Heliconius In A New Light: The Effects of Light Environments on Mimetic Coloration, Behavior, and Visual Systems

by

Brett M. Seymoure

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved May 2016 by the
Graduate Supervisory Committee:

Ronald Rutowski, Co-Chair
Kevin McGraw, Co-Chair
W. Owen McMillan
Stephen Pratt
Jürgen Gadau

ARIZONA STATE UNIVERSITY

August 2016

ABSTRACT

Although mimetic animal coloration has been studied since Darwin's time, many questions on the efficacy, evolution, and function of mimicry remain unanswered. Müller (1879) hypothesized that unpalatable individuals converge on the same conspicuous coloration to reduce predation. However, there are many cases where closely related, unpalatable species have diverged from a shared conspicuous pattern. What selection pressures have led to divergence in warning colors? Environmental factors such as ambient light have been hypothesized to affect signal transmission and efficacy in animals. Using two mimetic pairs of *Heliconius* butterflies, Postman and Blue-white, I tested the hypothesis that animals with divergent mimetic colors segregate by light environment to maximize conspicuousness of the aposematic warning signal under their particular environmental conditions. Each mimetic pair was found in a light environment that differed in brightness and spectral composition, which affected visual conspicuousness differently depending on mimetic color patch. I then used plasticine models in the field to test the hypothesis that mimics had higher survival in the habitat where they occurred. Although predation rates differed between the two habitats, there was no interactive effect of species by habitat type. Through choice experiments, I demonstrated that mimetic individuals preferred to spend time in the light environment where they were most often found and that their absolute visual sensitivity corresponds to the ambient lighting of their respective environment. Eye morphology was then studied to determine if differences in total corneal surface area and/or facet diameters explained the differences in visual sensitivities, but the differences found in *Heliconius* eye morphology did not match predictions based upon visual sensitivity. To further understand how eye

morphology varies with light environments, I studied many tropical butterflies from open and closed habitats to reveal that forest understory butterflies have larger facets compared to butterflies occupying open habitats. Lastly, I tested avian perception of mimicry in a putative *Heliconius* mimetic assemblage and show that the perceived mimetic resemblance depends upon visual system. This dissertation reveals the importance of light environments on mimicry, coloration, behavior and visual systems of tropical butterflies.

DEDICATION

To my parents, Denise and Michael, who fostered my curiosity in the natural world. To Kevin McCarthy whose curiosity has led me to better understand biology, science, and philosophy. And to Lindsey Seymoure, who's love, support, and sacrifice throughout this journey made this dissertation possible.

ACKNOWLEDGMENTS

I have been privileged to work with so many creative and brilliant mentors. My history of strong mentors began at Alma College with Dave Clark, whose research interests and passion led me to my own path of scientific enquiry. Of course, I have learned immensely from my chairs: Ronald L. Rutowski and Kevin J. McGraw. Words cannot express my appreciation for their hard work and guidance throughout this process and I must thank them for pushing me both intellectually and in productivity.

It has been a complete pleasure to work with W. Owen McMillan at the Smithsonian Tropical Research Institute and Ellis R. Loew at Cornell. Both of whom have greatly influenced my career path and research interests. Jürgen Gadau has been an excellent member of my committee and I appreciate his challenging questions and thought provoking thoughts on my research. I owe everything I know about statistics and modeling to Stephen Pratt (that may be a bit of an exaggeration – but it is close to the truth), and without his guidance in experimental design and statistical analyses, this research would have difficulty being published.

So many of my colleagues invested intellectually into my research resulting in a much stronger and relevant body of work. Mike Butler, Lisa Taylor, Melissa Meadows, Melissa Amarello, Matt Toomey, Scott Davies, Jake Brashears, Karla Moller, Martin Bergman, Susan Finkbeiner, Mathieu Chouteau, Melanie McClure, Lucie Queste, Richard Merrill, Pierce Hutton, and Melinda Weaver all greatly enhanced the research presented here through hours of conversations, edits, and field work. Richard Simpson, Nikos Lessios, Robert Orpet, Beryl Jones and Timothy Thurman all have sculpted me

into the biologist I am today. Lastly, I am grateful for all of the mentoring I received from Kim Pegram and Russell Ligon throughout the six years that we spent together at ASU.

Without the support of so many talented and hardworking undergraduates, I would have never finished my dissertation. To Michael Folk, Bharvi Rajyaguru, Raniya Rashid, McKenzie Liberty-Bibbens, Janelle Matura, Ryan Schmoll, Olivia van Vianen, Krisjanis Malins, Amanda Justice, Jennifer Armstrong, Britney Southers, Betsy Spencer, Nicole Hamlin, Tyler Mello, Kaci Fankhouser, Andre Szejner Sigal, Tamara Cherwin, Kayla Seltzer, Emily Brodie, Collin Whitset, Ben Rice, Michael Neimeyer, and Claire Johnson, I am forever grateful for their contributions. And to Andrew Raymundo and Rachel Olzer, I thank you for your commitment and dedication throughout this process.

My dissertation was supported by the Smithsonian Tropical Research Institute and Arizona State University partnership. I greatly appreciate the use of infrastructure and resources from both institutions. Adriana Bilgray, Adriana Tapia and Raineldo Urriola were both amazing administrators whom made conducting research in the tropics much easier and less stressful. I thank Wendi Simonson and Yvonne Delgado for always being patient with me and helping me with the administrative tasks throughout my dissertation. Furthermore, I am indebted to the Panamanian government, for access to their priceless biodiversity.

Biological research in the tropical rainforest of Central and South America is expensive. I am grateful to the National Science Foundation, the Society of Integrative and Comparative Biology, Sigma Xi, ASU Graduate and Professional Student Association, SoLS Graduate Programs and RTI, and the Research on Lepidoptera Foundation, all of whom contributed much appreciated funding.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

PREFACE

Bright coloration has often evolved as a means of warning potential predators of a prey's defense mechanisms (e.g. toxins, spines). Mimicry is a classic example of the beneficial role of aposematic coloration, such that several species converge on the same appearance to distribute the cost of predator education and reduce individual risk of predation (Woodruff 1972; Sherratt 2008). A driving hypothesis of sensory ecology is that signals are modified by selection to improve their efficacy and transmission through the environment and their perception by intended receivers (Endler 1992; Stevens 2013). However this has rarely been studied for warning coloration or mimetic groups of animals. Therefore I tested this hypothesis in an aposematic group of butterflies in ecologically relevant contexts.

Animal colors that advertise unprofitability may be mimicked by other species, whether they are palatable or unpalatable (Bates 1862, Müller 1876, Cott 1940, Edmunds 1976, Ruxton et al. 2004). This mimetic coloration can benefit the mimic only (Batesian mimicry) or both mimic and model (Müllerian mimicry; Bates 1862, Müller 1876, Ruxton et al. 2004). Müllerian mimicry reduces the costs and maximizes the benefits of warning signals in two or more species. Poison dart frogs, bees and wasps, Danaid butterflies, Appalachian millipedes, and coral snakes are a few examples of Müllerian mimics (Brower 1969; Dressler 1979; Darst and Cummings 2006; Marek and Bond 2009; Kikuchi et al. 2014).

Theory predicts that closely related, sympatric, brightly colored, unpalatable species should converge on a similar color and pattern that facilitates learning and reinforcement, forming a Müllerian mimicry complex (Müller 1876, Ruxton et al. 2004).

However, contrary to this expectation, there are clear examples of diversity in the colors of Müllerian mimics, even within relatively small geographic areas (Papageorgis 1975, Mallet and Gilbert 1995, Noonan and Wray 2006, Marek and Bond 2009). In the neotropics, there are poison dart frogs and *Heliconius* butterflies, both of which display a diversity of visual warning signals that range from red and yellow to blue and black. Other diverse warning colors include the millipedes of the Appalachian mountains (Marek & Bond 2009) and bumblebees of south-central Asia (Hines & Williams 2012).

The question remains: what ecological and evolutionary factors have produced this diversity? One hypothesis is that diverse warning colors have evolved because they are presented to potential predators in different light environments. Endler (1990, 1993, 1997) proposed that light environments vary within and across habitats, which can affect the efficacy and utility of color signals under different environmental conditions (Fleishman et al. 1993, Endler & Théry 1996). For example, due to the spatial arrangement of the sun, clouds, blue sky, and vegetation, there may be up to four different distinct types of light environments within a forest (Endler 1993): (1) small gap (SG) (light is bright and rich in long wavelengths due to blue sky being occluded by vegetation), (2) large gap (LG) (light is bright and rich in all wavelengths found in sunlight), (3) forest shade (FS) (light is dim and rich in mid wavelengths due to vegetation filtering), and (4) woodland shade (WS) (light dim but rich in short wavelengths due to the sun being occluded by vegetation). Thus, the lighting that illuminates a warning signal can be very dynamic in the rainforest as wavelength composition can drastically differ (Hutton et al. 2015).

In addition to general solar illumination conditions, warning signal efficacy also can depend upon contrast within the animal's warning signal pattern as well as contrast of the colored animal against the background. This contrast is affected by reflectance of the colors in the pattern, ambient light illuminating the pattern (irradiance), transmission properties of the medium through which the light is traveling, and visual perception of the viewer (Endler 1990, Endler & Mielke 2005). These elements can be combined to form many different viewer perceptions, and therefore one warning signal may work best under one set of conditions (i.e. one predator, one irradiance, and one background) while an entirely different warning signal may be naturally selected by a different predator's perception of the signal in a different light environment with a different background.

Most research on the effects of light environment on efficacy of signals in general has been done with vertebrates. For example, three species of lekking birds in Guyana selectively perform courtship displays in specific light environments, which heightens conspicuousness to mates. Conversely, during non-courtship bouts, the birds avoid light environments where they are conspicuous to reduce predation risk (Endler & Théry 1996). Furthermore, light environments drive color divergence and speciation of cichlid fishes in the Rift Lakes Basin in Africa (Seehausen et al. 1997). When light environment diversity decreases due to eutrophication, many cichlid fishes cannot reproduce due to the difficulty in perceiving and recognizing sexual signals of conspecifics. Species of sympatric *Anolis* lizards have also been shown to segregate by microhabitats that vary in light environment (Leal and Fleishman 2001). Although there are several strong cases of support in vertebrate systems for the idea that light environment plays a pivotal role in visual communication systems, little is known about the effects of light environments on

visual signals in invertebrates. Invertebrates comprise most prey items and most cases of mimicry (Seymoure and Pegram, unpublished data). Thus by investigating light environment effects on invertebrates, a better understanding predator-prey interactions will emerge. Therefore, the central aim of my dissertation is to better understand the causes of diversity in warning signals and visual systems in sympatric species, especially the effects of diverse light environments.

Study System

To determine the effect of light environment on warning signal efficacy and diversity, I utilized a well-studied system of nymphalid butterflies in the genus *Heliconius*. *Heliconius* butterflies occur throughout the neotropics and their natural history and genetics are well known (Brown 1981, De Moura et al. 2011; Legrand et al. 2011). Also, within Soberania National Park in Panama, there are at least nine species of *Heliconius* and each of them falls into one of four mimicry rings: 1) Postman or Red-Yellow, 2) Blue-White, 3) Blue-Yellow, 4) Tiger or Orange-Yellow (figure 1) (Devries et al. 1997). This system is ideal for addressing effects of light environments on signaling because previous research shows that the mimetic groups occur in different areas of the jungle and ecological data suggest that these different areas differ in forest composition (Pike et al. 2001, Estrada et al. 2002, Santiago et al. 2004) and thus putatively in lighting conditions.

Previous research on *Heliconius* in Central and South America has shown in behavioral experiments with birds that avian predators are able to discern amongst but not within the different mimetic groups (Chai 1986, Chai & Srygley 1990, Langham, 2004, 2005). When wild-caught jacamars were enclosed with many different species of

butterflies, *Heliconius* species were not attacked (Chai 1986). Furthermore, starved jacamars attacked more *Heliconius* species that had their pattern manipulated or were non-local, than the local morph (Langham 2004). Also, flight pattern and height along with roosting sites of mimics are more similar to their co-mimics than they are to non-mimetic but closely related species (Mallet and Gilbert 1995; Srygley and Ellington 1999).

For most of this dissertation, I have focused on two different mimicry rings that occur in Panama: the Postman and the Blue-white. The Postman mimicry ring is comprised of *Heliconius erato* and *Heliconius melpomene,* which in Panama are yellow, red and black. *Heliconius sapho* and *Heliconius cydno* comprise the Blue-white mimicry ring, which are blue, white, and black. These two mimetic rings offer an ideal system to study the effects of light environments on warning coloration efficacy because previous research has suggested that these mimetic pairs occur in different microhabitats (Mallet and Gilbert 1995; Estrada and Jiggins 2002). Also, the evolutionary relationships among four well-studied *Heliconius* species allow some control for phylogeny. *Heliconius cydno*, a member of the blue-white ring, is more closely related to *H. melpomene*, a member of the postman ring. Similarly, the blue-white *H. sapho* is more closely related to the postman, *H. erato.* Ultimately, this means that the different mimetic rings are a result of convergent evolution and not shared ancestry.

Hypotheses and Tests

I use an adaptationist's approach to test the overall hypothesis **that the optimal warning coloration, behavior, and vision of comimics is a function of the**

characteristics of the light environment in which comimics occurs. Through this dissertation I test these specific hypotheses:

Chapter 1) **Microhabitat segregation of two mimetic pairs affects predator perception of warning coloration.**

Chapter 2) **Warningly colored and mimetic individuals have evolved coloration to reduce predation in their respective environment.**

Chapter 3) **Individual mimetic butterflies prefer the light environment in which they naturally occur.**

Chapter 3) **Individual mimetic butterflies have visual sensitivities that match their behavioral preference for light and their respective light environment in nature.**

Appendix A) **Co-mimics have converged on similar eye morphology that is adapted to shared environmental conditions including lighting.**

Addendum) **Butterfly eye morphology has evolved to effectively capture photons under different lighting conditions.**

Appendix B) **Butterflies that have converged on coloration match their predator's spectral sensitivities.**

The work described in the following chapters and appendices test these hypotheses and predictions. I first examined mimetic groups are segregated by habitat and how light environment affects mimicry conspicuousness in the eyes of predators (Chapter 1). Then following from the results, I tested if predation differed between microhabitat and mimetic coloration (Chapter 2). For chapter 3 I tested if mimetic individuals differ in their preference for light environments and if visual sensitivity

between *Heliconius* mimics differs. I further investigated the differences in visual sensitivity between mimetic pairs by studying the effects of light environment on eye morphology (Appendix A). To better understand how light environment has driven eye morphology in tropical butterflies in general, I measured eye morphology for many species in both open and closed habitats (Addendum). Lastly, I conducted a study to determine if one *Heliconius* species is involved in mimicry and if the mimetic coloration is perceived better by natural predators (Appendix B).

Chapter One

BUTTERFLY MICROHABITAT SEGREGATION BY LIGHT ENVIRONMENT AFFECTS PREDATOR PERCEPTION OF MIMETIC BUTTERFLIES

Introduction

Animal colors that advertise unprofitability may be mimicked by other species, whether they are palatable or unpalatable (Bates 1862, Müller 1876, Cott 1940, Edmunds 1976, Ruxton et al. 2004). This mimetic coloration can benefit the mimic only (Batesian mimicry) or both mimic and model (Müllerian mimicry; Bates 1862, Müller 1876, Ruxton et al. 2004). Müllerian mimicry reduces the costs and maximizes the benefits of warning signals in two or more species. Poison dart frogs, bees and wasps, Danaid butterflies, Appalachian millipedes, and coral snakes are a few examples of Müllerian mimics (Brower 1969; Dressler 1979; Darst and Cummings 2006; Marek and Bond 2009; Kikuchi et al. 2014).

Theory predicts that closely related, sympatric, brightly colored, unpalatable species should converge on a similar color and pattern that facilitates learning and reinforcement, forming a Müllerian mimicry complex (Müller 1876, Ruxton et al. 2004). However, contrary to this expectation, there are clear examples of diversity in the colors of Müllerian mimics, even within relatively small geographic areas (Papageorgis 1975, Mallet and Gilbert 1995, Noonan and Wray 2006, Marek and Bond 2009). In the neotropics, there are poison dart frogs and *Heliconius* butterflies, both of which display a diversity of visual warning signals that range from red and yellow to blue and black. Other diverse warning colors include the millipedes of the Appalachian mountains (Marek & Bond 2009) and bumblebees of south-central Asia (Hines & Williams 2012).

An animal's fitness relies on effective and accurate signaling to mates, conspecifics, or predators. A driving hypothesis of sensory ecology is that signals are selected to be optimally transmitted through the environment and perceived by intended receivers (Endler 1992; Stevens 2013). One hypothesis to explain the diversity in warning coloration is that different warning colors have evolved because they are presented to potential predators in different light environments. Endler (1990; 1993; 1998) proposed that different light environments occur within habitat types and that these different light environments can affect color-signal efficacy (Fleishman et al. 1993; Endler and Thery 1996). A visual signal is perceived under ambient illumination against a specific background (e.g. forest vegetation or blue sky) and these factors can alter the conspicuousness of the signal and therefore lead to behavioral differences in the intended receiver of the signal (Bergman et al. 2015). The ambient illumination is dependent upon the spatial arrangement of the sun, clouds, blue sky, and vegetation (Endler 1993; Hutton et al. 2015a). Thus, there may be up to five different distinct types of light environments within a forest at a given time (Endler 1993; Hutton et al. 2015a). These light environments range from bright and full spectrum light in open habitats to dim and middle wavelength rich light in forest understory.

Warning signal efficacy depends upon, among other things, contrast within the animal's warning signal pattern as well as contrast of the colored patches against the background. This contrast is affected by reflectance of the colors in the pattern, ambient light illuminating the pattern (irradiance), transmission properties of the medium through which the light is traveling, and visual perception of the viewer (Endler 1990; Endler and Mielke 2005). These elements can be combined to form many different viewer

perceptions, and therefore one warning signal may work best under one set of conditions (i.e. one predator, one irradiance, and one background) while an entirely different warning signal may be naturally selected by a different predator's perception of the signal in a different light environment with a different background.

Most research on the effects of light environment on signaling efficacy has been studied in sexual selection contexts between male and female conspecifics. Three species of lekking birds in Guyana selectively choose to court in specific light environments that heighten conspicuousness to mates. Conversely, during non-courtship bouts, the birds avoid light environments where they are conspicuous to reduce risk of predation (Endler and Thery 1996). Furthermore, light environments drive the diversity of species of cichlid fishes in the Great Lakes Basin in Africa (Seehausen et al. 1997; Seehausen 2015). Furthermore, *Anolis* lizards have also been shown to segregate by disparate light environments where multiple species are sympatric (Leal and Fleishman 2001; Fleishman et al. 2006; Leal and Fleishman 2013). Although there are several strong cases of support for the hypothesis that light environment plays a pivotal role in sexually selected visual signals (Endler and Thery 1996; Uy and Endler 2004; Maan and Seehausen 2011), little is known about the effects of light environments on the diversity of naturally selected aposematic signals. Thus, investigating how light environments affect conspicuous warning signals in diverse mimetic assemblages will increase our understanding of environmental factors sculpting predator-prey evolution.

Heliconius butterflies are known for their diverse warning coloration, which has led to multiple mimetic assemblages that occur in Central and South America (Brown 1981; Flanagan et al. 2004; Merrill et al. 2015). Within one lowland forest in Panama,

there are at least nine species of *Heliconius* and each of them falls into one of four mimicry rings: 1) Postman (yellow, red and black), 2) Blue-White, 3) Blue-Yellow, 4) Tiger (orange, yellow, and black, see figure 1 (Devries et al. 1997). This system is ideal for addressing effects of light environments on signaling because previous research shows that the mimetic groups occur in different areas of the forest and ecological data suggest that these different areas differ in forest composition, which could lead to differences in light environment between mimetic groups (Pyke et al. 2001; Estrada and Jiggins 2002; Santiago et al. 2004).

Previous research on *Heliconius* in Central and South America has shown that the members of different mimicry groups are discernible by predators and that *Heliconius* individuals have converged on not only color pattern but also flight behavior (Chai 1986, Chai & Srygley 1990, Langham, 2004, 2005). The avian predators of *Heliconius*, such as tyrant flycatchers and jacamars (Chai 1996; Langham 2004; Pinheiro 2011), are likely viewing the different mimetic pairs in habitats that have different lighting conditions. When wild-caught jacamars were enclosed with many different species of butterflies, *Heliconius* species were not attacked (Chai 1986). Furthermore, starved jacamars attacked more *Heliconius* species that had their pattern manipulated or were non-local, than the local morph (Langham 2004). Also, flight pattern and height along with roosting sites of mimics are more similar to their co-mimics than they are to non-mimetic but closely related species (Mallet and Gilbert 1995; Srygley and Ellington 1999). Furthermore, the evolutionary relationships among four species allow some control for phylogeny, figure 1. *Heliconius cydno*, a member of the blue-white ring, is more closely related to *H. melpomene*, a member of the postman ring. Similarly, the blue-white *H.*

sapho is more closely related to the postman, *H. erato*. Ultimately, this means that the different mimetic rings are a result of convergent evolution and not shared ancestry. Lastly, Estrada and Jiggins (2002) documented that the Postman butterflies occupy more open habitats while the Blue-white butterflies are found in closed canopy habitats. Therefore

Here I tested the hypothesis that microhabitat segregation of two *Heliconius* mimetic pairs affects predator perception of warning coloration. Specifically, I predicted that the Blue-white mimics would occupy forested habitats comprised of dim and middle-wavelength-rich light, while the Postman mimics would occupy open habitats comprised of bright and full-spectrum light. Furthermore, I predicted that the respective light environment would increase conspicuousness of each mimetic pair as seen by avian predators. I tested these predictions through distribution surveys of the four butterfly species along a neotropical rainforest transect and then quantified the light environment along the same transect. Butterflies were caught and reflectance was measured, enabling for use of avian visual models to test for the effects of light environments on conspicuousness and for differences in conspicuousness within and between mimetic pairs.

