyffeler, M., Şekercioğlu, Ç.H. & Whelan, C.J. (2018). Insectivorous birds consume an estimated 400–500 million tons of prey annually. *Sci Nat*, 105, 47.

Postema, E.G. (2022). The effectiveness of eyespots and masquerade in protecting artificial prey across ontogenetic and seasonal shifts. *Curr Zool*, 68, 451-458.

Postema, E.G., Lippey, M.K. & Armstrong-Ingram, T. (2022). Color under pressure: how multiple factors shape defensive coloration. *Behav Ecol*, 34, 1-13.

Prudic, K.L., Skemp, A.K. & Papaj, D.R. (2007). Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behav Ecol*, 18, 41–46.

Richards, L.A, & Coley, P.D. (2008). Combined effects of host plant quality and predation on a tropical lepidopteran: a comparison between treefall gaps and the understory in Panama. *Biotropica*, 40, 736-741.

Romero, G.Q., Gonçalves-Souza, T., Roslin, T., Marquis, R.J., Marino, N.A.C., Novotny, V., *et al.* (2022). Climate variability and aridity modulate the role of leaf shelters for arthropods: A global experiment. *Glob Change Biol,* 28, 3694–3710.

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Anim Behav*, 58, 921–931.

Rowland, H.M., Speed, M.P., Ruxton, G.D., Edmunds, M., Stevens, M. & Harvey, I.F. (2007). Countershading enhances cryptic protection: an experiment with wild birds and artificial prey. *Anim Behav*, 74, 1249–1258.

Ruxton, G.D., Speed, M.P. & Broom, M. (2009). Identifying the ecological conditions that select for intermediate levels of aposematic signalling. *Evol Ecol*, 23, 491–501.

Schaedelin, F.C. & Taborsky, M. (2009). Extended phenotypes as signals. *Biol Rev*, 84, 293–313.

Seymoure, B.M., Raymundo, A., McGraw, K.J., McMillan, W.O. & Rutowski, R.L. (2018). Environment-dependent attack rates of cryptic and aposematic butterflies. *Curr Zool*, 64, 663–669.

Skelhorn, J., Holmes, G.G., Hossie, T.J. & Sherratt, T.N. (2016a). Eyespots. *Curr Biol*, 26, 52–

54.

Skelhorn, J., Holmes, G.G. & Rowe, C. (2016b). Deimatic or aposematic? *Anim Behav*, 113, 1–3.

Stevens, M. & Ruxton, G.D. (2018). The key role of behaviour in animal camouflage. *Biol Rev*, 94, 116-134.

Stuart-Fox, D. (2022). Defensive coloration as a multivariate optimum: a comment on Postema et al. *Behav Ecol*, arac065.

Stuart-Fox, D. & Moussalli, A. (2008). Selection for social signalling drives the evolution of chameleon colour change. *PLoS Biol,* 6, 22–29.

Tan, E., Reid, C., Symonds, M., Jurado-Rivera, J. & Elgar, M. (2017). The role of life-history and ecology in the evolution of color patterns in Australian chrysomeline beetles. *Front Ecol Evol*, 5, 1-15.

Tvardikova, K. & Novotny, V. (2012). Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. *J Trop Ecol*, 28, 331–341.

Umbers, K.D.L., De Bona, S., White, T.E., Lehtonen, J., Mappes, J. & Endler, J.A. (2017).

Deimatism: a neglected component of antipredator defence. *Biol Lett*, 13, 20160936.

Umbers, K.D.L., Lehtonen, J. & Mappes, J. (2015). Deimatic displays. *Curr Biol*, 25, 58–59.

Umbers, K.D.L. & Mappes, J. (2015). Postattack deimatic display in the mountain katydid, *Acripeza reticulata*. *Anim Behav*, 100, 68–73.

Umbers, K.D.L. & Mappes, J. (2016). Towards a tractable working hypothesis for deimatic displays. *Anim Behav*, 113, 5–7.

Umbers, K.D.L., White, T.E., De Bona, S., Haff, T., Ryeland, J., Drinkwater, E., *et al.* (2019). The protective value of a defensive display varies with the experience of wild predators.

Sci Rep, 9, 463.