Methods

Distributions of the mimicry rings

To test and confirm the findings that the two mimetic pairs occupy different habitats along Pipeline Road in Central Panama (Estrada and Jiggins 2002), I applied mark and release methods in the summer of 2011 and 2012. I walked along Pipeline Road

from 0 km (beginning) to 10.5 km (hereafter referred to as end) and with insect nets I caught any *Heliconius* individuals I could. In 2011, each individual was caught, had distance along Pipeline Road recorded using a Garmin 60CSx GPS (Garmin International, Olathe, Kansas), was marked on the ventral surface of the forewing wing with a fine tip permanent sharpie marker (Downers Grove, IL) and then released. Recaptured individuals were rare (less than 20%) and ignored in the survey because recaptured individuals were recaptured within the same section as the initial capture location. For analysis the 10.5 km along Pipeline Road was divided into three 3.5 km sections comparable to Estrada and Jiggins (2002). Furthermore, I sampled for a total of 40 hours in each 3.5 km section between the two field seasons. Lastly, individuals were grouped by respective capture location. Chi square analyses were used to test if the species differed in where they occurred.

Habitat Light Measurements

To investigate if there were differences in the light environment between where the mimetic pairs occurred, I implemented two complimentary techniques: canopy cover analysis and absolute irradiance with spectroradiometry. For canopy cover, I divided the 10.5 km of Pipeline Road into .5 km sections beginning at 0 km and ending at 10.5 km, see figure 2. Within each .5 km section, I measured both canopy cover with two photographs at three locations separated by 50 meters within each half kilometer section, see figure 2.

Canopy cover is a common surrogate for light environment (Rich 1990; Frazer et al. 1999; Frazer et al. 2001) and is an advantageous technique when clouds are frequent, as in the rainforest. I used a fisheye lens (.18X Ultra-Wide Fisheye Converter Lens,

HDSales, Monsey, NY.) affixed to a Nikon D70 camera (Nikon Corporation, Tokyo, Japan) pointed skyward, which produced 180 degree circular images that records the size, shape, and location of gaps in the forest canopy (Frazer et al. 1999). Photographs were taken at dawn and dusk to prevent any images from being saturated by direct illumination from the sun. These images were then analyzed using the Gap Light Analyzer program (Frazer et al. 1999), which transforms images into white (sky) or black (vegetation) pixels and then calculates the proportion of black pixels, which produces the percentage of canopy cover for the full hemisphere. I further refined the images to restrict the pixel measurements to only a 45 degree region directly above. This refinement was needed as the light entering the canopy through gaps will illuminate an area as a function of the cosine of the entry angle, therefore, light entering within the horizon and 45 degrees above the horizon, will have a minimal effect on overall light environment. A linear regression was calculated for the percentage of canopy cover as a function of distance along Pipeline Road.

The light environment of the habitats was also characterized by measuring the downwelling irradiance. Irradiance is the number of photons at each wavelength that strike a surface, weighted by the cosine of the angle of incidence (Johnsen 2012). Measuring irradiance enables for quantifying both the absolute intensity of photons as well as the spectral composition of light in an environment. Furthermore, irradiance is required for calculating conspicuousness of the different mimetic individuals, see below. I only measured irradiance under clear skies because clouds can greatly affect irradiance (Endler 1993) and I was concerned with capturing the variation of light environment between habitat types, not the effects of weather. Lastly, measured irradiance for two

different groups of measurements: 1) irradiance in the three different sections of Pipeline Road (i.e. 0-3.5km, 3.5-7km, 7-10.5km); 2) for a selected group of 10 individuals for each mimetic group. For group 1, I measured the irradiance on the road at distances of 2 km on three separate days between 7:30 am and 11:30 am, which is peak activity time for *Heliconius* butterflies (Devries 1987). For each 2 km section, I measured irradiance every 1 m for 200 meters, see figure 2. For group 2, I found *Heliconius* individuals and measured the irradiance of where they perched. This usually included following individuals until they perched on a nectar source and I was restricted to measuring irradiance of individuals that perched within 3 meters of the ground. Irradiance was measured three times for each individual and only during clear skies.

Using an Ocean Optics USB 2000 spectrometer (Dunedin, FL) connected to a 400μm fiber optic cable (Ocean Optics, Dunedin, FL) with a cosine corrected irradiance probe (CC3, Ocean Optics, Dunedin, FL) pointed towards the sky, I measured irradiance from 300 nm to 700 nm for each group of measurements. Measurements were recorded with Spectrasuite software (Ocean Optics, Dunedin, FL). For each irradiance measurement I set an appropriate integration time that increased the signal to noise ratio as well as reducing the saturation of the spectrometer. The integration time ranged from 3 milliseconds in bright gap environments to over 2 seconds under full canopy.

The irradiance data were first converted to photons/cm^2/s/nm and brightness and the hue extracted from the spectra with *pavo* in R (Maia et al. 2013; Team 2014). Brightness was calculated as the integral under the spectral curve from 300 nm to 700 nm. Hue was calculated as the wavelength at the midpoint between the maximum and minimum photon flux of the spectrum, (Montgomerie 2006). For group 1, the Pipeline

Road irradiance, I ran a linear regression on both brightness and hue as a function of distance along Pipeline Road. For group 2, the individual butterfly irradiance, I ran ANOVAs for both brightness and hue for each mimetic pair.

Mimetic Wing Reflectance and Quantification

Eight males and eight females with little wing wear were collected from Soberania National Park for each species during 2011 and 2012. Individuals were euthanized by freezing and then stored in glassine envelopes. I measured the reflectance of the dorsal surface of both the hindwing and forewing for eight males and eight females for each of the four species: *H. cydno*, *H. sapho*, *H. erato*, and *H. melpomene*. The dorsal surface was measured because these species are day-flying and the dorsal surface is likely the most visible to avian predators. Reflectance was measured three times for each dorsal color patch on both the hindwing and forewing. For Postman individuals, I measured the red and black of the dorsal forewing, and the yellow and black of the dorsal hindwing, see figure 3. For the Blue-white butterflies, I measured the blue, white, and black of the forewing and the blue and white of the hindwing, see figure 3.

The color patches were measured with a USB 2000 spectrometer (Ocean Optics, Dunedin, FL) with a PX-2 pulsed xenon light source (Ocean Optics, Dunedin, FL, USA) to measure a circular reflectance area of approximately .8 cm^2. For all non-blue patches, reflectance spectra were measured in a dark room with the light path of the collecting probe normal to the wing surface and were measured relative to a Spectralon diffuse reflectance white standard (Ocean Optics, Dunedin, FL, USA). I captured reflectance spectra from 300nm to 700nm using Spectrasuite software (Ocean Optics, Dunedin, FL,

USA) to collect reflectance. The blue coloration of the Blue-white individuals is iridescent and therefore the reflectance of the iridescent blue patch is a function of the angle of illumination and collection (Meadows et al. 2011). The same spectral equipment and software were used for measuring the blue iridescence, however, I used an optical table designed to measure iridescence that allows for controlling both the angle of illumination and collection. To standardize for angle among all individuals, I measured the blue reflectance at its brightest signal for all individuals, see Rutowski et al. 2010b and Meadows et al. 2011 for specific protocols.

All butterfly reflectance spectra were processed in the R package *pavo* (Maia et al. 2013). Each reflectance spectrum was smoothed and any negative values were set to zero, which occurred only in the black reflectance patches near 300 nm. Each butterfly patch was averaged among the three measurements. To determine if color patches differed between species and sex, I extracted brightness (B1), hue (H4), and chroma (S5). Brightness (B1) was calculated as the integral of the spectrum from 300nm to 700nm. Hue (H4) and chroma were calculated with H4 and S5, respectively, because these metrics use segment analysis from 300nm to 700nm and are less affected by noisy spectra or multiple peaks (see Montgomerie 2006 for equations). Negative values of H4 indicate hues rich in short wavelengths (e.g. UV and blue), while positive values indicate hues rich in longer wavelengths (e.g. yellow and red). Chroma is a measurement of spectral purity and the values of S5 range from 0 (low chroma, e.g. white) to 1 (high chroma, e.g. monochromatic red). Differences within the three color metrics were tested using ANOVAs with sex and species as between factors. All metrics were normally distributed

and had similar variances between factors as was revealed by qqplots and Shapiro-Wilk test.

Background Vegetation Reflectance

To quantify the visual background for *Heliconius* butterflies, leaves from known nectar sources of *Heliconius* were collected for reflectance measurements. I collected leaves from C*ephaelis* species and from *Lantana* species along Pipeline Road in the wet season of 2011. *Heliconius* butterflies frequent flowers from the genera *Cephaelis* and *Lantana* and it is common to find *Heliconius* individuals perched on these species of plants (Brown 1981; Estrada and Jiggins 2002). The reflectance methods above were used to quantify leaf spectral reflectance composition and I measured three locations on each of 41 leaves from separate plants. These reflectance measurements were then incorporated into the avian visual models.

Perceived Contrast of Color Patches with Avian Visual Color Space

Several models have been constructed to understand how colors are perceived by an individual's visual system (Chittka et al. 1993; Vorobyev and Osorio 1998; Endler and Mielke 2005). These models calculate how a given color would stimulate photoreceptors with different spectral sensitivities. Furthermore, these visual models assume that the achromatic (brightness) component and the chromatic component (hue and chroma) are processed independently and thus these models produce estimates of both achromatic perception and chromatic perception. Here I used the Vorobyev and Osorio (1998) receptor noise threshold model.

For full modeling details, see Vorobyev and Osorio (1998); here I briefly describe the model. Visual models calculate the quantum catch for each photoreceptor, of the

patch reflectance spectrum illuminated by a specific light environment. Once each photoreceptor's quantum catch has been calculated, color distances (Just Noticeable Differences (JND)) are calculated by weighting the Euclidean distance of the photoreceptor quantum catches by the Weber fraction of the cones. These color distances represent the ability for an individual to perceive two color patches as different; a JND of less than one indicates than an individual would not be able to perceive a difference between two colors under optimal viewing conditions, while high JNDs indicate that two colors would be conspicuous and have high contrast (Vorobyev and Osorio 1998; Siddiqi et al. 2004). Furthermore, the visual models calculate both the chromatic JND (ΔS) and the achromatic JND (ΔL).

All birds are tetrachromats, that is, they have four different cone types. The short (S), the mid (M), and the long (L) wavelength cones vary little between bird species. However, birds have a fourth cone that is either sensitive to ultraviolet (UV) or is sensitive to violet but not ultraviolet (V). Among the various putative avian predators of tropical butterflies (Pinheiro 2011), flycatchers and jacamars have violet-sensitive color vision (V/VIS), while other insectivorous birds may have ultraviolet-sensitive color vision (UV/VIS) (Hart 2001; Endler and Mielke 2005; Endler et al. 2005). Therefore, I used two different avian visual system models for chromatic contrast: V/VIS and UV/VIS. For achromatic contrast, birds rely on the double-cones, which are responsible for perception of brightness-contrast (Hart 2001; Hart and Hunt 2007). The V/VIS and UV/VIS systems have the same double-cone sensitivity and therefore, achromatic contrast will be the same between visual systems.

To determine if the mimetic butterflies might be perceived differently in their respective habitats by the two different visual systems, I calculated chromatic and achromatic contrasts for each patch against a leaf background and I also calculated internal chromatic and achromatic contrast by comparing two color patches of an individual. For the Postman individuals I calculated the internal contrast for both the red patch and the yellow patch compared to the black, as the black surrounds the yellow, and the red patches. The internal contrast for the Blue-white butterflies consisted of the blue and black patches compared to the white patch, as the white borders the black, and blue patches. Thus, internal contrast is the contrast between neighboring patches of each mimetic color pattern.

All visual modeling was performed using the R package *pavo* (Maia et al. 2013). The quantum catches for each photoreceptor were calculated for each wing patch and each leaf for both an average UV/VIS visual system and an average V/VIS visual system for two different light environments: open (the beginning of PLR) and closed (end of PLR). Then the chromatic and achromatic JNDs were calculated for each comparison: color patch against leaves and color patch against color patch.

To determine if there were differences between visual system (UV/VIS and V/VIS), I ran linear regressions on the normally distributed data for both the chromatic contrasts and achromatic contrasts. And to test if there were differences between the perceived chromatic and achromatic contrasts of the different patch comparisons, I ran ANOVAs and then Tukey's Post Hoc comparisons.

Effects of Light Environment on Perceived Contrast

Each contrast was calculated with both an open light environment irradiance and with a closed light environment irradiance enabling for tests of light environment differences on perceived contrast. Each pair of contrasts (e.g. Postman red patch against vegetation in open habitat light and Postman red patch against vegetation in closed habitat light) was tested to determine if light environment affected perceived achromatic and chromatic contrasts. The differences between the contrasts from open and closed habitats were normally distributed, allowing for paired t-tests.

Results

Mimicry Distribution

During 120 hours of surveying along Pipeline Road, I caught 298 butterflies and species composition differed among the three sections along Pipeline Road ($X^2_{4,298}$=7.9, p<.05, figure 4). For each species, the sex ratio (males/females) of collected individual was male biased: 1.67 for *H. sapho,* 1.74 for *H. erato*, 2.58 for *H. cydno*, and 1.77 for *H. melpomene*. Furthermore, the sex ratio was consistent among the PLR sections. The Postman ring was found mostly within the first 3.5 kilometers of the road and the Blue-white was found mostly after 7 kilometers.

Habitat Light Environment Differences

Both canopy openness and irradiance differed as a function of distance along Pipeline Road. The canopy was significantly more open at the beginning of the road and then exponentially decreased with distance (the best fit model is openness = 17.06 + $67.95^{(-0.81)(\text{distance})}$, the difference between road beginning and end p<0.001, figure 5).

Furthermore, the first 2 km along Pipeline Road were more open (40-80% openness) relative to the last 8.5 km (12%-25% openness).

The irradiance differed in overall brightness and spectral composition among the sections of Pipeline Road (Brightness: $F_{2,1197}$=1362, p<0.001; Hue: $F_{2,1197}$=454, p<0.001, figure 6). For brightness (B1), the beginning of Pipeline Road was significantly brighter than the other sections (p<0.001 for both comparisons), while the brightness did not differ between the mid and end sections (p=0.956). For hue (H3), each section of the habitat was significantly different from each other with the beginning section having the longest wavelength composition and the end having the shortest wavelength composition (p<0.001 for all comparisons, figure 6).

Heliconius Individual Light Differences

I was able to measure the irradiance at capture location for 10 individuals of each mimicry ring; however, the species and sex for each mimicry ring were not equally sampled. For the blue-white mimicry ring I measured irradiance for five male *H. cydno*, one female *H. cydno*, and 4 male *H. sapho.* For the postman mimicry ring I measured irradiance for four female *H. erato*, five male *H. erato*, and one male *H. melpomene.* Irradiance brightness (B1: $F_{1,18}$=12.91, p=0.002), but not hue (H3, $F_{1,18}$=1.231, p=0.282), at capture location was greater for the Postman mimics than the Blue-whites.

Mimetic Wing Color Reflectance

The reflectance of color patches differed significantly among the mimetic groups, see figure 3 and table 1. The Blue-white mimicry ring differed from the postman in overall brightness ($F_{1,318}$=28.24, p<.001), hue ($F_{1,318}$=279.9, p<.001), and chroma ($F_{1,318}$=232.9, p<.001). Specifically, the white patches of the Blue-white mimics were the

brightest, followed by the yellow and then red patches of the Postman mimics, see figure 3. The Blue-white blue patches were only brighter than the black patches.

The color patch reflectances within each mimicry ring were not significantly different from each other for species and sex, with the exception of the chroma for the red patch between Postman male and females, the hue of the black patches between the Postman species, and the chroma of the black patches between the Blue-white species, see figure 3.

Perceived Conspicuousness of Mimicry Rings

The avian visual models revealed that the UV/VIS visual system perceived the butterflies with higher internal chromatic contrast than the V/VIS visual system in both the open habitat ($F_{1,254}$=5.29, p=0.022) and the closed habitat ($F_{1,254}$=4.343, p=0.038). The visual systems also differed in the ability to perceive the chromatic contrast of the wing patches against vegetation with the UV/VIS system having much higher chromatic contrast values than the V/VIS in both open ($F_{1,638}$=367.8, p<0.001) and closed ($F_{1,254}$=375.0, p<0.001) environments. As expected, both the internal and patch-against-vegetation achromatic contrasts were the same between visual systems because the same double cone photoreceptor absorbance spectrum was used.

The perception of wing color patches viewed against forest vegetation differed, regardless of habitat, among patches for both chromatic ($F_{9,630}$=405.1, p<.0001) and achromatic contrast ($F_{9,630}$=82.9, p<0.001), see figures 7-10. Post hoc comparisons revealed that, for both visual systems, the red patch had the highest chromatic contrast, followed by the blue, the yellow, the white and then the black, see figures 7-8. The achromatic contrast was greatest for the blue and black patches, which did not

significantly differ from one another. The Postman yellow patch and Blue-white white patch had intermediate levels of achromatic contrast, while the Postman red patch had very low achromatic contrast. These trends did not change with habitat lighting.

Effects of Light Environment on Conspicuousness

The habitat lighting affected perceived contrast for both visual systems in 47/56 comparisons, see tables 2 and 3; figures 7-10. However, not all habitat lighting increased perceived contrast and was dependent upon the contrast type (e.g. patch vs. vegetation), and the visual system. The Blue-white chromatic contrast was higher in the closed habitat for 5/7 comparisons for the UV/VIS system and only 4/7 for the V/VIS system, see tables 2 and 3, and figures 7-10. The achromatic contrast of the Blue-white mimics was higher in the closed habitat for 4/7 comparisons for both visual systems. The Postman mimics were less conspicuous in their respective environment with having only 2/7 and 3/7 contrasts increased by the open environment, for the V/VIS and UV/VIS systems respectively. Achromatically, the Postman mimics had increased conspicuousness in only 2/7 comparisons for both visual systems, see tables 2 and 3, and figures 7-10.

Discussion

Overall

Previous research has shown that diversity in warning signals among individuals will lead to greater predator confusion, mistakes, and thus higher mortality (Müller 1878; Mallet and Barton 1989; Kapan 2001; Rowland et al. 2010). However, much diversity in warning signals does occur and has led to much controversy on the evolutionary implications of Müllerian mimicry (Papageorgis 1975; Mallet and Gilbert 1995; Joron

and Mallet 1998; Joron and Mallet 1999). Here I demonstrate that although different warningly colored species may have the same geographical distribution on a large scale, species are distributed in different habitats that differ in forest composition and light environment. Furthermore, this study has revealed that *Heliconius* mimetic pairs segregate by habitats that increase conspicuousness of their warning colors.

Mimicry Ring Distribution

I first investigated whether mimicry complexes were segregated by microhabitat along Pipeline Road to reveal that the Postman mimics were most abundant along the first 3.5 km, while the Blue-white mimics were most common after 7.5 km on Pipeline Road. These findings confirm previous results and imply that individuals within mimetic pairs are more ecologically similar than between mimicry rings (Papageorgis 1975; Mallet and Gilbert 1995; Estrada and Jiggins 2002). Previous research on these two mimetic pairs has revealed that these mimetic pairs also converge on behavior including roosting gregariously with co-mimetic individuals (Mallet and Gilbert 1995). Thus, it is likely that within the microhabitat that each mimetic pair occurs, specialist avian predators are more familiar with the specific warning coloration of that microhabitat – e.g. open habitat birds would be more averse to attack the Postman mimics than the Blue-white mimics.

The specific mechanism for the observed differences in abundances was not investigated in this study and there are several possibilities. The community of avian predators in edge habitats is different from that in forested habitats, and the different avian predators could be selecting against the less abundant mimetic form in each habitat (Angehr and Dean 2010). Mallet and Barton (1989), and Kapan (2001) both

demonstrated that mimetic *Heliconius* individuals translocated to an area with non-mimetic *Heliconius* experience higher predation rates compared to translocated *Heliconius* individuals that matched the local mimetic pattern. Alternatively, larval and adult foodplant distributions may be at play. *Heliconius* larvae feed on *Passiflora*, and coevolution between the host plant and larvae has resulted in *Passiflora* specialists among the different species of *Heliconius* (Brown 1981; Cardoso and Gilbert 2013a). *Heliconius* individuals within mimetic pairs do not compete for *Passiflora* hostplants, while there is competition between species from the different mimetic pairs (Brown 1981; Devries 1987). Thus it is unlikely that hostplant distribution explains the disparate distributions in these mimetic pairs. However, Estrada and Jiggins (2002) found that although the *Heliconius* species studied here all fed from the same species of flowers (*Cephaelis spp* and *Lantana spp*), mimetic pairs differed in the relative proportion of different nectar sources and nectar foraging appears to be innate (Salcedo 2011). The floral species proportion did differ along the Pipeline Road gradient and therefore, it is plausible that the distribution of *Heliconius* is due to differences in nectar resources between microhabitats. Lastly, abiotic factors such as temperature, humidity, and lighting, may explain the abundance differences as these are likely different between the microhabitats.