Vidal-García, M., O'Hanlon, J.C., Svenson, G.J. & Umbers, K.D.L. (2020). The evolution of startle displays: a case study in praying mantises. *Proc R Soc B: Biol Sci,* 287, 20201016.

Wagner, D.L. (2005). *Caterpillars of Eastern North America: A Guide to Identification and Natural History*. Princeton University Press, Princeton, N.J.

Whiting, M.J., Noble, D.W.A. & Qi, Y. (2022). A potential deimatic display revealed in a lizard. *Biol J Linn Soc,* 136, 455–465.

SUPPLEMENTARY INFORMATION FOR CHAPTER II

Table S2.1. Avian species visually observed at study sites during the experimental trials.

Bird Species	**Locations Observed**
Agelaius phoeniceus	All
Aphelocoma californica	All
Buteo jamaicensis	A, B, C, D
Buteo lineatus	C, D, E
Colaptes auratus	A, B
Corvus brachyrhynchos	C, D, E
Meleagris gallopavo	E
Melospiza melodia	C, D
Mimus polyglottos	A, B, C, D
Molothrus ater	C, D
Myiarchus cinerascens	A, B
Pica nuttalli	A, B
Picoides nuttallii	C, D, E
Pipilo maculatus	C, D
Sayornis nigricans	All
Sitta carolinensis	A, B, E
Sitta pygmaea	A, B
Spinus psaltria	All
Tachycineta bicolor	All
Troglodytes aedon	A, B, C, D
Zenaida macroura	A, B, C, D