Habitat Light Environment Differences

The canopy cover along the Pipeline Road change substantially as distance from the start increased. This finding is likely due to the road following a precipitation gradient that results in twice as much rain on the Caribbean coast than on the Pacific coast (Pyke et al. 2001; Santiago et al. 2004). The data reveal that there is a sharp decrease in canopy

openness near 2 km and then the canopy cover only slightly increases with distance. The fourfold decrease in canopy openness from the beginning of Pipeline Road to the end is likely a significant factor affecting not only predation pressures, but also behavior of *Heliconius* butterflies. Although little is known about how shade and sunlight directly affect *Heliconius* species, research in other butterflies demonstrates the importance of shade and access to sunlight in thermoregulation and mating (Kingsolver 1985; Bergman and Wiklund 2009; Kleckova et al. 2014). Thus, the much greater shade in the habitat of the Blue-white butterflies compared to the Postman is likely to be biologically relevant regardless of predator avoidance due to different thermal and humidity requirements.

The changes in the brightness and spectral composition of the ambient lighting from the beginning of the road to the end correlate with the changes canopy cover. As distance increased along Pipeline Road the irradiance becomes darker and rich in middle wavelengths of light (i.e. green). In the sample of irradiance measurements for individuals, the Postman individuals were found in brighter light environments than the Blue-white individuals. However, there was no difference in the spectral composition between mimetic pairs. The small sample sizes presented here may have not been able to detect spectral composition differences between mimetic pairs. Light environments can be ephemeral in the rainforest and the small sample size presented here is preliminary. To further understand the specific preference and role that light plays into the lives of these butterflies requires more field and laboratory experiments.

Mimetic Wing Reflectance

Overall, individuals within each mimetic pair match the spectral reflectance well and are unlikely to be perceived differently dependent upon sex or species. However, the

red patch of the Postman mimicry ring differed in chroma (i.e. spectral purity) between sexes and the black patches differed in both brightness and hue. The findings that the black patches were different between mimetic species is not necessarily indicative of perceptual differences as black (i.e. low brightness across all wavelengths) is difficult to accurately assess with color metrics and the significant findings here may be non-biologically relevant (see Dalrymple et al. 2014). The precise mimicry within mimetic pairs is rare in nature as many mimetic assemblages resemble each other imprecisely (Kikuchi and Pfennig 2013; Thurman and Seymoure 2016). *Heliconius* mimics are strongly similar and research has shown that the same developmental pathways and pigment production mechanisms are shared within the genus (Beldade and Brakefield 2002; Reed et al. 2011).

Perception of wing reflectance

Overall, the achromatic and chromatic contrast depended upon the viewer, with the UV/VIS perceiving higher chromatic contrast than the V/VIS. The contrasts also depended upon the patch color and the background (i.e. vegetation or, black or white wing). Both mimetic pairs had high chromatic contrasts by the UV/VIS system. The colorful patches (i.e. red, yellow, and blue) all had high chromatic contrast, while the non-colorful patches (i.e. black and white) had high achromatic contrast.

Previous work has shown correlations between the coloration of aposematic butterflies and the light environments in which they are found. Butterflies that fly in open habitats tend to have chromatic signals (e.g. red and yellow) while butterflies from closed habitats have achromatic signals (e.g. black and white) (Douglas 2013). These data support Douglas (2013) when considering the red and yellow Postman patches, and

the black and white patches of the Blue-white individuals. However, an exception is the blue patch which is highly chromatic but found on butterflies that live in a closed habitats. Perhaps the directional nature of the blue reflectance and may be perceived as blue or black depending on the lighting and viewing angles (Stevens 2013). Thus, natural viewers may rarely perceive the iridescent patch at its highest chromatic contrast which is what I measured. Further investigation into the role of iridescence as a warning signal is needed, but see Rutowski et al. 2013; Pegram and Rutowski 2014; Pegram et al. 2015.

Our results show that the discrimination of different color patches of both mimetic pairs depends upon the viewer's visual system. The ultraviolet-sensitive avian visual system was better at discriminating between both the wing patches and the vegetative background, and the colorful wing patches against the black or white wing patches. The increased discrimination by the ultraviolet system is due to the ultraviolet components of color patches of *Heliconius*. The findings here confirm previous research demonstrating that ultraviolet sensitive individuals are better at discriminating other species of *Heliconius* and their non-*Heliconius* mimics than violet sensitive viewers (Llaurens et al. 2014; Thurman and Seymoure 2016). The best documented predators of *Heliconius* butterflies are jacamars (*Galbula spp*) and tyrant flycatchers (Tyrannidae) (Chai 1986; Langham 2004), and both of these avian groups are expected to have a violet-sensitive visual system (Hart 2001; Odeen and Hastad 2003; Endler and Mielke 2005; Llaurens et al. 2014). Thus, the mimetic butterflies are perceived with less contrast to their environment and within the wing color patterns by their avian predators.

The color patterns and visual signals of *Helicionius* butterflies have evolved in the context of predator avoidance as well as through mate recognition (Jiggins et al. 2001;

Jiggins et al. 2004; Merrill et al. 2011; Finkbeiner et al. 2014). The higher discriminability of wings by ultraviolet sensitive viewers is most likely very important in the context of mate recognition as *Heliconius* butterflies have been shown to have a duplicated UV-sensitive visual pigment (Briscoe et al. 2010; Bybee et al. 2012). Thus, *Heliconius* conspecifics are likely able to recognize one another better than either avian visual system, a beneficial adaptation to increase individual mating success (Bybee et al. 2012; Finkbeiner et al. 2014). Of course, more research is needed to delve into the visual perception of *Heliconius* butterflies and how their increased ultraviolet sensitivity functions to detect mates.

Effects of Light Environment on Conspicuousness

Habitat lighting affected conspicuousness for most of the calculated contrasts reported here; however, not all habitat lighting affected the conspicuousness in the predicted direction. I predicted that the Blue-white individuals would be more conspicuous in their respective environment of forested shade, while the Postman individuals would be more conspicuous in the open habitat. However, the analysis revealed that only the Postman yellow against vegetation and the Postman internal red had higher achromatic contrast in the open habitat compared to the closed. The Blue-white mimics had greater achromatic contrast for all non-white patches against vegetation and for the internal black and white comparison. However, the fact that the coloration of *Heliconius* individuals is not more achromatically conspicuous in their relative environments is not surprising because the Postman individuals have much higher chromatic contrast independent of habitat compared to the higher achromatic contrast of the Blue-white individuals.

The effects of light environment on the chromatic component of the warning signal also had mixed support for my predictions. The color patches against green foliage were mostly more chromatically conspicuous in the closed habitat. However, the internal chromatic contrast was higher in the open habitat for the red Postman patch and the blue patch of the Blue-whites. Thus, each mimetic pair has internal patches that are strengthened in each habitat type. The Postman red is more chromatically conspicuous in the open habitat, while the yellow is more chromatically conspicuous in the closed habitat. The Blue-white blue patch is more chromatically conspicuous in the closed habitat while the black patch is more conspicuous in the open habitat.

Our predictions were tested strictly using known physiological parameters of avian perception that assesses contrast between two objects for both chromatic and achromatic channels. However, these two channels are not mutually exclusive of each other and likely interact (Renoult et al. 2015). Furthermore these models are conservative and to fully understand the role of the habitat lighting on predator perception of mimetic individuals, experiments in the field and with predators and prey in captivity are needed.

Diversity of Mimicry

The diversity of coloration in Müllerian mimicry is paradoxical as individuals should converge on the same warning signal to reduce individual costs of predator education. However, many examples of diverse mimetic assemblages occur throughout the world and here I proposed and tested the hypothesis that microhabitat segregation of two mimetic pairs affects predator perception of warning coloration. The Blue-white mimics occupied forested habitats comprised of dim and mid-wavelength rich light, while the Postman mimics occupied open habitats comprised of bright and full spectrum light.

The two different mimicry rings matched the predictions that open habitat individuals would be chromatically conspicuous, while closed habitat individuals would be achromatically conspicuous. Also, the different light environments did affect the perceived conspicuousness of the wing coloration although, each mimetic pairs had wing patches that were more conspicuous in each environment.

The modeling data presented here supports that different coloration is more conspicuous in different light environments. However, to test if differently colored individuals receive a fitness benefit by occurring in an environment where their warning signal is most conspicuous, tests with predators and potential mates are necessary. In chapter two, I test if predation differs for each mimicry ring dependent upon habitat to show that the habitat resident is attacked more in its respective habitat. Thus, predation may not be a major selective force for diverse warning coloration, nor the different distribution that was shown here. It is likely that mate choice may be selecting for conspicuous signals in different environments as I show in chapter three that male *Heliconius* individuals seek the light environment in which they are found in nature (e.g. Postman in open). Furthermore, recent research has shown that *Heliconius* wing coloration is used for mate choice (Finkbeiner et al. 2014). Thus, future investigation into the conspicuous of *Heliconius* coloration as seen by conspecifics is needed in both modeling and behavioral studies. I conclude that microhabitats are a factor contributing to the diversity of mimetic assemblages and that more research is needed into predator and conspecific behavior in the context of light environment and microhabitats.

Table 1. ANOVA results for three wing-reflectance metrics (see text for description of abbreviations) for each mimetic group. Highlighted p-values indicate significance at an alpha level of 0.05. Brightness (B1), hue (H4), and chroma (S5), were all significantly different for the different color patches, but sex and species did not differ for the three metrics except for Blue-white species chroma (S5) and Postman sex chroma (S5) and species hue (H4).

	B1			H4			S5		
Blue-white mimicry ring	F	df	p	F	df	p	F	df	p
Patch	1145	4,155	<0.001	48.43	4,155	<0.001	61.87	4,155	<0.001
Sex	0.025	1,158	0.873	0.96	1,158	0.328	2.25	1,158	0.135
Species	0.002	1,158	0.970	0.40	1,158	0.524	8.53	1,158	0.004
Postman mimicry ring	F	df	p	F	df	p	F	df	p
Patch	573.9	4,155	<0.001	143.4	4,155	<0.001	69.4	4,155	<0.001
Sex	0.026	1,158	0.873	2.482	1,158	0.117	5.48	1,158	0.02
Species	0.114	1,158	0.736	5.522	1,158	0.02	0.09	1,158	0.7703

Table 2. T-tests and confidence intervals for achromatic comparisons of JNDs between open and closed habitat for the UV/VIS visual system. Ave Diff represents the average difference in JNDs between the two patches being compared. Conf Int represents the lower and higher bounds, respectively. Highlighted p-values indicate significance at an alpha of 0.05. Highlighted directions indicate that the direction matches the mimetic pair's respective habitat (i.e. the Postman butterflies are more conspicuous in the open habitat or the Blue-whites are more conspicuous in the closed habitat). All comparisons have degrees of freedom of 31.

			UV/VIS Achromatic					
Mimicry	**Patch 1**	**Patch 2**	**t**	**Ave Diff**	**Conf Int**		**p**	**Direction**
Postman	**HW Yellow**	**Leaves**	70.68	-0.38	-0.3904	-0.3685	0.000	open
Postman	**FW Red**	**Leaves**	1.56	0.07	-0.0209	0.1576	0.129	closed
Postman	**FW D Black**	**Leaves**	61.58	0.44	0.4293	0.4588	0.000	closed
Postman	**FW P Black**	**Leaves**	87.60	0.44	0.4302	0.45078	0.000	closed
Postman	**HW Black**	**Leaves**	89.13	0.45	0.4357	0.45617	0.000	closed
BW	**FW Blue**	**Leaves**	35.97	0.34	0.3212	0.3599	0.000	closed
BW	**FW Black**	**Leaves**	40.29	0.39	0.3737	0.4135	0.000	closed
BW	**FW White**	**Leaves**	123.85	-0.35	-0.351	-0.34	0.000	open
BW	**HW White**	**Leaves**	48.45	-0.32	-0.3347	-0.3077	0.000	open
BW	**HW Blue**	**Leaves**	39.77	0.34	0.3182	0.3526	0.000	closed
	Internal Contrast							
Postman	**HW Yellow**	**HW Black**	11.49	0.05	0.0403	0.05775	0.000	closed
Postman	**FW Red**	**FW P Black**	15.41	-0.40	-0.4497	-0.3446	0.000	open
BW	**FW Black**	**FW White**	17.24	0.07	0.0636	0.0807	0.000	closed
BW	**FW Blue**	**FW White**	0.70	0.00	-0.0183	0.0089	0.490	open

Table 3. T-tests and confidence intervals for chromatic comparisons of JNDs between open and closed habitat for the UV/VIS visual system. Ave Diff represents the average difference in JNDs between the two patches being compared. Conf Int represents the lower and higher bounds, respectively. Highlighted p-values indicate significance at an alpha of 0.05. Highlighted directions indicate that the direction matches the mimetic pair's respective habitat (i.e. the Postman butterflies are more conspicuous in the open habitat or the Blue-whites are more conspicuous in the closed habitat). All comparisons have degrees of freedom of 31.

			UV/VIS Chromatic					
Mimicry	**Patch 1**	**Patch 2**	**t**	**Ave Diff**	**Conf Int**		**p**	**Direction**
Postman	**HW Yellow**	**Leaves**	5.14	-0.08	-0.1118	-0.0483	0.000	open
Postman	**FW Red**	**Leaves**	1.96	-0.01	-0.0212	0.0004	0.058	open
Postman	**FW D Black**	**Leaves**	7.16	0.20	0.1448	0.2603	0.000	closed
Postman	**FW P Black**	**Leaves**	7.48	0.21	0.1499	0.2623	0.000	closed
Postman	**HW Black**	**Leaves**	7.45	0.22	0.1581	0.2772	0.000	closed
BW	**FW Blue**	**Leaves**	7.50	0.11	0.0803	0.1403	0.000	closed
BW	**FW Black**	**Leaves**	7.81	0.09	0.067	0.115	0.000	closed
BW	**FW White**	**Leaves**	22.06	0.05	0.04818	0.058	0.000	closed
BW	**HW White**	**Leaves**	24.62	0.05	0.0442	0.05223	0.000	open
BW	**HW Blue**	**Leaves**	7.73	0.11	0.08285	0.1422	0.000	closed
	Internal Contrast							
Postman	**HW Yellow**	**HW Black**	11.69	-0.42	-0.4916	-0.3455	0.000	open
Postman	**FW Red**	**FW P Black**	3.13	-0.05	-0.076	-0.016	0.004	open
BW	**FW Black**	**FW White**	1.93	0.04	-0.0025	0.0887	0.060	open
BW	**FW Blue**	**FW White**	4.11	0.07	0.03489	0.103	0.000	closed

Table 4. T-tests and confidence intervals for achromatic comparisons of JNDs between open and closed habitat for the V/VIS visual system. Ave Diff represents the average difference in JNDs between the two patches being compared. Conf Int represents the lower and higher bounds, respectively. Highlighted p-values indicate significance at an alpha of 0.05. Highlighted directions indicate that the direction matches the mimetic pair's respective habitat (i.e. the Postman butterflies are more conspicuous in the open habitat or the Blue-whites are more conspicuous in the closed habitat). All comparisons have degrees of freedom of 31.

			V/VIS Achromatic					
Mimicry	**Patch 1**	**Patch 2**	**t**	**Ave Diff**	**Conf Int**		**p**	**Direction**
Postman	**HW Yellow**	**Leaves**	70.68	-0.38	-0.3904	-0.3685	0.000	open
Postman	**FW Red**	**Leaves**	1.56	0.07	-0.0209	0.1576	0.120	closed
Postman	**FW D Black**	**Leaves**	61.58	0.44	0.4293	0.4588	0.000	closed
Postman	**FW P Black**	**Leaves**	87.60	0.44	0.4302	0.45078	0.000	closed
Postman	**HW Black**	**Leaves**	89.13	0.45	0.4357	0.45617	0.000	closed
BW	**FW Blue**	**Leaves**	35.97	0.34	0.321	0.3599	0.000	closed
BW	**FW Black**	**Leaves**	40.29	0.39	0.3737	0.4135	0.000	closed
BW	**FW White**	**Leaves**	123.85	-0.35	-0.3514	-0.34	0.000	open
BW	**HW White**	**Leaves**	48.45	-0.32	-0.3347	-0.3077	0.000	open
BW	**HW Blue**	**Leaves**	39.77	0.34	0.3182	0.3526	0.000	closed
	Internal Contrast							
Postman	**HW Yellow**	**HW Black**	11.49	0.05	0.040344	0.0577	0.000	closed
Postman	**FW Red**	**FW P Black**	15.41	-0.40	-0.449	-0.344	0.000	open
BW	**FW Black**	**FW White**	17.24	0.07	0.0636	0.0807	0.000	closed
BW	**FW Blue**	**FW White**	0.70	0.00	-0.018	0.0089	0.490	open

Table 5. T-tests and confidence intervals for chromatic comparisons of JNDs between open and closed habitat for the V/VIS visual system. Ave Diff represents the average difference in JNDs between the two patches being compared. Conf Int represents the lower and higher bounds, respectively. Highlighted p-values indicate significance at an alpha of 0.05. Highlighted directions indicate that the direction matches the mimetic pair's respective habitat (i.e. the Postman butterflies are more conspicuous in the open habitat or the Blue-whites are more conspicuous in the closed habitat). All comparisons have degrees of freedom of 31.

			V/VIS Chromatic					
Mimicry	**Patch 1**	**Patch 2**	**t**	**Ave Diff**	**Conf Int**		**p**	**Direction**
Postman	**HW Yellow**	**Leaves**	16.22	0.29	0.2543	0.3274	0.000	closed
Postman	**FW Red**	**Leaves**	4.48	0.05	0.02778	0.0742	0.000	closed
Postman	**FW D Black**	**Leaves**	0.99	0.00	-0.0031	0.00897	0.330	closed
Postman	**FW P Black**	**Leaves**	2.35	-0.01	-0.0151	-0.0011	0.025	open
Postman	**HW Black**	**Leaves**	0.57	0.00	-0.008	0.0047	0.570	open
BW	**FW Blue**	**Leaves**	11.03	0.08	0.0655	0.0952	0.000	closed
BW	**FW Black**	**Leaves**	3.90	0.02	0.00754	0.02405	0.000	closed
BW	**FW White**	**Leaves**	0.24	0.00	-0.0067	0.0053	0.810	open
BW	**HW White**	**Leaves**	1.91	0.00	-0.00757	0.00025	0.060	open
BW	**HW Blue**	**Leaves**	9.95	0.09	0.0693	0.1051	0.000	closed
	Internal Contrast							
Postman	**HW Yellow**	**HW Black**	12.85	0.35	0.296	0.4077	0.000	closed
Postman	**FW Red**	**FW P Black**	-12.49	-0.10	-0.118	-0.0848	0.000	open
BW	**FW Black**	**FW White**	-5.68	-0.04	-0.0525	-0.0247	0.000	open
BW	**FW Blue**	**FW White**	4.71	0.07	0.041	0.1046	0.000	closed

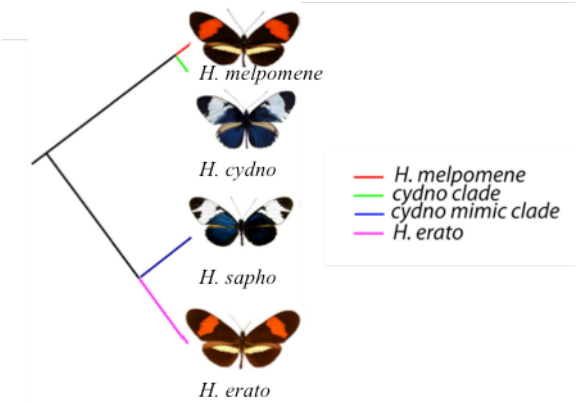

Figure 1. Abbreviated phylogeny of the four species of *Heliconius* species studied in this dissertation and appendix A. These four species comprise two mimicry rings in Panama. The phylogeny is restricted and many other sister species occur within each clade, see Kozak et al. (2015). *H. melpomene* is a comodel with *H. erato* and together they comprise the Postman mimics. The middle two species, each of which are most closely related to Postman species, are *H. sapho* and *H. cydno* and together they comprise the Blue-white mimicry ring.

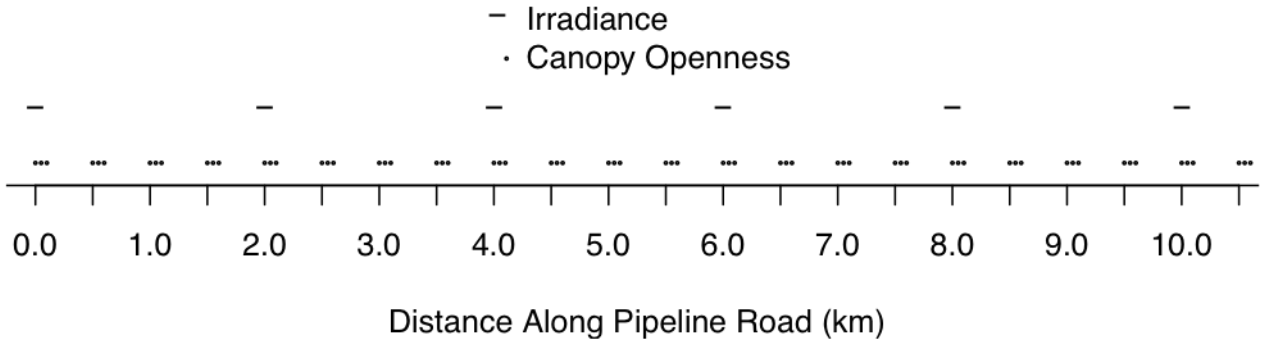

Figure 2. Schematic of sampling locations along the Pipeline Road for canopy openness and irradiance measurements. I measured canopy openness in three locations separated by 50 meters for each 0.5 kilometer area from 0 kilometers to 10.5 kilometers along Pipeline Road. Irradiance was measured at 200 spots separated by 1 meter for each location at 0, 2, 4, 6, 8, and 10 kilometers along Pipeline Road.

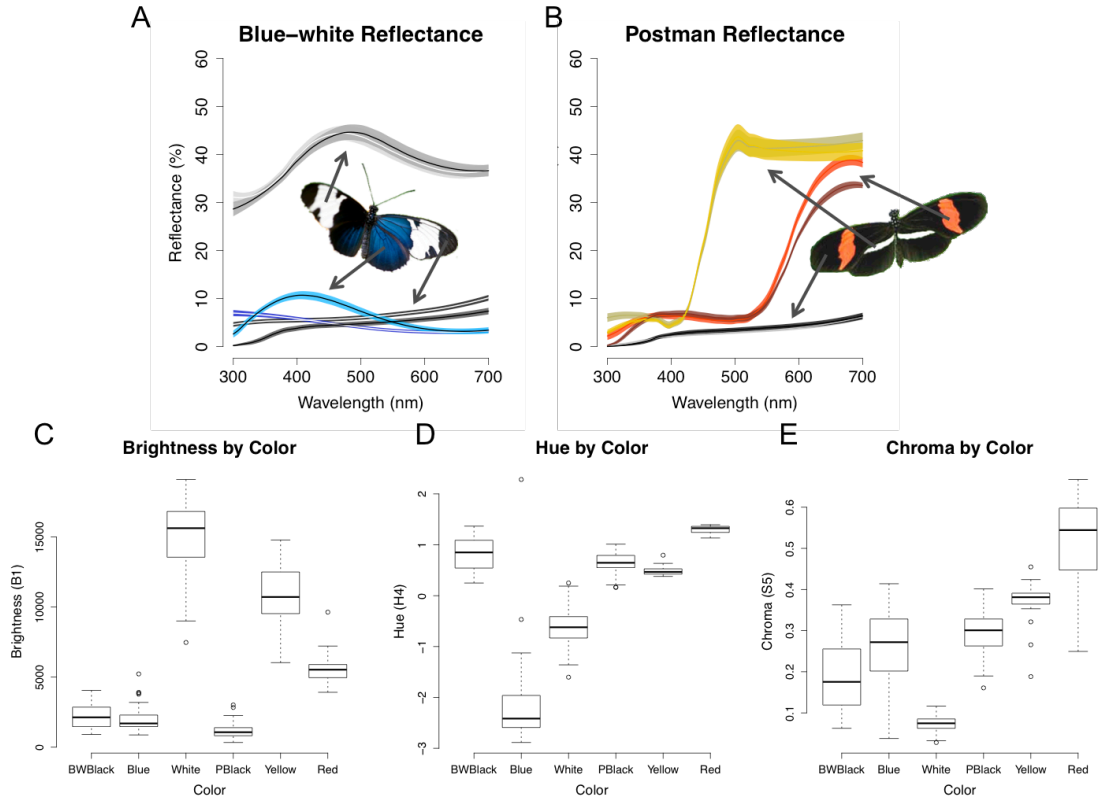

Figure 3. Reflectance and color metrics of color patches of each species for each mimicry ring. A) The reflectance spectra for the white, iridescent blue, and black patches of Blue-white individuals. The darker shaded spectra represent *H. cydno* and the lighter shaded spectra represent *H. sapho*. B) The reflectance spectra for the red, yellow, and black patches for Postman individuals. The darker shaded spectra represent *H. melpomene* and the lighter shaded spectra represent *H. erato.* In both A and B, the shaded region represents 95% confidence intervals and the lines are the means. For figures C-E, the box and whisker plots represent specific color patches for all individuals within each mimicry ring. The three leftmost factors (i.e. BWBlack, Blue, and White) are for the Blue-white mimicry ring while the three rightmost factors (i.e. Black, Yellow, and Red) are for the Postman mimicry ring. The color metrics for each mimetic group's color patches: C) brightness (B1), D) Hue (H4), and E) Chroma (S5).

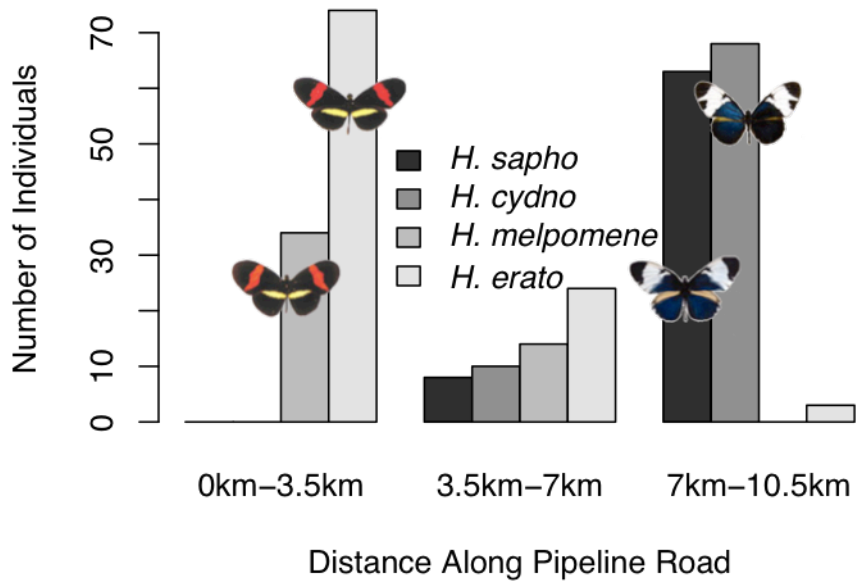

Figure 4. The distribution of individuals for each species at each 3.5 km section along Pipeline Road. The first 3.5 km section only had Postman individuals while the last 3.5 km section had predominately Blue-white individuals. The middle section had lower abundance of each species relative to where each species occurred most, but had the highest species richness.

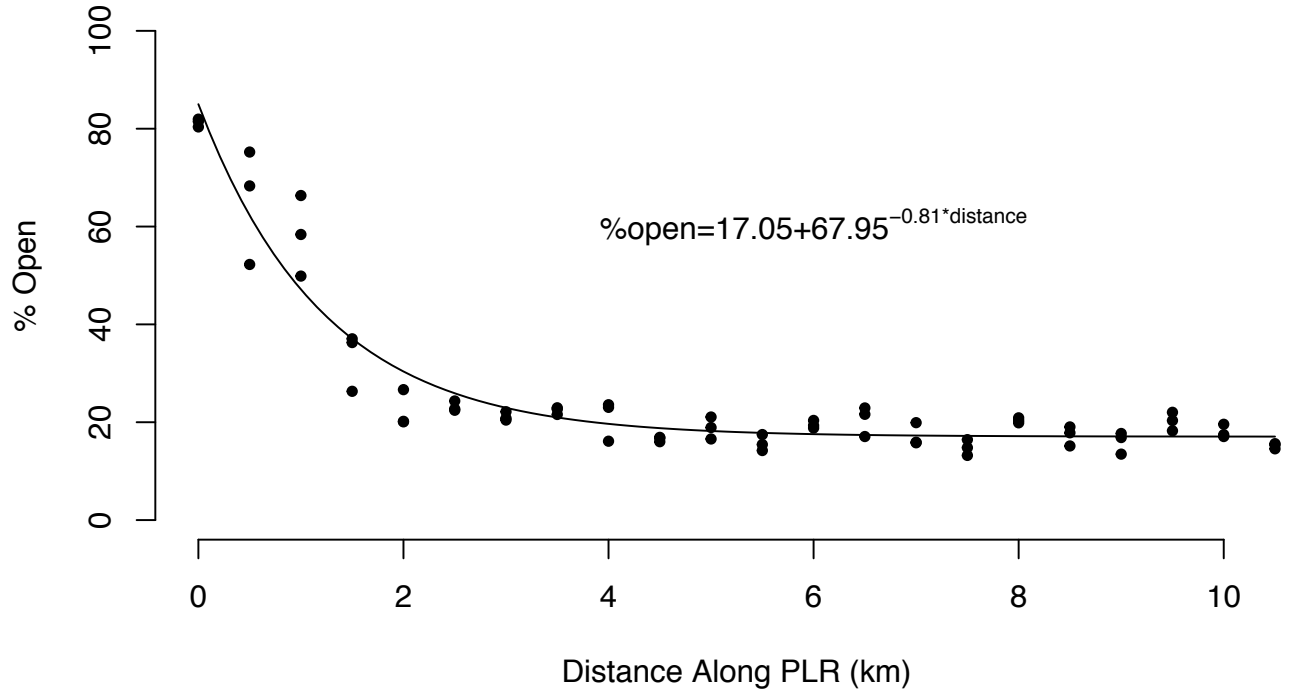

Figure 5. Canopy openness along Pipeline Road (PLR) from 0-10.5 km. The first two kilometers were relatively open compared to the last 8.5 km, which were consistently between 15% and 30% open. The best fit for the canopy openness was exponential and the equation is presented within the figure.

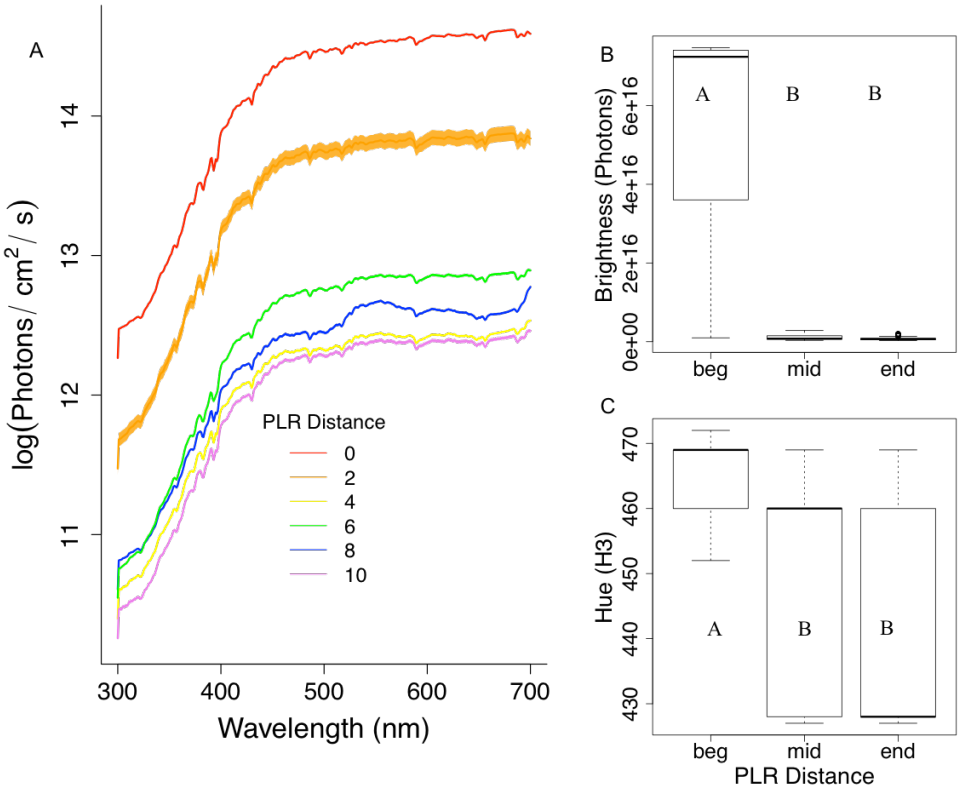

Figure 6. Absolute irradiance and irradiance metrics (brightness and hue) for each habitat section along Pipeline Road. A) The irradiance in log photon for each of the 6 distances along Pipeline Road. For B and C, the distances along Pipeline Road were binned with 0 and 2 km as beginning, 4 and 6 k as middle, and 8 and 10 km as end. Letters within the plots represent significantly different groups. B) The brightness for each section along PLR. The beginning section had significantly greater brightness than the middle and the end, which did not differ from one another. C) The spectral hue for each section along Pipeline Road. Again, the beginning section differed compared to middle and end, which did not differ from each other.

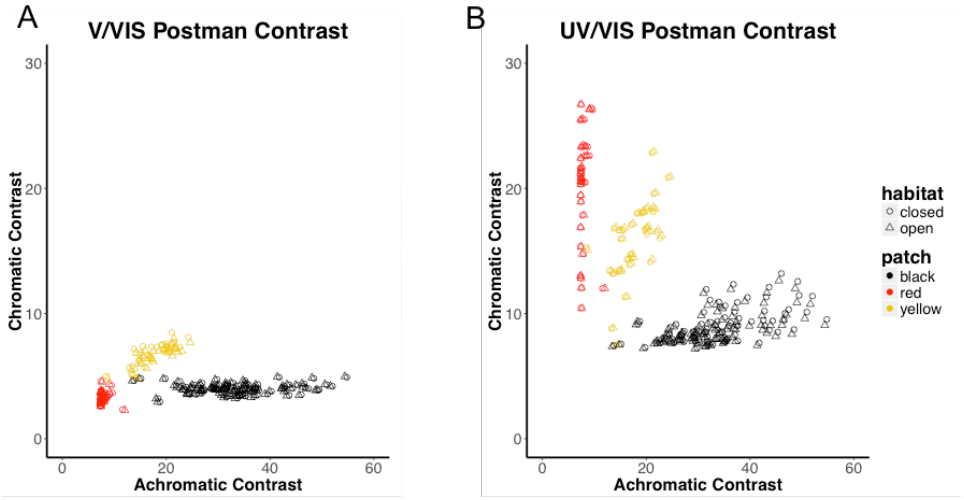

Figure 7. Visual contrast for color patches against vegetation for Postman mimics in the two different habitats for each visual system. The V/VIS visual system contrasts (A) and the UV/VIS visual system contrasts (B) for the red, yellow, and black patches against green vegetation. In each figure, the further a point is from the origin the more conspicuous it is relative to a green background. Thus, the conspicuousness of the patch is dependent on patch color and visual system. The V/VIS system has low chromatic contrast for all patches, but high achromatic contrast for the black patch. The UV/VIS system has high chromatic but low achromatic contrast for the red and yellow patches. Lastly, the different habitats affect the conspicuousness of the perceived patches as is demonstrated by the circles (closed habitat) and the triangles (open habitat) not matching. The red and yellow patches are more conspicuous in the open environment, while the black patch is more conspicuous in the closed.

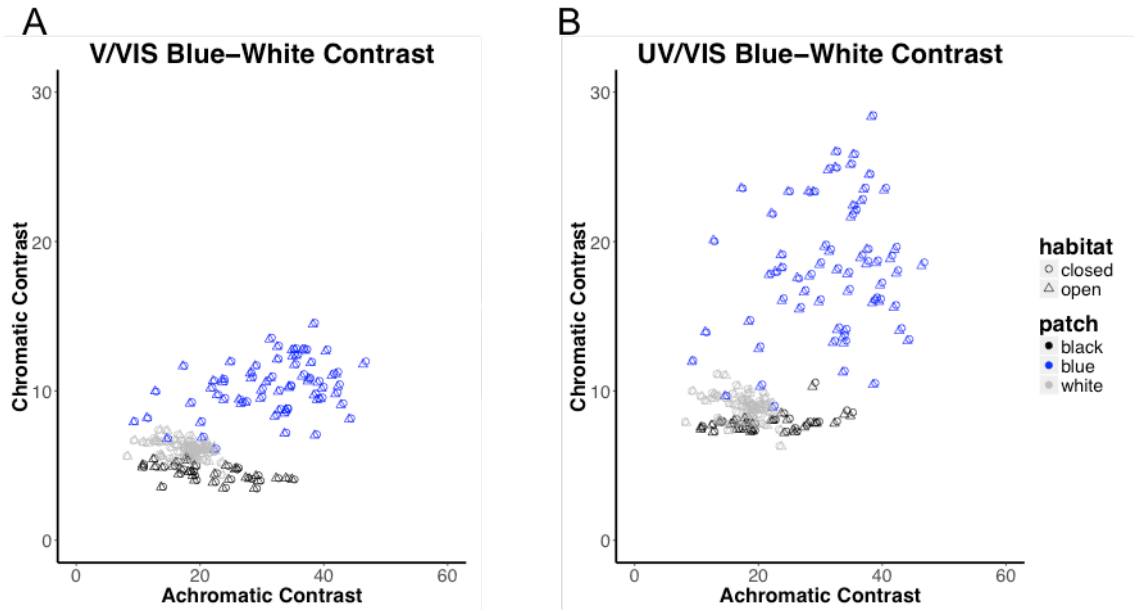

Figure 8. Visual contrast for color patches against vegetation for Blue-white mimics in the two different habitats for each visual system. The V/VIS visual system contrasts (A) and the UV/VIS visual system contrasts (B) for the blue, white, and black patches against green vegetation. In each figure, the further a point is from the origin the more conspicuous it is relative to a green background. Thus, the conspicuousness of the patch is dependent on patch color and visual system. The V/VIS system has low chromatic but high achromatic contrast for the black and white patches, and high achromatic and chromatic contrast for the blue patch. The UV/VIS system has high chromatic and achromatic contrast for the blue patch, while the white and black have low chromatic contrast. Lastly, the different habitats affect the conspicuousness of the perceived patches as is demonstrated by the circles (closed habitat) and the triangles (open habitat) not matching. All patches are more conspicuous in the closed habitat.

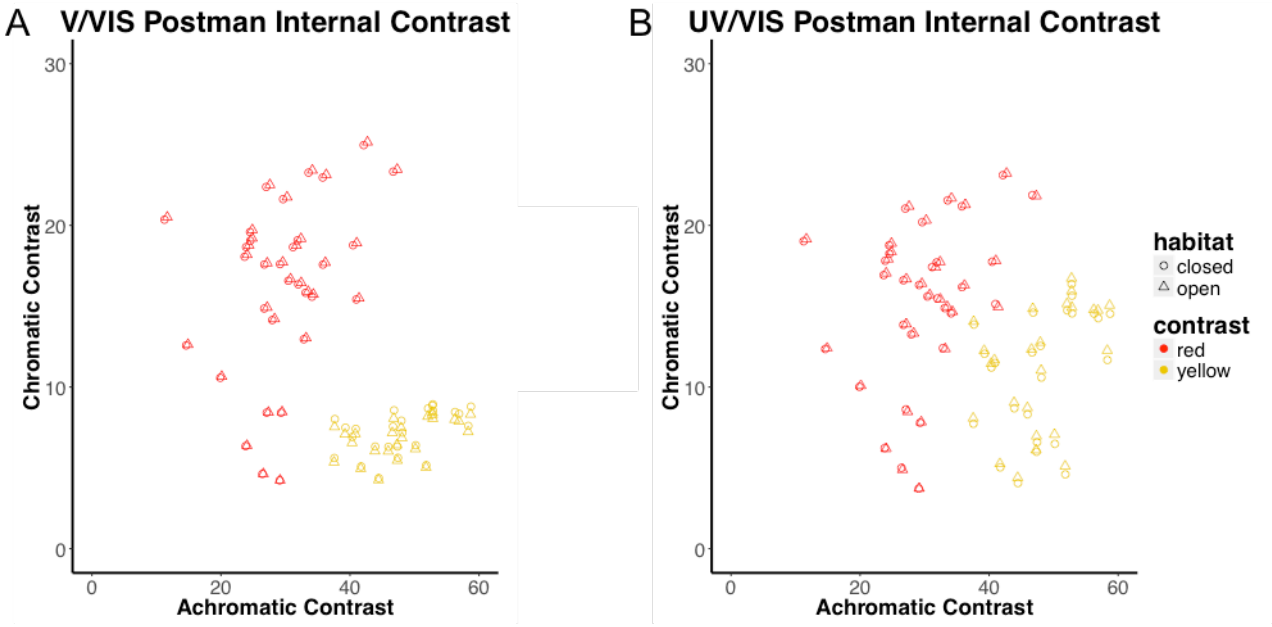

Figure 9. The V/VIS visual system contrasts (A) and the UV/VIS visual system contrasts (B) for the red and yellow patches against the black patch for Postman mimics in two different habitats. In each figure, the further a point is from the origin the more conspicuous the internal contrast is. Thus, the conspicuousness of the patch is dependent on patch color and visual system. Both visual systems have high chromatic and achromatic contrasts for both internal patches. Lastly, the different habitats affect the conspicuousness of the perceived patches as is demonstrated by the circles (closed habitat) and the triangles (open habitat) not matching. The internal contrasts are more conspicuous in the open habitat chromatically.