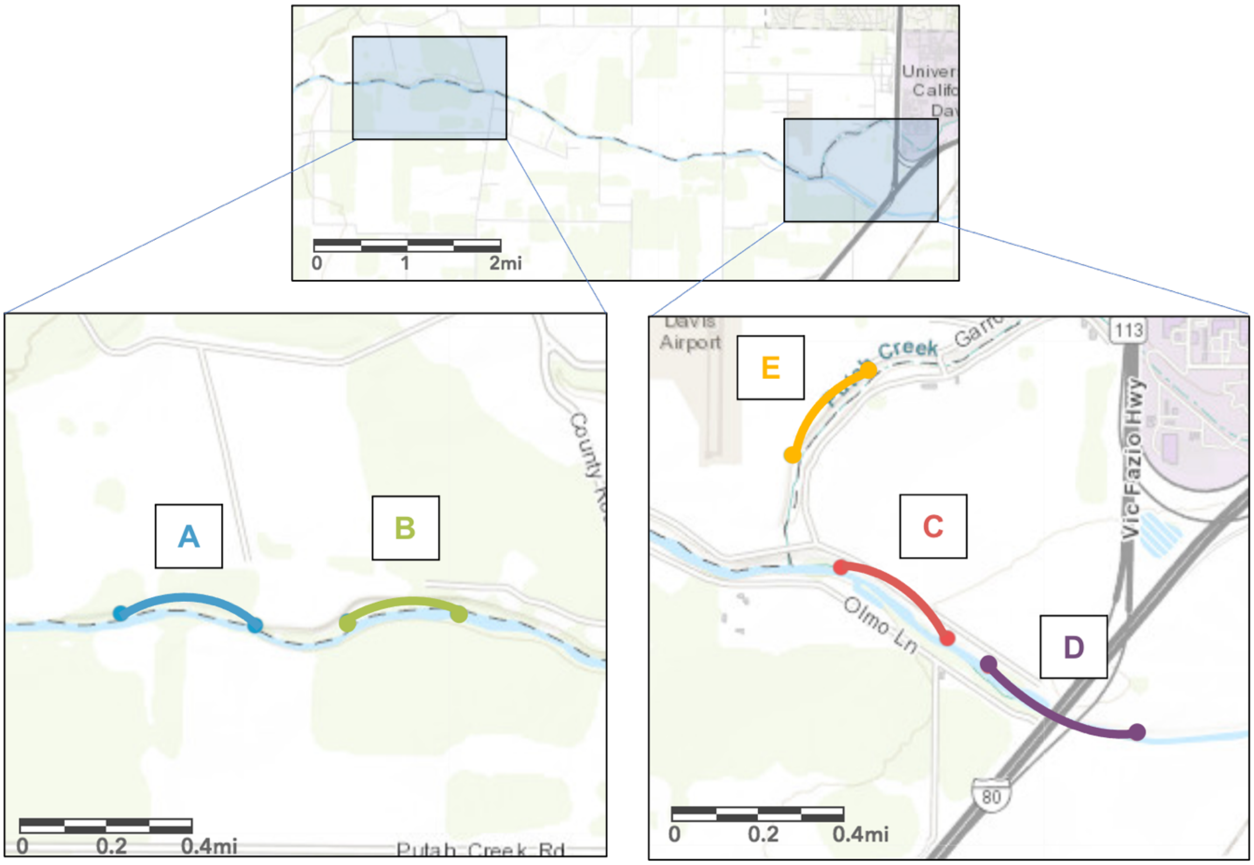


Figure S2.1. Map of the five creek-side transects in Davis, CA used for the field predation experiment. Transects are each 200m long and separated by at least 150m.

Figure S2.2. Examples of visual scoring of background greenness, from 100% green (far left) to 0% green (far right).

Figure S2.3. Examples of marks made by avian (top) and mammal (bottom) predators.

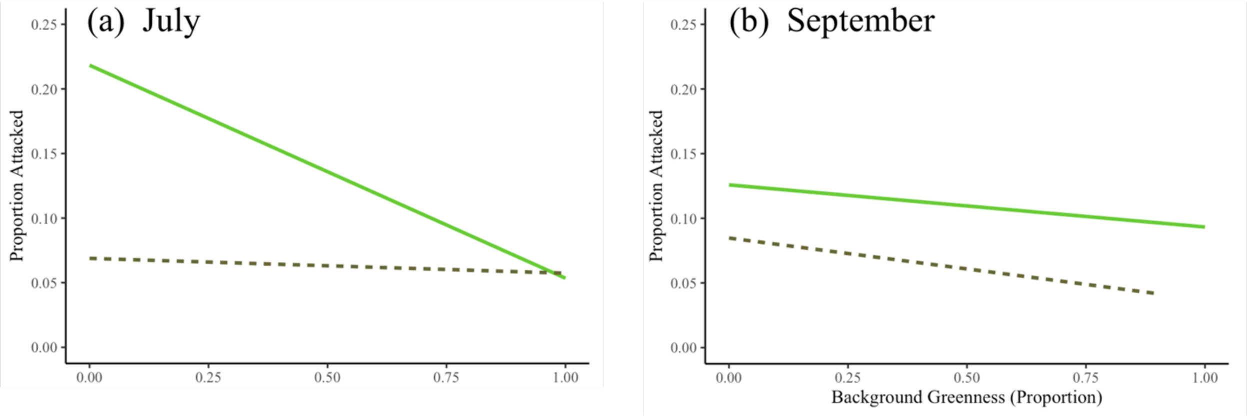


Figure S2.4. Comparison of the average proportion of model larvae attacked by avian predators across different background greenness levels (a visual estimate from 0.0 = 0% green to 1.0 = 100% green) between (a) July and (b) September.

SUPPLEMENTARY INFORMATION FOR CHAPTER III

Table S3.1. Bird species surveys conducted at Bird Hills Nature Area (BH) and Nichol's Arboretum (Arb) directly after each Trail (post-trial 1: 7/14, post-trial 2: 7/30). EGP conducted 30-minute visual and aural surveys for 5 total subsites (200m^2 rectangles; 3 in BH, 2 in Arb). Counts for each species are recorded below, but are a rough estimate, as it was not always possible to tell if individuals had been repeatedly observed. *Plausible predators of *P. troilus*, based on size and diet information listed on allaboutbirds.org.

Common Name	Scientific Name	BH 7/14	Arb 7/14	BH 7/30	Arb 7/30
*American robin	*Turdus migratorius*	11	11	7	8
*Black-capped chickadee	*Poecile atricapillus*	15	2	6	4
*Blue jay	*Cyanocitta cristata*	7	4	10	3
*Cedar waxwing	*Bombycilla cedrorum*	0	0	0	1
*Eastern towhee	*Pipilo erythrophthalmus*	0	2	0	0
*Eastern wood-peewee	*Contopus virens*	8	2	4	2
*European starling	*Sturnus vulgaris*	2	0	0	0
*Gray catbird	*Dumetella carolinensis*	0	1	0	0
*Northern cardinal	*Ailuroedus jobiensis*	3	1	9	4
*Scarlet tanager	*Piranga olivacea*	2	0	0	0
*Song sparrow	*Melospiza melodia*	0	1	0	0
*Tufted titmouse	*Baeolophus bicolor*	1	0	0	0
*Yellow warbler	*Setophaga petechia*	0	1	0	0
American goldfinch	*Spinus tristis*	2	3	9	6
Chimney swift	*Chaetura pelagica*	0	1	0	0
Downy woodpecker	*Dryobates pubescens*	4	0	3	1
Field sparrow	*Spizella pusilla*	0	1	0	0
House finch	*Haemorhous mexicanus*	1	0	1	0
Northern flicker	*Colaptes auratus*	3	0	4	0

Pileated woodpecker	*Dryocopus pileatus*	1	1	0	0
White-breasted nuthatch	*Sitta carolinensis*	3	2	0	2
Tree swallow	*Tachycineta bicolor*	0	0	1	1
Red-bellied woodpecker	*Melanerpes carolinus*	0	0	0	1
Red-tailed hawk	*Buteo jamaicensis*	0	0	4	0

Table S3.2. Observations and measurements of live *P. troilus* larvae and their host plants. In total, I observed n = 17 live larvae throughout the experiment. I observed some larvae multiple times; date observed is recorded in parentheses within the column. *Height is measured from the ground to the position of the larva in its host plant. **Larval orientation within the leaf roll: up = towards leaf petiole, down = away from leaf petiole, towards the ground.

ID	Host Plant	*Height (cm)	Larva Length (cm)	**Orientation
1	spicebush	121	2.5 (6/17), 3.5 (6/24)	up (6/17)
2	sassafras	-	-	up
3	sassafras	164	3.4 (6/24)	up (6/24)
4	sassafras	54	2.45 (6/26), 3.3 (6/28), 4.3 (7/2)	up (6/26), up (6/28), up (7/2)
5	sassafras	136	0.7 (6/28)	up (6/28)
6	sassafras	103	0.5 (6/28), 1.7 (7/7)	up (6/28), up (7/7)
7	sassafras	70	3.0 (6/28)	up (6/28)
8	sassafras	35	1.7 (6/28), 2.0 (7/2)	up (6/28)
9	sassafras	71	2.4 (6/28)	up (6/28)
10	sassafras	34	3.0 (6/28), 3.5 (7/2)	up (6/28), up (7/2)
11	sassafras	35	1.0 (6/28), 2.1 (7/2), 2.2 (7/7)	down (6/28), up (7/2), up (7/7)
12	sassafras	69	0.8 (6/28), 1.9 (7/7)	up (6/28), up (7/77)
13	sassafras	96	0.8 (6/28)	up (6/28)
14	sassafras	25	4.0 (7/8)	up (7/8)
15	sassafras	119	2.0 (7/11), 2.8 (7/19), 1.2 (7/26)	up (7/11)
16	sassafras	99	1.2 (7/11)	up (7/11)
17	sassafras	91	2.1 (7/12)	up (7/12)