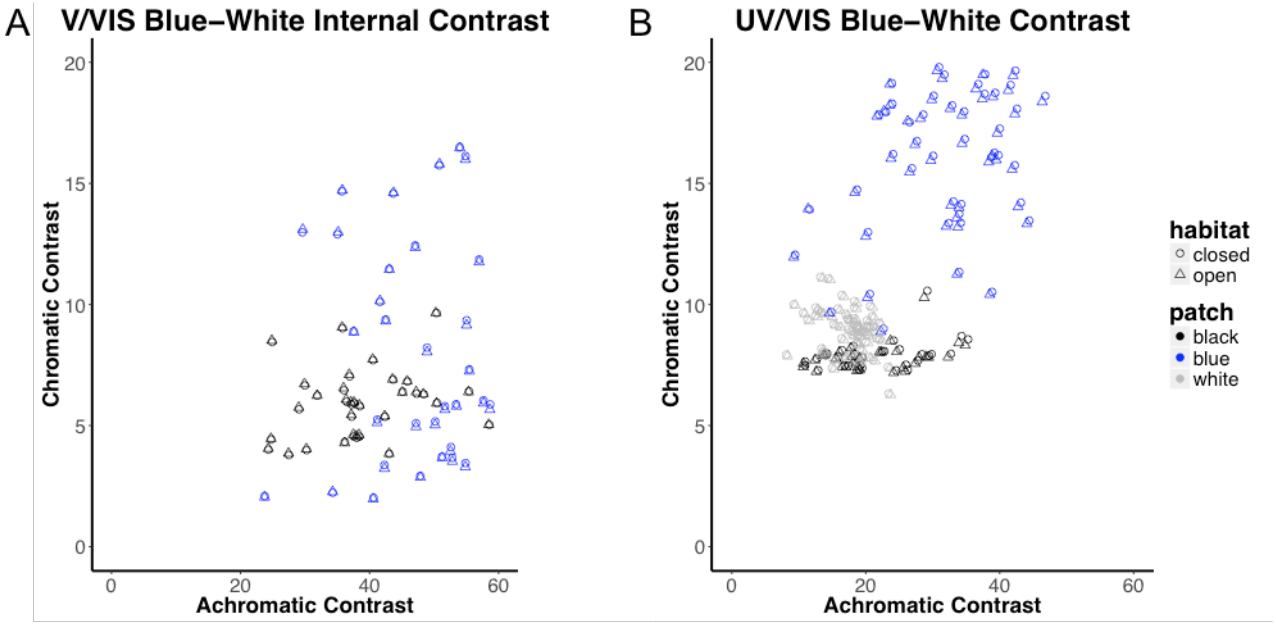

Figure 10. The V/VIS visual system contrasts (A) and the UV/VIS visual system contrasts (B) for the blue and black patches against the white patch for Postman mimics in two different habitats. In each figure, the further a point is from the origin the more conspicuous the internal contrast is. Thus, the conspicuousness of the patch is dependent on patch color and visual system. Both visual systems have high chromatic and achromatic contrasts for the blue against white, but low chromatic contrast for the black against white. Lastly, the different habitats affect the conspicuousness of the perceived patches as is demonstrated by the circles (closed habitat) and the triangles (open habitat) not matching. The internal contrasts are slightly more conspicuous in the closed habitat.

Chapter 2
ENVIRONMENT DEPENDENT SURVIVAL OF CRYPTIC AND APOSEMATIC BUTTERFLIES

Introduction

Many animals face high rates of predation in the wild and have evolved a diverse array of defenses to increase survival (Poulton 1890; Cott 1940; Ruxton et al. 2004; Stevens and Merilaita 2009). One adaptation to avoid detection by predators is through camouflage, in which a prey's color pattern blends with that of the background (i.e. crypsis) against which they are viewed, thus rendering that individual difficult for potential predators to detect (Edmunds 1974; Endler 1984; Cuthill et al. 2005; Stevens and Merilaita 2011; Seymoure and Aiello 2015). Another common defensive adaptation is aposematism, in which the characteristics of potential prey animals that are potentially damaging to predators (e.g. with stings, toxins, armor, etc.) are coupled with conspicuous signals to facilitate predator recognition of unprofitable prey (Wallace 1867; Poulton 1890; Ruxton et al. 2004). The functional benefits of both crypsis and aposematism are well documented (Endler 1981; Heiling et al. 2005; Mappes et al. 2005; Speed et al. 2010; Summers et al. 2015).

Avian predators are important agents of selection on coloration of many prey organisms (e.g. arthropods, amphibians, other birds) due to their keen color vision and widespread use of color-based cues and signals (e.g. feeding, mating; Endler 1978; Chai 1986; Langham 2004; Endler and Mielke 2005; Finkbeiner et al. 2014). Indeed, previous research shows that both camouflage and aposematism are effective strategies for reducing avian predation (Speed 2000; Stevens et al. 2006; Halpin et al. 2008; Skelhorn

and Rowe 2009), but aposematism is expected to reduce predation better than camouflage due to mutual benefits to both the prey (i.e. survival) and predator (i.e. avoiding noxious characteristics; Papageorgis 1975; Guilford 1990; Guilford and Dawkins 1993; Mappes et al. 2005; Saporito et al. 2007). However, until recently there was no direct comparison of predation on cryptic and aposematic prey by wild predators in the field. Carroll and Sherratt (2013) used pastry baits with paper model wings of artificial winged decoys and found that contrary to expectation aposematic prey and cryptic prey had the same overall predation rates, but that aposematic prey were less often fully consumed less than cryptic prey. Hence, there appear to be opportunities for aposematic, but not cryptic, prey to be taste-rejected by predators, leading to higher survival of aposematic prey (Wiklund and Järvi 1982; Pinheiro 1996; Nokelainen et al. 2014).

The intensity of selection from visually hunting predators will not only be a function of unpalatability but also how coloration and backgrounds are perceived by the visually hunting predators. Perception of prey depends upon several factors including the reflectance of the prey's surface, the behavior of both prey and predator, the ambient lighting, transmission properties of the environment, and predator visual sensitivity (Endler 1990; Endler 1993; Stevens 2013; Hutton et al. 2015b). These various determinants of trait perception have led to the hypothesis that the nature of selection on cryptic and warning coloration will be different in different environments. Furthermore, specific features of cryptic and aposematic colorations should be different in different environments (Endler 1990; Endler 1992; Stevens and Merilaita 2011). Camouflage depends on the ambient illumination and visual background, therefore one phenotype may be cryptic in one set of conditions and very conspicuous in another (Endler and

Greenwood 1988; Rojas 2014). Also, Douglas (2013) demonstrated that aposematic butterflies differ in coloration depending on the habitat in which they are found, with tropical understory butterflies exhibiting high achromatic contrast (i.e. black and white), while butterflies that occupy open habitats exhibited highly chromatic contrasts (e.g. yellow and red). However, no study to date has tested survival rates of naturally cryptic individuals and of aposematic species in different habitats. Different habitats should affect predation rates due to visibility of prey (e.g. dense forest vs open fields), local abundance of predators, environmental effects on conspicuousness (i.e. lighting and visual background), as well as differences in prey abundance and predator experience with specific warning color patterns. Therefore, the environmental context must be considered when assessing the survival advantages of particular "conspicuous" aposematic and "inconspicuous" cryptic phenotypes.

Lepidoptera offer excellent opportunities to comparatively test the environmental factors that affect the adaptive value of crypsis and aposematism (Endler 1984; Nokelainen et al. 2014). Many Lepidoptera, such as the common buckeye butterfly *(Junonia ceonia*), are profitable prey and display an arguably cryptic coloration when perched (Silberglied et al. 1979; Devries 1987; Pinheiro 1996; Camara 1997), whereas other species such as *Heliconius* butterflies sequester host plant toxins and display a conspicuous warning coloration(Chai 1986; Devries 1987). Both *Junonia coenia* and *Heliconius* butterflies, occur in Panama (Brown 1981; Kozak et al. 2015). Unlike the palatable *J. coenia*, *Heliconius* butterflies contain cyanogenic glycoside toxins (Cardoso and Gilbert 2013b), which combined with their conspicuous color patterns leads avian predators to avoid consuming them (Chai 1986; Langham 2005; Finkbeiner et al. 2014).

Furthermore, *Heliconius* butterflies exhibit immense color diversity both within and between species and may have up to five different aposematic color patterns that are segregated by habitat in one forest (Papageorgis 1975; Devries 1987; Mallet and Gilbert 1995; Thurman and Seymoure 2015). In the lowland rainforest of Panama, two aposematic coloration patterns are segregated by habitat, the Postman (yellow, red, and black; comprised of *H. melpomene* and *H. erato*) occur in open-canopy, disturbed habitats and the Blue-white (blue, white, and black; comprised of *H. cydno* and *H. sapho*) occur in closed-canopy, undisturbed forest (Estrada and Jiggins 2002). Therefore, these two different aposematic groups live in areas with different ambient illumination (brighter and broad spectrum in open-canopy, while darker and rich in green light in closed-canopy), as well as with different avian predators. Due to the habitat segregation of these aposematic patterns, tests of environmental effects on the effectiveness of aposematic coloration are possible.

Here I utilized clay models of a cryptic species (*Junonia coenia*), and the two species with aposematic color patterns (Postman and Blue-white) to test three sets of hypotheses and predictions: 1) cryptic and aposematic individuals have evolved coloration to reduce predation and therefore will have similar and high overall survival; 2) the cryptic species has evolved to be undetected at rest and therefore the cryptic species will have similar survival across both habitats; 3) the aposematic species' warning signals are most effective in their respective habitats and therefore I predict that the Postman will survive better in open-canopy while Blue-white will survive better in closed-canopy habitats.

Methods

Model Construction

I collected three males each of *Heliconius melpomene* (Postman pattern), *Heliconius cydno* (Blue-white pattern) and *Junonia coenia* in lowland rainforest habitats of central Panama in July of 2012 using aerial nets. I then used these males to develop artificial models following the methods of Finkbeiner et al. (2012) and Seymoure & Aiello (2015). The models were constructed using scanned images (Brother MFC-J4510DW Scanner, Brother Industries, Nagoya, Japan) of ventral wing surfaces of each species because individuals of *Heliconius* and *Junonia* perch with their wings closed unless they are thermoregulating or involved in courtship (Brown 1981; Devries 1987). High resolution models were printed onto Whatman filter paper (GE Healthcare Life Sciences, Pittsburgh, PA) with a Brother MFC-J4510DW printer (Brother Industries, Nagoya, Japan) and then cut and inserted into the "body", a 2.5 cm long piece of black, non-toxic plastalina modeling clay (Craftsmart, Irving, TX), which remains malleable in the field and thereby shows beak marks when attacked by the bill of avian predators (Finkbeiner et al. 2012; Merrill et al. 2012; Seymoure and Aiello 2015).*Model Color Measurements*

To confirm that each model type was visually indistinguishable from the natural butterfly wings, I quantified full-spectrum reflectance and incorporated the data into avian visual threshold models (Vorobyev and Osorio 1998; Maia et al. 2013). I measured the ventral reflectance of the main color patches for each species using three male individuals and then measured the same color patches of three of each printed model type

using a USB2000 spectroradiometer (Ocean Optics, Dunedin, FL) and Xenon standardized light source (Ocean Optics, Dunedin, FL). Wing color reflectance was measured as the proportion of a white reference standard (WS-1-SL, Ocean Optics, Dunedin, FL) using a coaxial fiber cable (QR400-7, Ocean Optics, Dunedin, FL). I used avian visual thresholds using the PAVO program within R (Maia et al. 2013; R Core Team 2014) to determine if the artificial wing models accurately represented the coloration of natural wings, as seen through the eyes of birds with both ultraviolet-sensitive (UVS) and violet-sensitive (VS) visual systems (Vorobyev *et al.*, 1998; Osorio &Vorobyev, 2005). Although the main predators of *Heliconius* are jacamars and tyrant flycatchers (Pinheiro, 2011), which have the VS visual system, the predators of *J. coenia* may include predators with either the VS or UVS visual system (DeVries, 1987). I applied von Kries transformation to account for receptor adaptation and used the default parameters for Weber's fraction (.05), illumination (D65 irradiance spectrum for standard daylight), background, and cone ratios of N1=1, N2=2, N3=2, N4=4 (Hart, 2001: Maia *et al.*, 2013). I calculated both achromatic and chromatic Just Noticeable Differences (JND) for each model to its respective natural butterfly: Postman red, Postman yellow, Postman black, Blue-white white, Blue-white black, Blue-white red, *Junonia* brown, and *Junonia* orange, see figure 11). JNDs represent the ability of a visual system to perceive two colors differently, with a JND value of less than one being indistinguishable in ideal conditions (Siddiqi *et al.*, 2004). All comparisons had JNDs of less than 1 for achromatic and chromatic comparisons for both the V/Vis and UV/Vis visual systems, see figure 12 and 13. Therefore, I inferred that in the eyes of birds the difference in coloration between the models and real butterflies would be minimal if not imperceptible. Furthermore,

spectral reflectance curves for each model fit within the natural color variation of each species, see figure 11).

Survival Experiments

I tested the survival of my model types in two different habitats in Soberania National Park in Central Panama (9.1° N, 79.7° W). Models were set out in blocks of three that included one of each color pattern (i.e. Postman, Blue-white, and *Junonia*). Within each block, models were arranged 1 to 3 m apart at heights ranging from .2 m to 2 m. I tied each model with black string to leaves and branches of rainforest plants. Though I did not specifically control for background, there is no evidence that *Heliconius* individuals or *J. coenia* choose a particular type of vegetation or background for resting (Devries 1987; Mallet and Gilbert 1995). Each block was placed 100 m from the nearest block to reduce the risk of the same bird attacking models in more than one block (Hurlbert, 1984; Finkbeiner *et al.*, 2012). Blocks of models were placed in each habitat type, open-canopy and closed-canopy, which were categorized by tree cover (less than 70% tree cover, while closed-canopy had more than 90% tree cover, Seymoure unpublished data). Each specific block site was only used once and there were fewer locations in the closed-canopy habitats to place models, so the overall sample size for open-canopy was 99 blocks while closed-canopy was 50 blocks, for a total placement of 447 models. I conducted 8 different three-day trials from February to April during the dry season in 2013. These experiments took place during the dry season for two reasons: because predation rates on insects are higher in the dry than in the wet season and to avoid the potential for rain damage to the models (Kricher, 1999). Each model was checked daily (11am-4pm) for 3 days for beak, teeth, and mandible marks (see

Finkbeiner et al. 2012; Seymoure and Aiello 2015). Attacked models were removed from the experiment and not replaced, to avoid inflating mortality rates among treatments (Cuthill et al. 2005; Finkbeiner et al. 2012; Merrill et al. 2012). I counted only beak marks (*i.e.,* triangular indentations, see figure 14) as predatory attacks. Models that disappeared were censored (i.e. included in the survival analyses until removed from the study for non-relevant reasons) in the statistical analysis, because it is impossible to know if the models were removed by an avian predator or a non-relevant force (e.g. curious people, rodents, wind) (Hurlbert, 1984). Models that showed evidence of non-avian attacks (*i.e.,* teeth marks and gashes of mammals; small holes of insects) were also censored in the statistical analysis since these attacks were unlikely to have been visually guided and are therefore not a good indicator of color-based predation (Finkbeiner *et al.*, 2012).

Statistical Analysis

Differences in survival probabilities after 72 hours were analyzed using Cox proportional-hazards regression ('survival' package) in R (R Development Core Team 2011). Missing models and non-avian attacks were censored in the Cox proportional-hazards regression. I also calculated the effect sizes with odds ratios (OR), where a value of 1.00 indicates that two models have identical survival probabilities.

Results

Over the eight different trials, all of which lasted three days, 12.1% (54/447) of the models showed evidence of attack by birds. Avian attack rates in the open habitat

were 14.8% (44/297) and in the closed habitat were 6.7% (10/150). Attacks by non-avian predators (e.g. rodents and insects) contributed another 2.2% (10/447), while 7.6% (34/447) of the models were missing (Table 4). Lastly, the open habitat had 10.8% (32/297) of the models missing while the closed only had 1.3% (2/150). The high rates of missing models in the open habitat is due to areas of forest being clear cut and removing 15 models, five of each model type. I included these missing models into my analysis because the clear cutting occurred after day one, thus allowing for the use of survival data from these models for at least one day.

Model survivorship curves differed significantly by species (Cox regression, F = 2.049, p = 0.040; figure 15A) and habitat (Cox regression, F = 2.536, p = 0.011; figure 15B), but not with placement date (Cox regression, F = 1.784, p = 0.074). Pairwise comparisons revealed that independent of habitat, *H. melpomene* models were attacked more often than *J. coenia* models (Wald = 10.18, d.f. = 2, p = 0.006, OR = 2.290), but *H. melpomene* had similar attack rates to *H. cydno* models (Wald = 5.26, d.f. = 2, p = 0.061, OR = 1.177). *Heliconius cydno* and *J. coenia* models also had similar attack rates (Wald = 4.73, d.f. = 2, p = 0.094, OR = 1.945). Also, the number of attacks on *H. melpomene* differed between habitat types with much higher predation in the respective, open habitat of *H. melpomene* (Wald = 4.48, d.f. = 1, p = 0.034, OR = 3.966; figure 16), while number of attacks on the other two species did not differ between habitats (*H. cydno*: Wald = .840, d.f. = 1, p =0.358, OR = 1.607; *J. coenia*: Wald = 1.38, d.f. = 1, p = 0.240, OR = 2.4; figure 16).

Discussion

Previous research has shown that both cryptic individuals and aposematic individuals have similar survival rates in artificial prey (Carroll and Sherratt 2013). Here, I demonstrate that attack rates on two different aposematic species (*Heliconius*) and cryptic (*Junonia*) individuals depend on the aposematic coloration as well as the environment. I found that the aposematic Postman models were attacked more than the cryptic model, yet the two aposematic color patterns had similar survival rates. Furthermore, the attack rates differed among habitats with more attacks occurring in the open habitat than in closed habitat. My results along with Carroll and Sherratt's (2013) indicate that aposematic theory may need to include factors other than just conspicuousness and unpalatability.

Survival of individuals with cryptic or aposematic coloration

Previous research has demonstrated repeatedly that aposematic models benefit from reduced attack rates when compared to non-aposematic models (Papageorgis 1975; Saporito et al. 2007; Stuart et al. 2012). In fact, a necessary criterion to test if an animal is aposematic is that the aposematic coloration survives more than an alternative non-conspicuous model, and most studies use the natural coloration of the aposematic organism and then alter the coloration to be “cryptic”, which is usually black, brown, or green (Papageorgis 1975; Halpin et al. 2008; Hegna et al. 2012; Arenas et al. 2015). Although this methodology works well at indicating if an organism receives protection from conspicuous coloration relative to a less conspicuous coloration, it does not directly test if an evolved aposematic coloration is more or less adaptive than an evolved cryptic coloration. I investigated the hypothesis that both crypsis and aposematism have evolved

to minimize predation, thus, I predicted that predation results would be similar between both morphological strategies. And although testing the adaptive value of putative conspicuous coloration functioning aposematically using the method mentioned above is important, here, my aims were to compare two different visual strategies in natural phenotypes and not artificial phenotypes.

Heliconius butterflies are aposematic and several studies have demonstrated that avian predators recognize the visual warning signals of *Heliconius* to avoid attacking individuals (Chai 1986; Chai and Srygley 1990; Chai 1996; Langham 2004; Langham 2005). The likely avian predators of *Heliconius* and other tropical butterflies are flycatchers and jacamars (Pinheiro 1996), which often attack prey at the thorax and then either consume palatable prey or taste reject chemically defended prey (Pinheiro 2011). Taste rejection by avian predators is likely an adaptation to find palatable mimics of aposematic prey and the act of taste rejection has been shown to leave butterflies intact and capable of flight (Wiklund and Järvi 1982; Sillen-Tullberg 1985; Pinheiro 1996; Pinheiro 2011). Therefore, although I found that the cryptic species had higher survival than the aposematic Postman species, I was not able to determine whether the aposematic species would have been taste rejected since the bodies were clay. It is likely that the survival rates of all three species are similar in wild butterflies due to taste rejection by birds. In fact, Carroll and Sherratt (2013) demonstrated that artificial models made to be unpalatable with quinine pastry baits, were attacked at the same rate as palatable, cryptic pastry bait models, but that the unpalatable pastry baits were taste rejected more often. I am currently designing studies to test taste rejection in these species of butterflies in the

wild to better understand the role of predator behavior in selecting for aposematic and cryptic phenotypes.

Our study replicated components of the study by Merrill et. al (2012) in that I used clay models of Postman and Blue-white butterflies in Panama to determine if predation rates differed between aposematic morphs in different habitats. Unlike Merrill et. al (2012), I found that attack rate did differ between forest edge and forest habitats, specifically for the Postman model. My findings may differ from Merrill et al (2012) because I tested predation during the dry season instead of the wet season. Avian predation has been reported to increase during the dry season due to lower availability of prey, which may mean that aposematic prey are attacked more during the dry season than in the wet season (Kricher 2011). In fact, I observed an attack rate that was three times that recorded by Merrill et. al (2012; 12% compared to 4%), even though the overall methods were very similar. Seasonal differences in attack rates have also been reported by Mappes et. al (2014), who found that the survival rates of cryptic and aposematic larvae in Finland varied with season. Specifically, Mappes et al (2014) attributed the seasonal survival differences between cryptic and aposematic larvae to seasonal differences in the prior experiences of avian predators. Naïve fledglings attacked more aposematic prey than cryptic prey, but later in the year when all birds were experienced, the cryptic prey was attacked more than aposematic prey. In my study, it is possible that differences in predation rates between aposematic and cryptic morphologies were due to bird age and experience. Both tyrant flycatchers and jacamars have breeding seasons that begin at the transition from wet season to dry season and thus naïve fledglings begin

foraging during the dry season and may have not learned to avoid aposematic species (Skutch 1968; Hoyo et al. 2004).