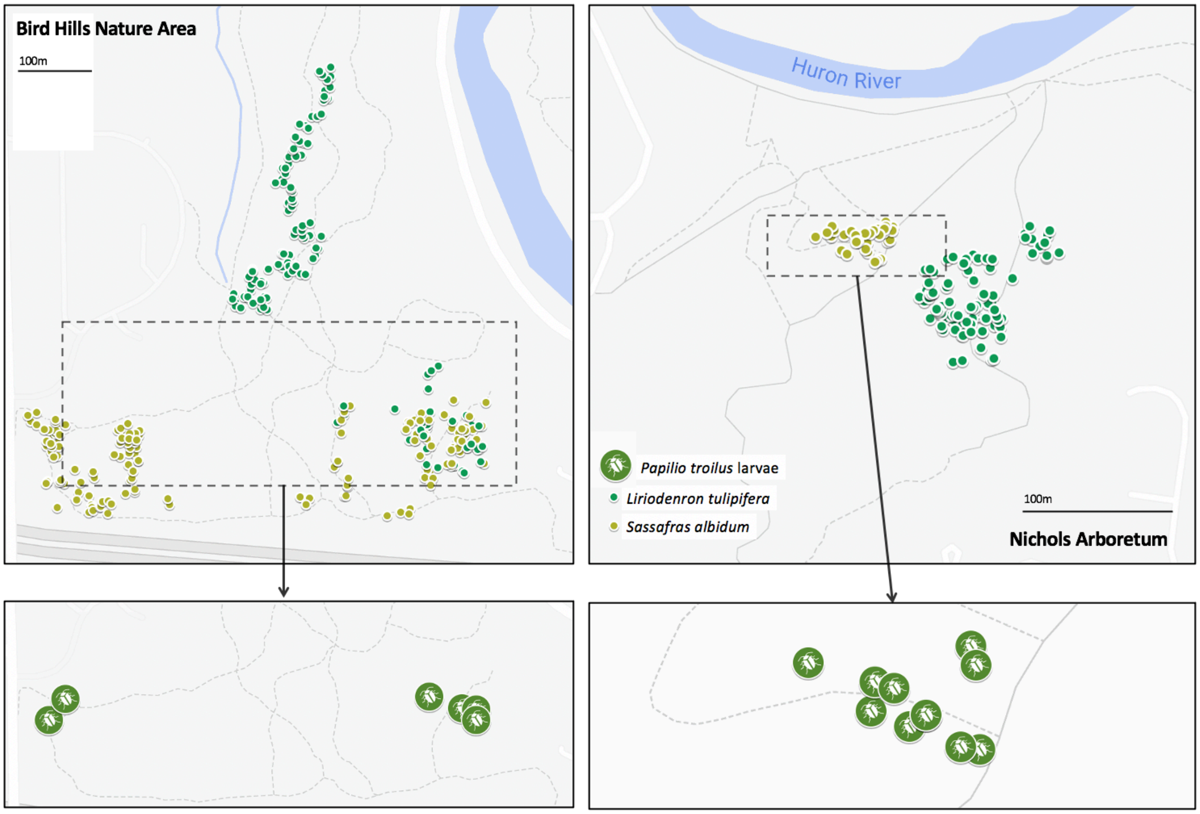


Figure S3.1. A map of the locations used in the field predation trials. On the left, individual plants used at Bird Hills Nature Area; on the right, plants used at Nichol's Arboretum. Individual host plants are marked with yellow-green (*L. tulipifera*) or dark green (*S. albidum*) circles. Below each map are close-up sections with circular icons. Each of these represents the locations of a live *P. troilus* caterpillar observed just before and during the experiment.

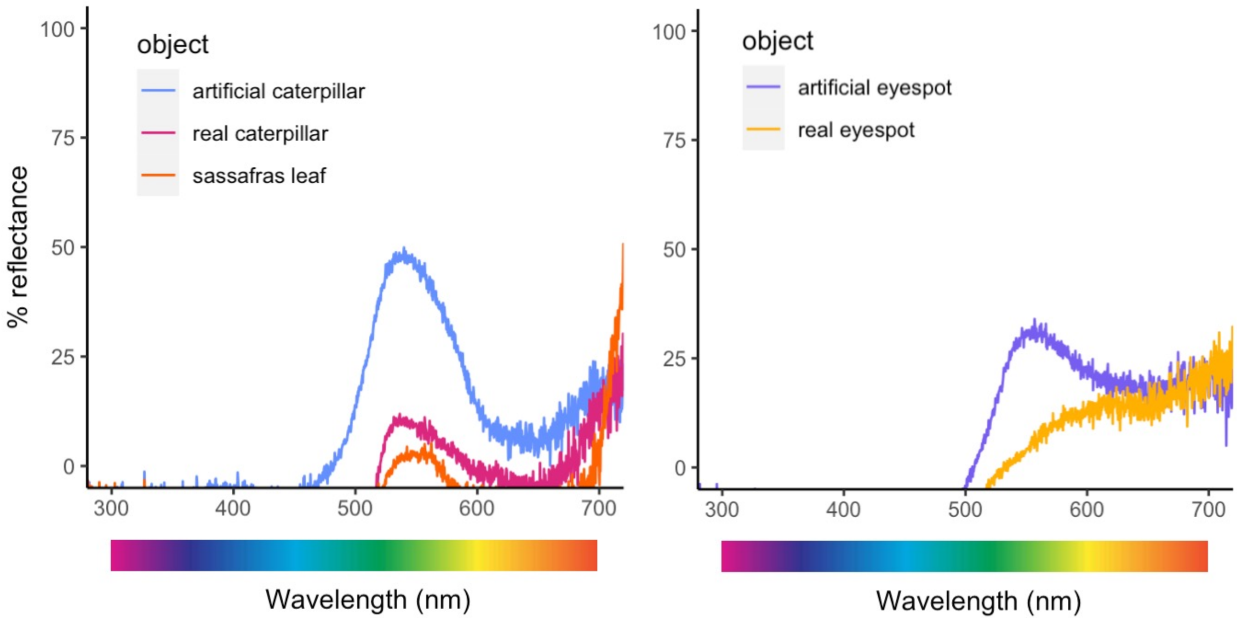


Figure S3.2. Reflectance curves of five different materials. On the left, the green areas of an artificial caterpillar, a real *P. troilus* caterpillar, and a sassafras leaf. On the right, an artificial caterpillar's eyespot and a real *P. troilus* caterpillar's eyespot. Each curve represents the average of three spectrometer measurements, taken from three different spots on the same object. Data collected by EGP and Hayley Crowell.

Figure S3.3. Locations of observed predator (avian, mammalian, and unknown) attacks on artificial prey at Bird Hills (left panels) and Nichols Arboretum (center panels), for trial 1 (top panels) and trial 2 (bottom panels). Panels on the right show examples of avian (top) versus mammalian (bottom) predation marks. Photographs by EGP.

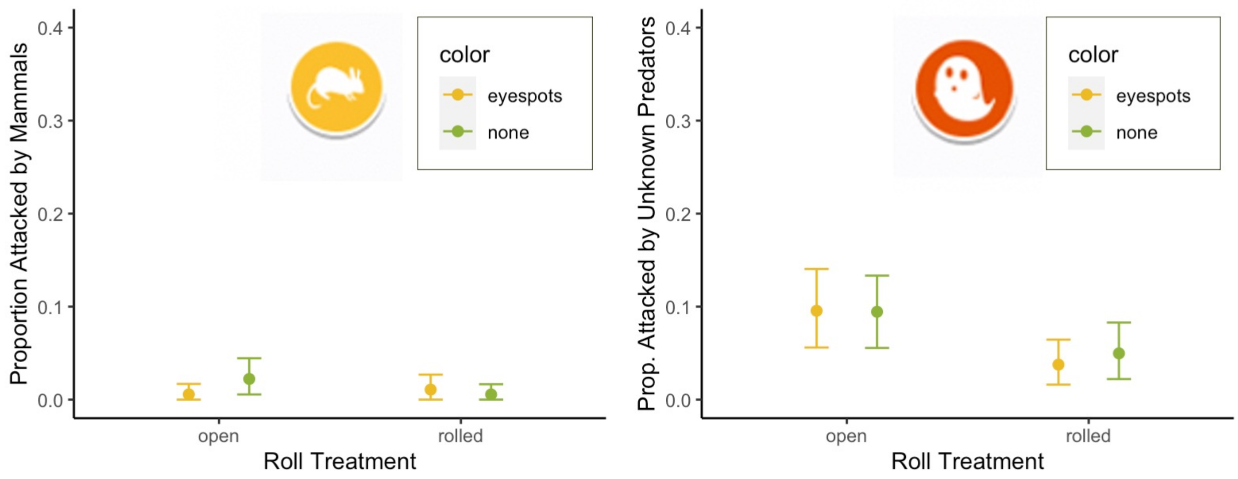


Figure S3.4. Proportion of rolled and unrolled artificial prey attacked by mammal (left) and unknown (right) predators, ± SE (n = 725). Yellow points represent eyespotted prey, while green points represent non-eyespotted prey.