Attack differences between habitats

There were more overall attacks for each species in the open habitat, there was only a significant difference for *H. melpomene.* This finding is most likely due to visibility and predator composition. The closed, forested site where models were placed was thick with vegetation and therefore it may have been harder for birds to detect even the conspicuous models. Also, predator composition varies between the two habitats and the forest edge habitat has high abundance of insectivorous birds such as tyrant flycatchers (Hoyo et al. 2004).

The Postman coloration was attacked more in its respective habitat than in the habitat where it does not reside. This is contrary to my predictions as I predicted that predation on aposematic models would be lower where the aposematic model is common due to experienced predators as has been supported in previous research (Mallet and Barton 1989; Merrill et al. 2012). As stated previously, this suggests that avian predators are likely attacking aposematic individuals and then deciding whether to consume or reject the prey dependent upon chemical defenses (Wiklund and Järvi 1982; Sillen-Tullberg 1985; Pinheiro 1996; Pinheiro 2011; Carroll and Sherratt 2013). *Heliconius* species have many palatable mimics that may be rewarding avian predators that test the palatability of prey items (Pinheiro 1996; Pinheiro 2007; Pinheiro 2011). And if the palatable mimics are segregated by habitat like their aposematic model (i.e. Postman butterflies), then predators may be searching for individuals with the Postman coloration. Furthermore, the Postman has high chromatic contrast (red, yellow, and black color

pattern) and thus is highly noticeable in well-lit environments like edge habitats and may be easier to detect by avian predators in the edge habitat (Douglas 2013). Further research into the rates of taste rejection in aposematic species is needed to understand the evolutionary processes behind warning coloration and mimicry.

Assumptions of plasticine models

Plasticine models have been used to test many hypotheses explaining differences in morphology, as well as hypotheses relative to the ecology and evolution of predator-prey interactions (Papageorgis 1975; Cuthill et al. 2005; Finkbeiner et al. 2012; Seymoure and Aiello 2015). However, in several such studies, the plasticine model manipulations done to address the questions they proposed are artificial and do not resemble any natural prey item (see Cuthill et al. 2005; Carroll and Sherratt 2013) or are drastically different from the natural coloration (see Finkbeiner et al. 2014; Seymoure and Aiello 2015). It is conceivable that this may lead to attack rates that are higher than would occur with natural coloration. Hence the comparatively low predator attack rates that I observed might be due to the relatively natural appearance of the plasticine models that I used.

My findings here suggest that both aposematism and cryptic coloration have high survival rates in the wild. However, the plasticine models are a surrogate for wild butterflies and may not be equally representative of the attack rates for living cryptic and aposematic individuals. Most prey items move, especially butterflies, and the models used in this study were static, so perhaps predation rates between cryptic and aposematic animals differ when movement is included. In fact, cryptic organisms are hypothesized to

move less than conspicuous organisms because predators can use movement to detect prey (Stevens and Merilaita 2011).

Conclusions

Overall, my study suggests that both aposematic coloration and cryptic coloration can be adaptive strategies for avoiding predation. The findings suggest that the form of aposematic coloration and the habitat (i.e. open-canopy vs. closed-canopy) in which an organism resides affects the predation rate. All three color forms were attacked more in the open habitat, which is most likely due to visibility and perhaps greater abundance of predators. Furthermore, the more chromatic aposematic species was attacked more than the cryptic species. This study indicates that the common approach to testing aposematic individuals against unnatural cryptic models is not biologically accurate, as many cryptic species have evolved a coloration that reduces predation. Lastly, this study highlights the need for further research into the tradeoffs of crypsis and aposematism. Why do some animals evolve crypsis while others evolve aposematism, if both have similar rates in survival? Future work studying the role of life history (e.g. dispersal, mobility, host plants) and predation risk in the context of crypsis and aposematism is needed to understand the selection pressures leading to crypsis or aposematism.

Table 6. Number of models that displayed evidence of avian and non-avian attacks, or went missing during the trials for each species and habitat. The number of models placed is represented by N.

	Open				Closed			
Species	N	Avian Attack	Non-avian Attack	Missing	N	Avian Attack	Non-avian Attack	Missing
H. melpomene	99	20	3	10	50	3	3	1
H. cydno	99	15	0	8	50	5	1	1
J. coenia	99	9	2	14	50	2	1	0

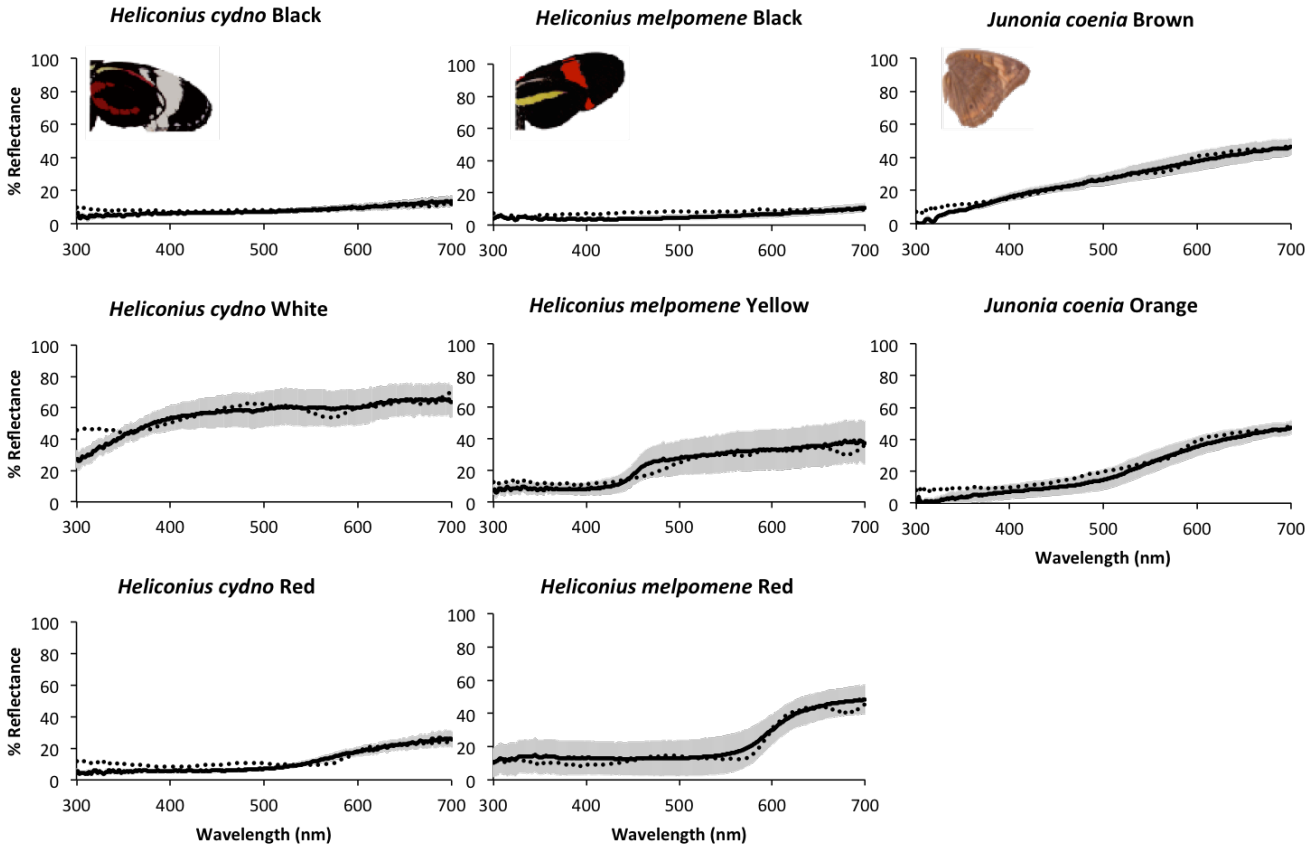

Figure 11. Reflectance spectra comparing the color of the ventral wing patches of natural wings to paper model wings. Black solid lines represent the mean natural spectra from ten individuals and the grey shading represents one standard deviation above and below the natural mean spectrum. The black dotted line is the mean reflectance of three models of each type. Each subfigure represents one color patch.

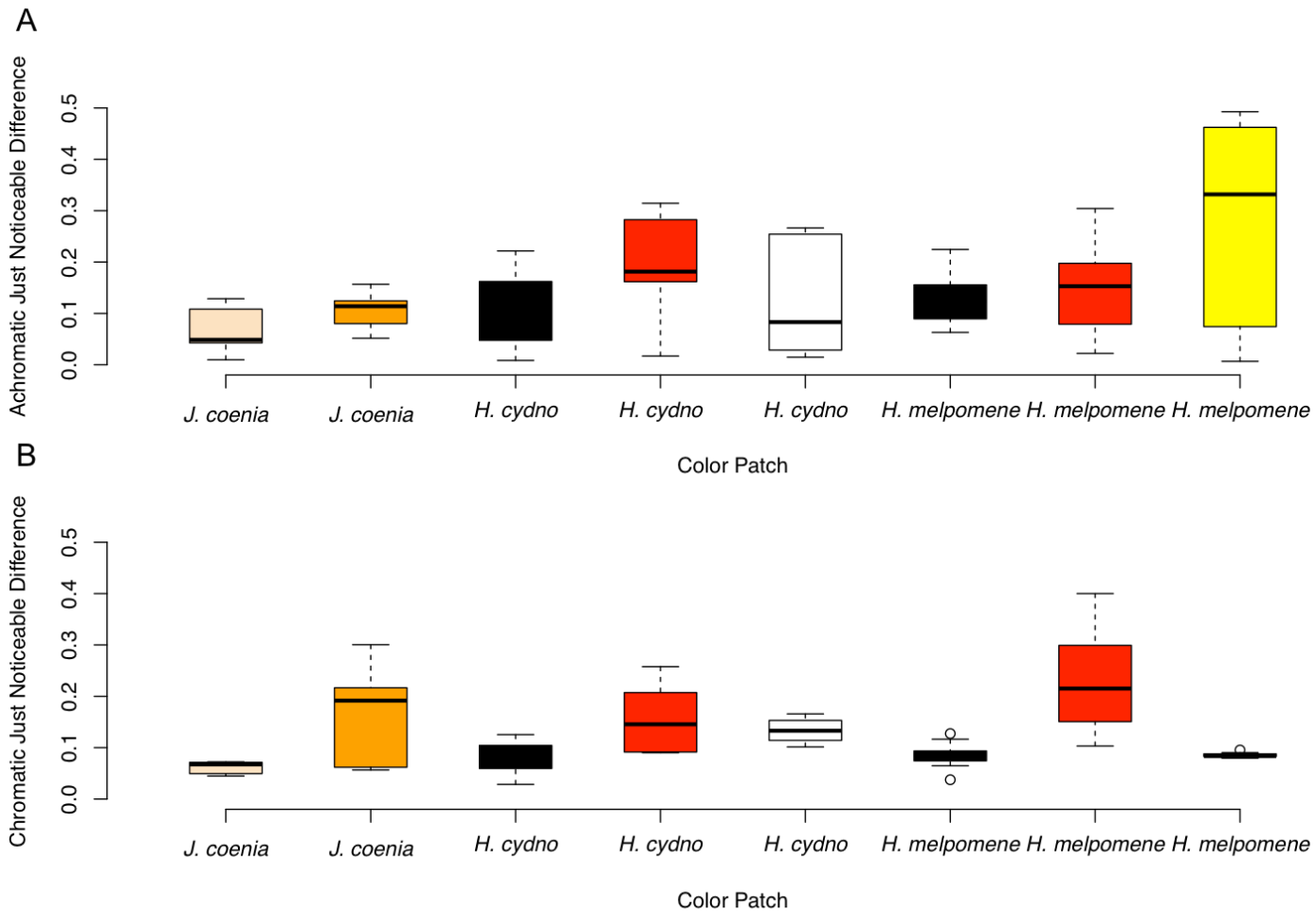

Figure 12. Just Noticeable Differences for the V/Vis avian visual system for the artificial models and real butterfly wings. A) Achromatic differences and B) Chromatic differences, between artificial models and real wings for the three different species. The color of the boxplot represents which color patch is being shown. All JNDs were less than 0.5 indicating that the V/Vis avian visual system would not be able to discriminate between the model color and the natural wing color.

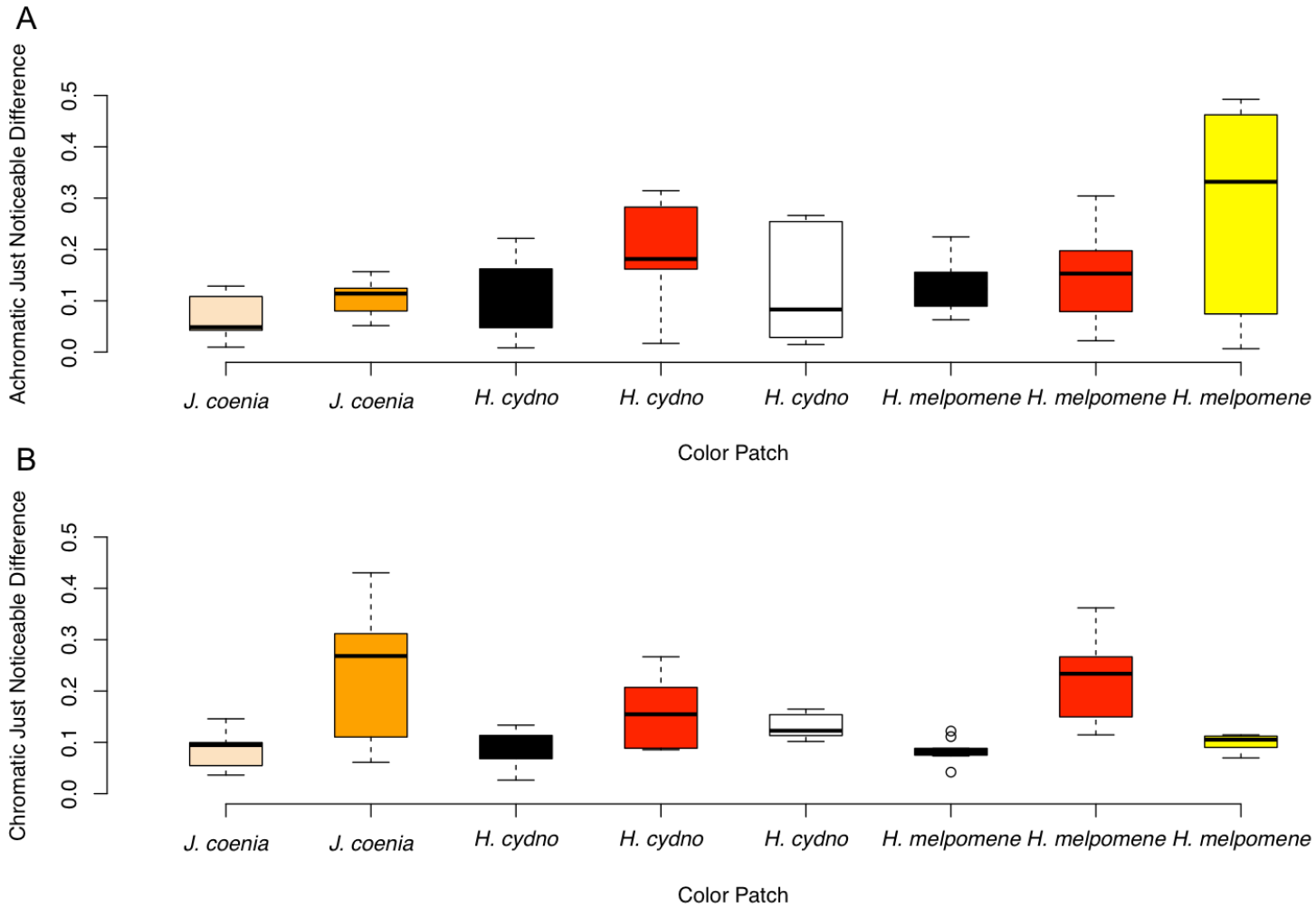

Figure 13. Just Noticeable Differences for the UV/Vis avian visual system for the artificial models and real butterfly wings. A) Achromatic differences and B) Chromatic differences, between artificial models and real wings for the three different species. The color of the boxplot represents which color patch is being shown. All JNDs were less than 0.5 indicating that the UV/Vis avian visual system would not be able to discriminate between the model color and the natural wing color.

Figure 14. Examples of marks interpreted as beak marks from attacks by avian predators on plasticine-paper models. Arrows point to beak marks. Left, a beak mark on the plasticine abdomen of a Postman model; right, a beak mark on the plasticine abdomen of a Blue-white model.

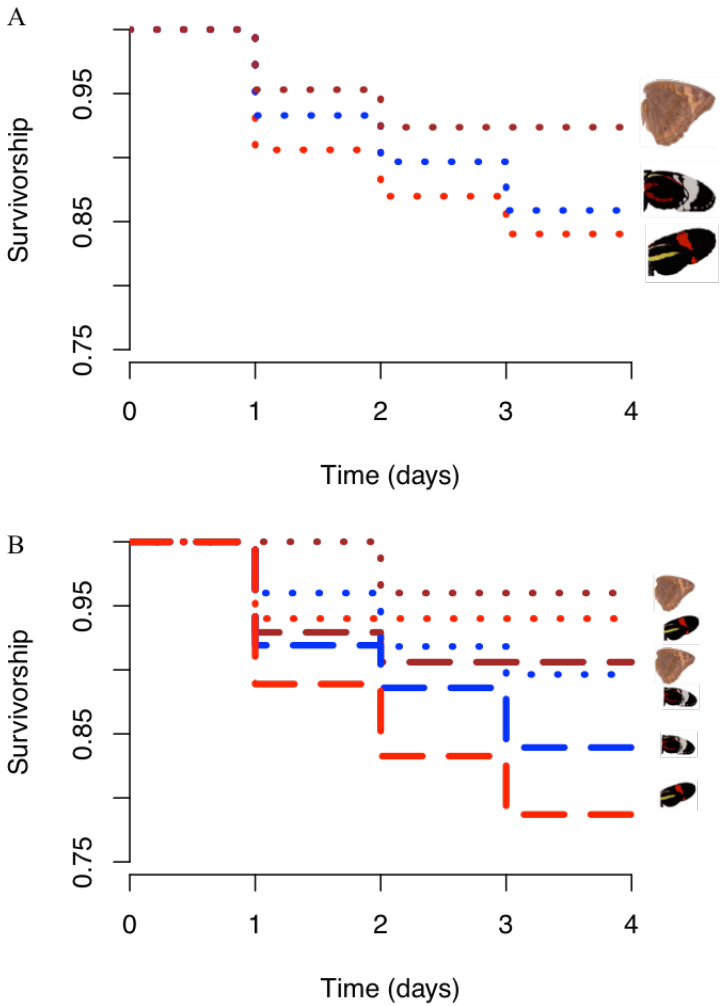

Figure 15. Survival curves for the three different models. Red represents Postman (*H. melpomene*), blue represents Blue-white (*H. cydno*), and brown represents the cryptic model (*J. coenia*). A) Combined habitat survival curves for each morph. B) Individual survival curves for each morph in each habitat. Long dashes represent survival in the open habitat while dots represent survival in the closed habitat.

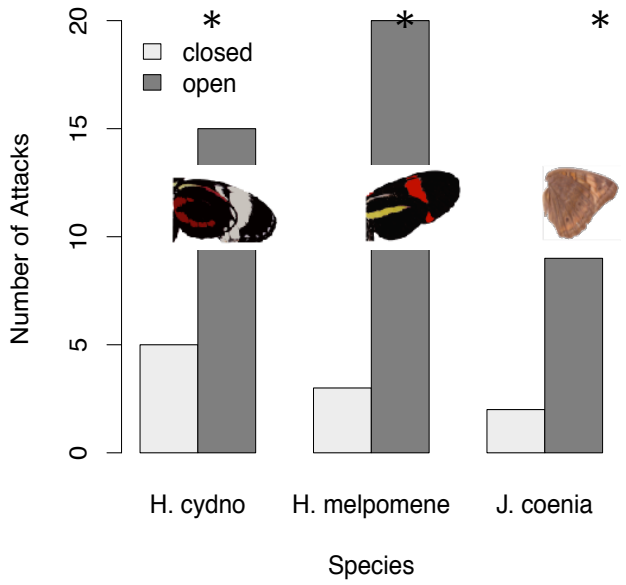

Figure 16. Number of attacks on each species in each habitat. Light grey bars represent closed habitat and dark grey bars represent open habitat. Asterisks represent significant difference in the number of attacks between habitat types for each species with p-value <0.05.

Chapter 3

MIMETIC BUTTERFLIES PREFER LIGHT ENVIRONMENTS THAT CORRESPOND TO VISUAL SENSITIVITIES AND MICROHABITAT

Introduction

Over 100 years ago, Fritz Müller hypothesized that aposematism (unpalatability coupled with a conspicuous warning signal) would lead to the convergence of warning signals among different species to reduce individual costs of predator education (Müller 1878; Ruxton et al. 2004). This evolutionary phenomenon is called Müllerian mimicry in honor of its discoverer and explains the convergence of warning signals ranging from amphibians (Twomey et al. 2013) to hymenopterans (Wilson et al. 2015). However, there are many examples in which closely related aposematic organisms diverged in conspicuous warning signals (Papageorgis 1975; Joron 2005; Noonan and Wray 2006; Marek and Bond 2009). This unpredicted divergence of warning signals has resulted in controversial hypotheses explaining the paradox of multiple Müllerian mimetic groups in closely related sympatric and/or parapatric species ranging from the benefits of cryptic coloration to visual illusions to microhabitat effects on visual perception of warning signals (Papageorgis 1975; Mallet and Gilbert 1995; Joron et al. 2001; Rojas et al. 2014). Following from the microhabitat hypothesis of diversity in warning signals of Müllerian mimicry, an increasing amount of evidence has revealed Müllerian mimics do occupy the same microhabitats (Papageorgis 1975; Mallet and Gilbert 1995). *Heliconius* butterflies, which have diverged in warning signals resulting in numerous mimetic assemblages in one location, have co-mimics that occupy the same microhabitats while non-comimics do not share microhabitats (Mallet and Gilbert 1995; Estrada and Jiggins 2002, see chapter

1). Furthermore, in chapter one of this dissertation, I have demonstrated that these different microhabitats differ in overall ambient lighting. This observation that co-mimics have the same microhabitats with the same ambient lighting bring into question what proximate mechanism(s) drive mimetic pairs to occupy the same light environments (see chapter 1). Here, I examine whether or not light-habitat preference or visual-system sensitivity can explain variation in microhabitat segregation by two mimetic rings of *Heliconius* butterfliy species.

Previous studies have shown that colorful animals may seek lighting conditions that enhance the efficacy of their visual signals for mate choice and conspecific communication (Théry and Vehrencamp 1995; Endler and Thery 1996; Long and Rosenqvist 1998; Leal and Fleishman 2001; Leal and Fleishman 2004). Similarly, *Heliconius* butterflies occupy habitats that affect the conspicuousness of their warning signals in their respective microhabitats, see chapter 1. Thus having a preference for an environment in which an aposematic individual is very conspicuous would also be adaptive, but no literature exists on this topic. I tested the hypothesis that the previous findings of microhabitat segregation between co-mimics are explained by behavioral preferences for different light intensities. I predicted that Blue-white individuals would select light environments that are dimmer, while Postman individuals would select brighter environments.

A main tenet of sensory ecology is that disparate environments select for very different visual systems (Stevens 2013; Cronin et al. 2014). The ability to visually perceive the world ultimately depends on the ability to catch photons by an individual's photoreceptors. Thus, nocturnal animals have very sensitive eyes relative to diurnal

animals, which have evolved acute eyes to increase resolution (Warrant 1999; Stevens 2013; Warrant and Johnsen 2013; Cronin et al. 2014). Behaviorally similar species occupying similar niches should have similar visual abilities to meet the visual demands of their respective environment. Previous research has shown that arthropod compound eyes vary depending upon light environment and behavior (Warrant 2006, Land & Nilsson 2012). Diurnal animals that evolve to be nocturnal have adaptations to increase photon capture and enhance visual sensitivity. These adaptations are morphological (i.e. larger eyes, larger facets, wider rhabdoms) and neurological (i.e. temporal and spatial summation) (Warrant et al 2004, Frederikson & Warrant 2008).

A technique that enables one to test for overall sensitivity to light is electroretinography (ERG). ERG measures electrical responses in the eye of an individual after a light is flashed (Brill et al. 2008; Horodysky et al. 2008). By controlling the brightness of the light, a minimal threshold sensitivity is measured. The minimal threshold sensitivity is the dimmest light that elicits an electrical response in the eye regardless of the physiological mechanism (e.g. larger eyes or neural summation). Thus, by utilizing ERG, I am able to test if butterflies from different light environments differ in the ability to detect light.

An understanding of how differences in diurnal light environments affect animal behavior and the ability of an animal to perceive its environment is lacking. Hence, I explored if these mimetic butterflies had differences in behavioral preferences for different light environments and if their visual sensitivities match the light environments in which they typically occur. I tested if the Postman butterflies preferred brighter environments and if the Blue-white butterflies preferred darker environments, as this is

where they are found in nature. Furthermore, I tested if Blue-white butterflies had greater visual sensitivity compared to Postman butterflies as an adaptation to living in much darker light environments.

Methods

Animal Collection and Husbandry

All individuals for both studies, the behavioral light preference and electroretinograms (ERGs), were wild-caught males. Wild caught individuals were used to test for natural differences in the behavior and visual system of individuals and to rule out effects from rearing conditions in a laboratory. I used only males in this study for two reasons: (1) to reduce the effect of terminally removing females from this heavily-collected population along Pipeline Road, and (2) because males were more heavily represented in my microhabitat distribution study (Chapter One). Males of the four species (*H. erato*, *H. melpomene*, *H. cydno*, and *H. sapho*) were collected during the dry season of 2014 (February-May) in central Panama in Soberania National Park and on the Caribbean Coast in Lorenzo National Park. Males were caught with insect nets and then transported to the Smithsonian Tropical Research Institute Insectaries in glassine envelopes with moist cotton to reduce overheating and dehydration. Males were released into insectary enclosures and fed a mix of pollen and sugar water *ad libitum*. Each individual was housed in an enclosure for at least one day but no more than three days before undergoing either a behavioral light preference trial or electroretinography.

Behavioral Light Preference Trials

To test if butterflies select for a light environment that corresponds to their respective light environment, I built an experimental enclosure with 99.9% transmittable insect screen (Econet B Insect Screen, US Global Resources, Seattle, WA) that was 2 m high, 2 m wide, and 5 m long. The sides were covered with black cotton sheets to block out sidewelling light. The enclosure was divided into two 2.5 m by 2 m light environment sections, one darker and one brighter. Both light sections were created using neutral density filters (LEE Filters, Burbank, CA). For the dark section I used a ND 1.2 filter (approx. 10% transmissive, see figure 17) and for the light section I used ND 0.15 filter(approx. 90% transmissive, see figure 17). These two light sections closely matches the respective illumination for the two habitat types that *Heliconius* individuals occupy, see figures 6 and 17. The enclosure was placed with its long axis perpendicular to solar azimuth so that the sun illuminated each light environment equally (i.e.). This condition was maintained throughout the day by shifting the enclosure's orientation. I confirmed that both light environments had the same ambient temperature using a non-contact thermal gun (AR550, Smart Sensor, Intell Instruments, Santa Clara, CA) and the light environment locations were randomized for each behavioral trial. All trials were run during the dry season of 2014.

Between 7 am and 11 am on days with little cloud cover and little wind, butterflies for the day's trials were transferred from the insectary enclosure to a small shaded holding tent (25 cm x 25 cm x 50 cm) with nectar sources available for *ad libitum* feeding. Before butterflies were individually released into the experimental enclosure, each was chilled in a cooler so it did not fly immediately when placed in the experimental enclosure. After cooling, a butterfly was placed into the enclosure at the border of the two

light sections. Once the butterfly was warmed by ambient temperatures and took flight, I began a 20-minute trial and recorded where the butterfly was in the enclosure every 15 seconds, resulting in 80 observations for each butterfly. The light preference trials were run for 11 male *H. erato*, and 9 males for each of the other three species. The proportion of time spent in the dark section was distributed normally and thus statistically analyzed with an ANOVA with species nested within mimicry ring.

Electroretinography

Electroretinography is a comprehensive method to measure summed retinal potentials that account for any optical filtering of light by ocular media and can be used to investigate an individual's ability to see under different lighting conditions (Brown 1968; Ali and Muntz 1975). I used ERG to test for luminance sensitivity between the species and mimetic pairs using wild-caught males during the dry season (February-June) of 2014. To control for any circadian changes in lumance sensitivity, I used a block design with each species equally represented in four different time periods (8am-10am, 10am-12:00pm, 12pm-2pm, 2pm-4pm). Furthermore, each individual was dark-adapted for 60 minutes before each ERG.

The whole-animal corneal ERG responses to broadband light stimuli were recorded using size 00 insect pins (Bioquip, Dominguez, CA). The recording electrode was placed at approximately 1 mm in the right eye in the dorsolateral region. The reference electrode was positioned approximately 1 mm in the left eye in the lateral region. All electrode placements and any further modifications in the experimental setup were conducted under a dim red LED light source.

Signal amplification and filtering, as well as data acquisition and processing followed Brill et al. (2008) and Horodysky et al. (2008). Briefly, retinal responses were recorded and controlled using custom-designed software developed by Eric Warrant written in LabVIEW graphical programming system (National Instruments, Austin, TX, USA). I assessed the retinal function in a dark-adapted state using light stimuli at an intensity (measured in volts) that elicited the minimal response relative to a control stimulus, in which the light stimulus was blocked from reaching the eye. As voltage increased, intensity also increased but not linearly, see figure 18. The ERG began with a low intensity at 2.5 volts and then increased by .1 volt steps up to when a response appeared, see figure 18 for a sample of light stimuli intensities. Then the intensity was increased by .02 volts to resolve the minimal threshold voltage to elicit a response. At each intensity step, the retinal responses were recorded from a train of five 100 ms flashes, each separated by 200 ms rest periods. This was repeated three times, see (Horodysky et al. 2008).

I tested minimal threshold voltages for 7 males for each of the four *Heliconius* species. The forewing length of each individual was used as a surrogate for body size (Seymoure et al. 2015) and was measured to the nearest tenth of a mm using digital calipers (6”/150MM Fractions Digital Caliper, ML Tools, Burlingame, CA). The minimal threshold voltages recorded were normally distributed and analyzed using an ANCOVA with forewing length as the covariate, and species nested within mimicry ring.

Results

Light Preference

Individuals varied in the percentage of time spent in the dark and light sections during the 20 minute trials and some individuals spent the entire time in one section while others spent close to an equal time in both sections. The mimetic groups differed from each other with the Blue-white mimetic group spending a greater proportion of time in the dark section, while the Postman group spent a greater proportion of time in the light section ($F_{1,34}$=4.01, p=0.004, figure 19). Thus, each pair preferred the light section which matched the intensity of their respective environment. However, within each mimetic group there were no significant differences in the time spent in the different sections ($F_{2,34}$=1.23, p=0.305).

Electroretinogram Minimal Thresholds

There were differences among but not within mimicry rings for minimal threshold sensitivity. Furthermore, forewing length did not explain differences in minimal threshold values regardless if individuals were grouped together ($F_{1,23}$=2.171, p=0.1542, figure 20) or only by species ($F_{1,7}$<1.46 and p-value>0.029 for all species trends), while mimicry and species did. As predicted, the Blue-white mimics had significantly greater sensitivity than the postman mimics ($F_{1,24}$=24.11, p<0.001, figure 21), and Blue-white mimics did not differ between species (p=0.995). Postman individuals also did not differ in minimal threshold sensitivities between species (p=0.221).

Discussion

Co-mimetic individuals preferred similar light environments compared to non-co-mimics and co-mimics had similar visual sensitivity at low light levels. The differences in behavioral preferences match each mimics respective natural microhabitat distribution. Furthermore, the visual sensitivity of each co-mimic matches the light level direction of their respective light environment with the Blue-white mimics preferring darker environments and having greater sensitivity than Postman mimics which preferred the brighter light environment.

Many animals seek out specific lighting environments and many animals exhibit phototaxis or negative phototaxis, see (Randel and Jékely 2016). Previous research has demonstrated that many vertebrate species behaviorally seek out specific light environments that increase signal efficacy in the context of mate recognition and courtship (Théry and Vehrencamp 1995; Endler and Thery 1996; Gomez and Théry 2004; Seehausen et al. 2012; Cole and Endler 2015). However, here I have demonstrated the importance of the behavior of aposematic individuals to seek light environments that theoretically increase warning signal efficacy. The Blue-white mimics have bright achromatic signals which are more conspicuous in dim environments and the Postman mimics have colorful chromatic signals which are more conspicuous in well lit and broadband environments (Douglas 2013, chapter 1), and each mimetic pair was shown to prefer their respective environment over the alternative. Thus, it is likely that the distribution of *Heliconius* butterflies that has been observed (Papageorgis 1975; Mallet and Gilbert 1995; Estrada and Jiggins 2002, chapter 1), is due to this behavioral preference. The selective pressures driving this behavior are unknown and may be due to

increased signal efficacy in the context of predator avoidance through warning signals (Mallet and Barton 1989; Finkbeiner et al. 2014), or in the context of mate choice (Jiggins et al. 2001; Finkbeiner et al. 2014). It is also possible that the behavior may be beneficial in the context of thermoregulation, although the experiment here controlled for temperature and previous research has shown that the different habitat types do not appear to affect the thermal properties of *Heliconius* individuals (Papageorgis 1975). Lastly, this behavioral difference between mimics is unlikely to have resulted from host plant search behavior, as the different species of *Passiflora*, the hostplants of *Heliconius*, are all able to grow in shaded light environments.

Like the behavioral preferences that matched the respective environments of each mimetic pair, here I support the prediction that mimetic pairs have evolved visual sensitivity that matches their respective light environment. However, all individuals had minimum visual thresholds that were much lower in intensity than their natural light environment. Of course, this is expected as I tested the minimum light levels that elicited a physiological response and at these light levels it is unlikely that vision will be able to function properly (Warrant 2004; Warrant 2015). All species of *Heliconius* studied here are inactive at low light levels as *Heliconius* roost before sunset and do not leave the roost until after sunrise (Finkbeiner et al. 2012). The overall intensities of the minimal thresholds found here are similar to light levels as the sun is setting and ranges from the sun being 2 to 6 degrees above the horizon for the Postman mimics or 1 to 6 degrees below the horizon for Blue-white mimics (Johnsen 2012). Thus, *Heliconius* are able to receive visual information from their environment as the sun is setting, albeit very little

information. To determine if the visual systems are tuned to the respective light intensities of each environment behavioral tests are needed at much lower light levels.

Although I have demonstrated that *Heliconius* individuals differ in their ability to detect light, the underlying mechanism remains unknown. Increased sensitivity to light can result from larger eyes, larger facets, larger rhabdoms, reduced screening pigment and/or less concentrated screening pigments around the photoreceptors, and neural summation of photoreceptors temporally and/or spatially (Warrant and Nilsson 2006; Land and Nilsson 2012; Cronin et al. 2014). Previous research has shown that although the four species of *Heliconius* butterflies investigated here differ in eye size and facet diameters, the trends in eye size do not match the trends in increased sensitivity (Seymoure et al. 2015). The two members of the Blue-whites, *Heliconius cydno* and *H. sapho,* had the largest eyes and facets and the smallest eyes and facets, respectively. Thus, the increased sensitivity of *H. sapho* is surprising due to the small eye morphology of the species and it is likely that *H. sapho* individuals compensate for the smaller eye morphology with several mechanisms listed above to increase sensitivity.

In this chapter and the previous two, I have found support that light is an important abiotic factor in the lives of *Heliconius* butterflies. The two different mimetic pairs have a preference for the light environment in which they are segregated by, are more conspicuous to predators in the light environment they prefer and inhabit and lastly, the mimetic pairs differ in the ability to detect light. The adaptive nature of these findings are obvious, however, the evolutionary rise of these adaptions are less apparent. Did the butterflies first evolve different visual sensitivities, which then led to behavioral and coloration differences between mimetic pairs? Or did the butterflies first diverge in

coloration and habitat use and then evolve behavioral preferences and visual sensitivities? With these data set, these questions are only proposed and further investigation is needed to understand the evolutionary mechanisms that resulted in the differences in coloration, vision and behavior documented here.

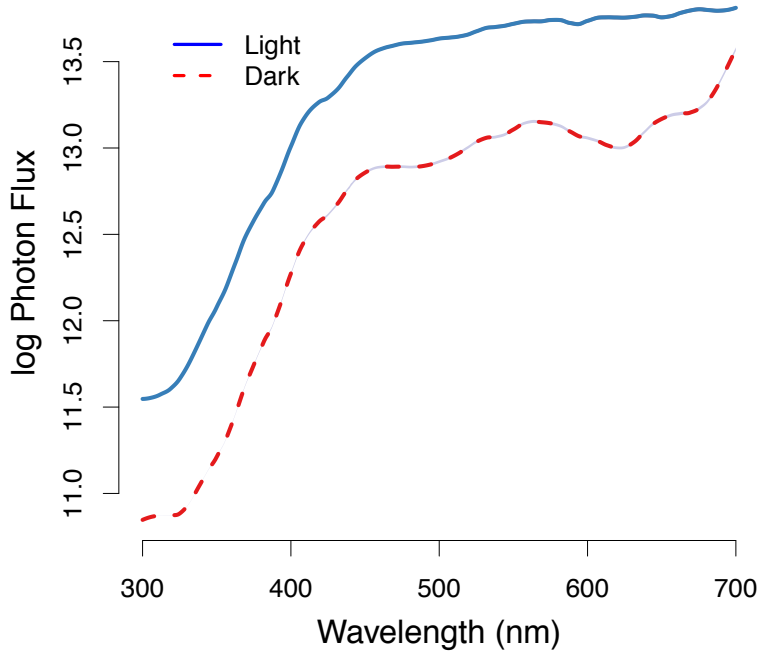

Figure 17. Irradiance spectra for the two light habitat sections in the behavioral study. Y-axis values have been log transformed for better spectral resolution. The light section (blue line) is greater in brightness by an order of magnitude compared to the dark section (red dashed line), while the spectral shape is similar between the two sections.

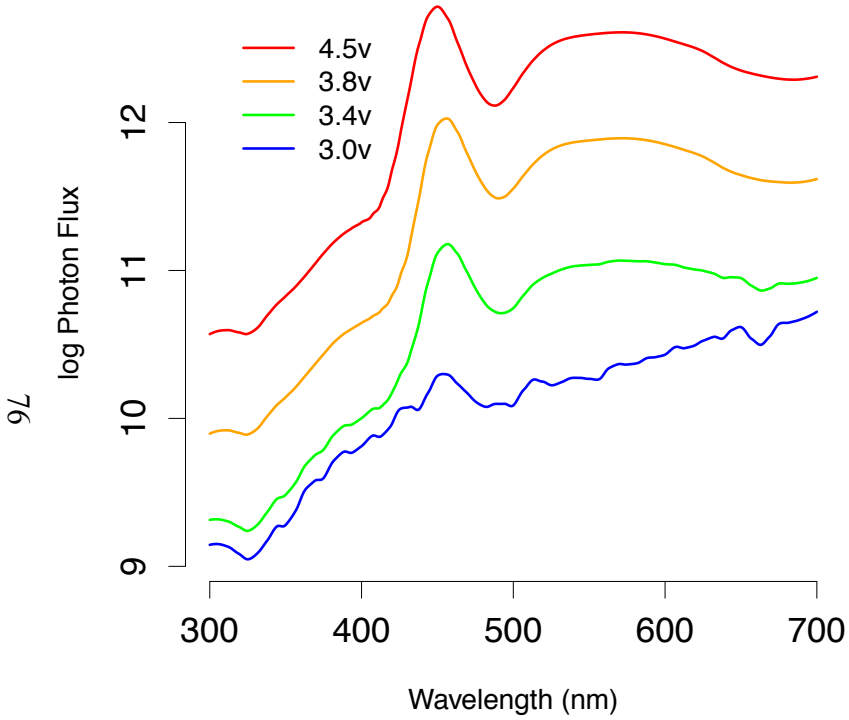

Figure 18. The irradiance of a sample of stimulus voltages ranging from 3 volts to 4.5 volts, which encompasses most minimal responses for individuals studied. Each spectrum represents the light that was flashed onto the right eye of the butterfly. The irradiance values have been log transformed to show both brightness and spectral composition. Thus the voltage that was used for each light flash is non-linearly related to the actual photon flux of the stimulus.

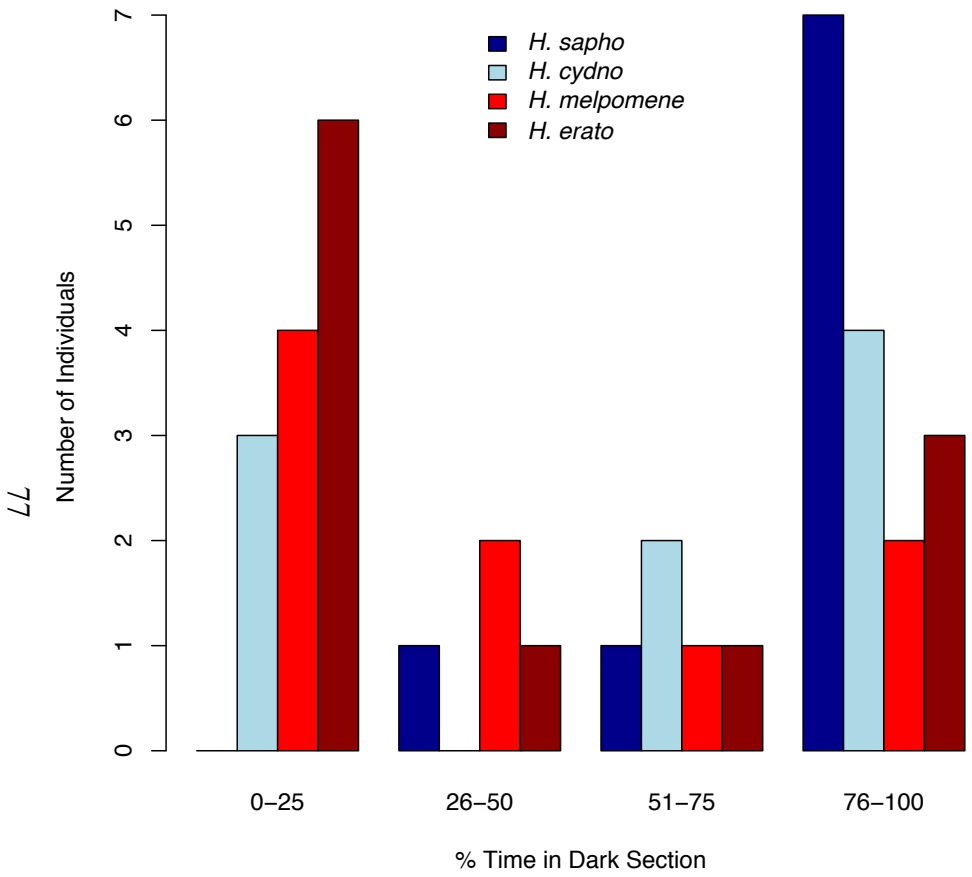

Figure 19. Percentage of time spent in the dark treatment for each species for each mimetic pair. The data are shown in 25% bins for each species. For each bin, the blue bars represent *H. sapho* and *H. cydno* of the Blue-white mimicry ring and the red bars represent *H. erato* and *H. melpomene* of the Postman mimicry ring. The Postman individuals spent less time in the dark section than the Blue-white individuals.

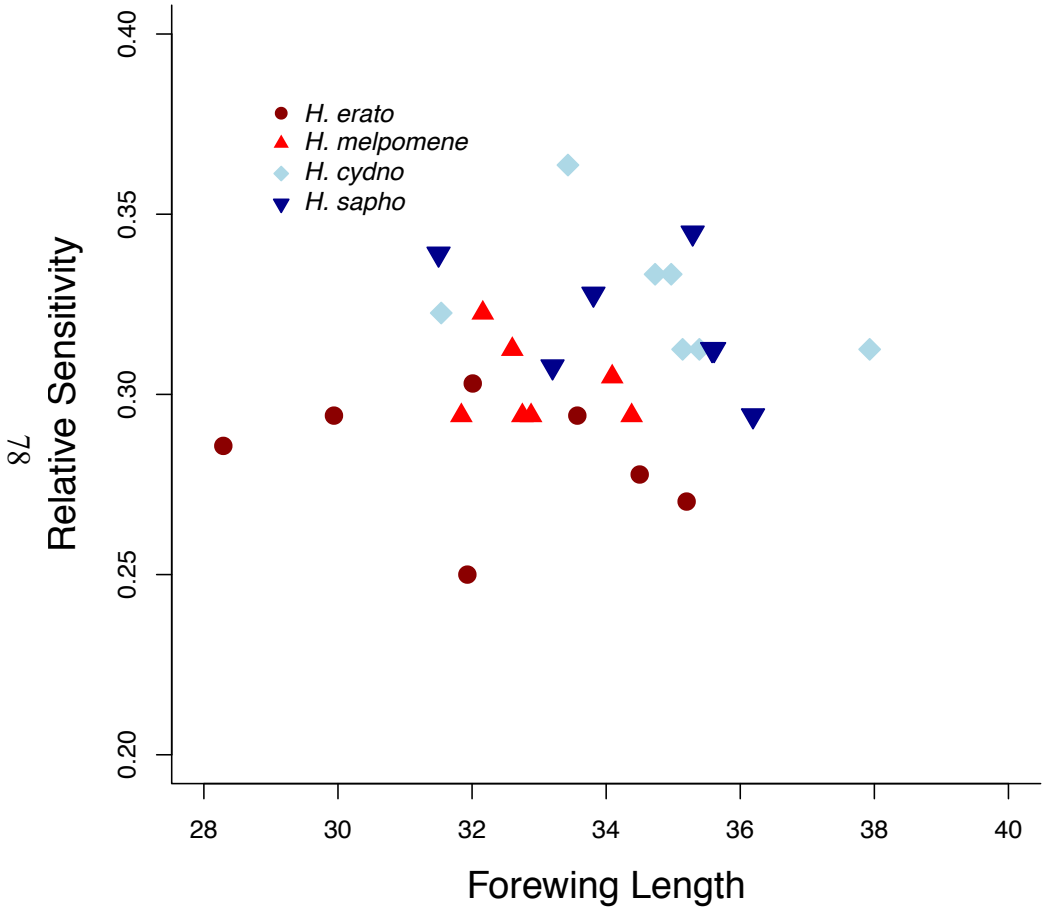

Figure 20. Minimum relative sensitivity as a function of forewing length and species. The minimum relative sensitivity is the inverse of the minimum voltage at which an individual had a retinal response. Forewing length is a surrogate for body size and this plot demonstrates that body size does not explain differences in minimal threshold sensitivity.

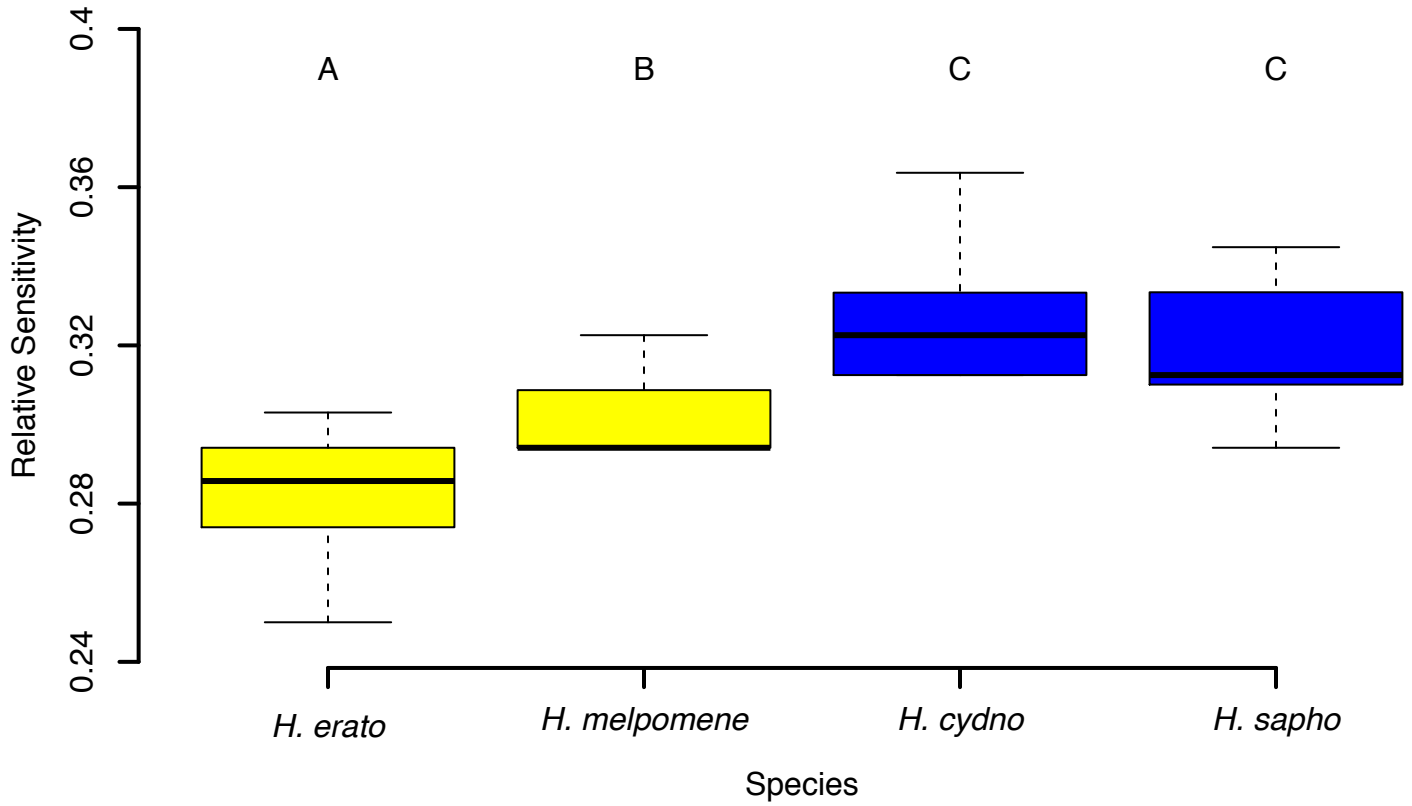

Figure 21. Relative minimal threshold sensitivity for each species in each mimetic pair. Relative sensitivities were calculated as the inverse of the minimal threshold voltages: 1/(voltage of minimal threshold). The Blue-white individuals (in blue) had significantly greater minimal sensitivity than the Postman individuals (in yellow) at an alpha level of 0.05. Furthermore, the minimal sensitivities differed between the Postman species.

ADDENDUM: EYE MORPHOLOGY OF NEOTROPICAL BUTTERFLIES DIFFERS BETWEEN LIGHT ENVIRONMENT

Introduction

The apposition compound eyes of butterflies may be constrained by their ability to capture photons (i.e. absolute sensitivity) in their environment. Previous research has shown that crepuscular butterflies have greater abilities of capturing photons than diurnal species with similar natural histories. There are several mechanisms for increasing absolute sensitivity including larger eyes, larger facets, wider and longer rhabdoms, and neural temporal summation of receptors as well as neural summation of neighboring photoreceptors (Warrant 2004; Warrant and Nilsson 2006; Frederiksen and Warrant 2008, see appendix B). Although much evidence exists demonstrating that large differences in light environment (e.g. several orders of magnitude) drive eye morphology and absolute visual sensitivity, little is known about how small differences in light environments (i.e. one order of magnitude) affect visual systems.

Data from my previous study with *Heliconius* butterflies suggests that eye morphology does not differ between species that occupy different light environments (Seymoure et al. 2015). However, *Heliconius* butterflies have unusually large eyes and perhaps may be a poor study system to understand the general trends of habitat light availability and eye morphology, see the discussion in appendix B. In this additional study, funded by a National Science Foundation Doctoral Dissertation Improvement Grant, I further investigated the effects of light environment on eye morphology of a broad and diverse group of butterflies spanning three different families.

Following from the background, hypotheses and predictions in appendix B, I hypothesized that the eye morphology of butterflies matches the light requirements of their respective environments. I predicted that butterflies occupying dim forest understory habitats will have larger facets than butterflies from bright open habitats. I predicted that total corneal surface area would not differ due to light environment because eye size will be selected by numerous pressures, see appendix B.

Methods

Seventy-three butterflies were collected during 2015 in Panama and Peru, see table 5 for species and location details, and figure 22 for an abbreviated phylogeny. I only used individuals that are open habitat or closed habitat specialists using previous research (Devries 1987) and my own personal observations. If a butterfly species was seen in both open and closed habitats, they were excluded from this study. I define open habitat as having little canopy cover (less than 20%) enabling for bright irradiance, while closed habitat was defined as having a closed canopy (greater than 80% canopy cover). Male butterflies were collected with insect nets and then transported to the field station in glassine envelopes. I then euthanized individuals by freezing. I recorded hind femur length and forewing length for each individual as a surrogate for body size.

Corneal preparations, photographs, and measurements were conducted as described in the methods in appendix B and so will not be repeated here. Briefly total corneal surface area, facet diameters from six regions of the eye, and hind femur length were measured. These data were analyzed in R. Facet diameters were averaged across regions. After confirming the normality of both total corneal surface area and average facet diameter with qqplots and the Shapiro-Wilks test, I analyzed the data with

ANCOVAs with hind femur length as a covariate to control for body size. Species were nested within the two habitat types and were not corrected for phylogeny.

Results

Both total corneal surface area and average facet diameter increased with hind femur length ($F_{1,60}$=471.67, p<0.001; t=4.92, p<0.001; respectively, see figures 23 and 24). Total corneal area was larger for individuals from the bright habitat ($F_{1,60}$=121.45, p<0.001) and corneal area differed between genera within the habitat types ($F_{9,60}$=43.52, p<0.001). There was not an interaction between femur length and habitat type although the p-value was close to the set alpha of .05 ($F_{1,60}$=3.41, p=0.070, figure 23). Average facet diameter also differed between habitat type with butterflies from closed understory having much larger facet diameters relative to body size than butterflies from open bright habitats ($F_{1,60}$=127.91, p<0.001) and facet diameter differed between genera ($F_{1,60}$=27.79, p<0.001, figure 24). However, there was not an interaction between hind femur length and habitat type ($F_{1,60}$= 1.03, p=0.316). Lastly, average facet diameter significantly differed due to total corneal surface area ($F_{1,69}$=235.33, p<0.001) and there was a significant interaction for facet diameter between habitat and corneal surface area ($F_{1,69}$=35.176, p<0.001, figure 25). Individuals with small corneal areas from the closed habitat had smaller facet diameters than small individuals from the open habitat, whereas closed habitat individuals with large corneal areas had larger facet diameters than similarly sized open habitat individuals, see figure 25.

Conclusion

In this addendum I tested if eye morphology differences between individuals were explained by habitat and found that average facet diameters were much larger for individuals that occupied closed, dimly lit habitats than for butterflies that occur in open, bright habitats. I also found a surprising difference in that open habitat individuals had larger total corneal surface area than closed habitat individuals. Furthermore, there was an interaction for facet diameter between corneal size and habitat type.

The findings here reveal that butterflies have evolved different eye morphology due to habitat type and light environment. This is an important finding because it reveals that even an order of magnitude difference in the brightness of light environments can affect eye morphology. My prediction that facet diameters would be larger in closed habitat was supported, however, the finding that open habitat butterflies have larger eyes is interesting. This finding suggests that many variables are at play for eye morphology and that perhaps closed habitat butterflies are constrained to increase both facet size and eye size. Furthermore, the interaction of corneal size and habitat type for facet diameter is intriguing and suggests that small eyes are not able to produce larger facets or that larger facets with a small eye are not as adaptive as a small eye with smaller but more facets.

This study is ongoing and, although the data collected to date suggest an interspecific correlation between eye morphology and light environment, the strength of the conclusions I can draw from this comparative study is limited by the lack of control for potential phylogenetic effects. As the phylogeny shows, figure 22, many of the closed-habitat genera are more closely related than they are to open-habitat genera. This lack of variation in habitat type within clades makes it difficult to test hypotheses about

the adaptive relationship between habitat and eye structure. Hopefully, the ability to control for phylogeny will improve as more species are added to this study.

These findings here add to the understanding of how light environments have affected eye morphology and vision in insects. Previous research has shown that disparate light environments (i.e. night vs. day) greatly affect visual systems in animals. However, little was known about if and how smaller differences in light environment that occur due to forest canopy cover have driven eye morphology and visual systems. These data shed light onto the importance of diurnal differences in light environments and future work will include understanding how the electrophysiology and behavior of butterflies differ between these two disparate light environments: closed, forest understory and bright, open gaps.

Table 7. The number and names of butterfly families and species for each habitat type for eye morphology. N is the number of individuals within each sample.

Habitat	Families	Species	n
Open	3	5	19
Closed	2	10	54

Habitat	Family	Species	n	Location
Open	Pieridae	*Phoebis sennae*	3	Panama
Open	Papilionidae	*Eurytides protesilaus*	3	Panama
Open	Papilionidae	*Parides arcas*	3	Panama
Open	Nymphalidae	*Anartia fatima*	8	Panama
Open	Nymphalidae	*Danaus gilppus*	2	Panama
Closed	Riodinidae	*Mesosemia judicialis*	2	Peru
Closed	Nymphalidae	*Cithaerias phantoma*	6	Peru
Closed	Nymphalidae	*Haetera piera*	4	Peru
Closed	Nymphalidae	*Napeogenes inachia*	12	Peru
Closed	Nymphalidae	*Oleria onega*	3	Peru
Closed	Nymphalidae	*Pierella astyoche*	7	Peru
Closed	Nymphalidae	*Pierella hortona*	1	Peru
Closed	Nymphalidae	*Pierella lena*	6	Peru
Closed	Nymphalidae	*Pierella luna*	8	Panama
Closed	Nymphalidae	*Pierella rhea*	5	Peru

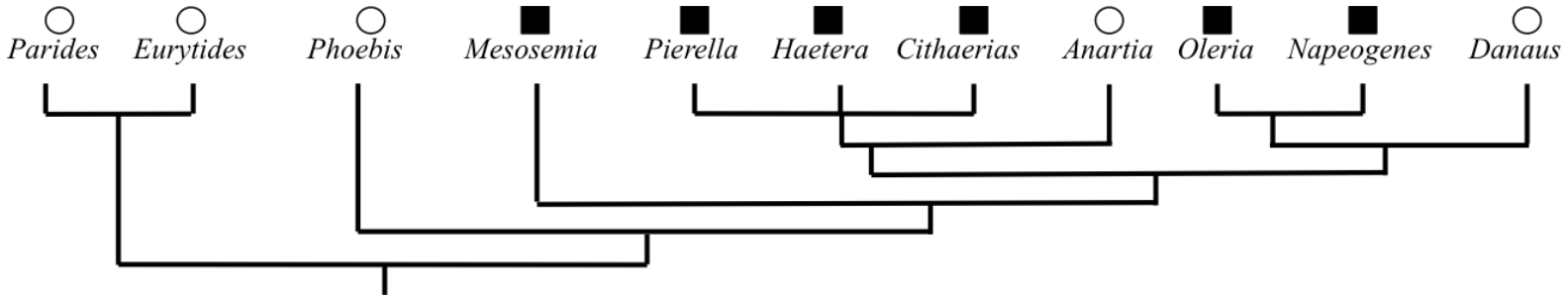

Figure 22. Abbreviated phylogeny of genera used to compare eye morphology between butterflies from open habitats (indicated with open circles) and closed habitats (black squares). Of the six genera that represent closed-habitat butterflies, three are all closely related and another two are closely related to each other. Of the five genera that represent open-habitat butterflies, two are closely related. Note that the branches are all set at a standard length because the evolutionary distances are unknown for many of these species (Ackery et. al 1999).

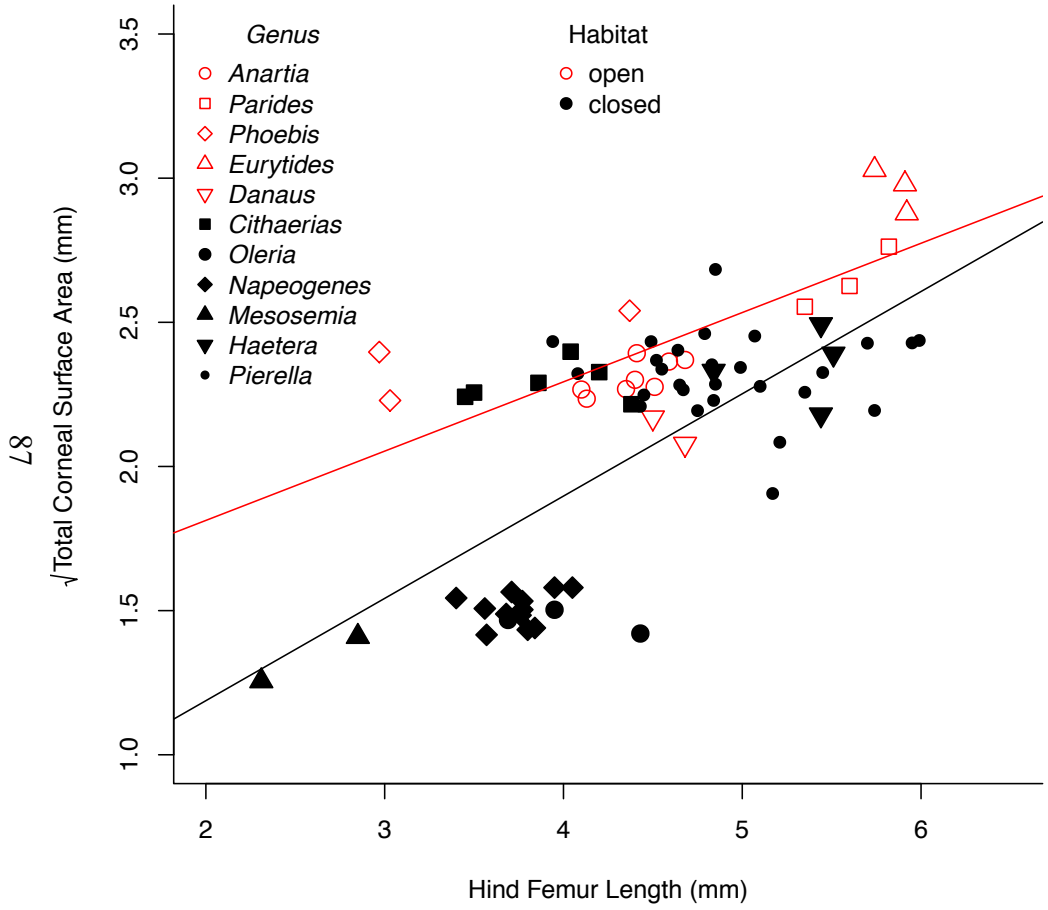

Figure 23. Square root of the corneal size relative to hind femur length for individuals from open (open shapes) and closed habitats (filled shapes). Corneal size increases with hind femur length and corneal size does not differ between the habitats.

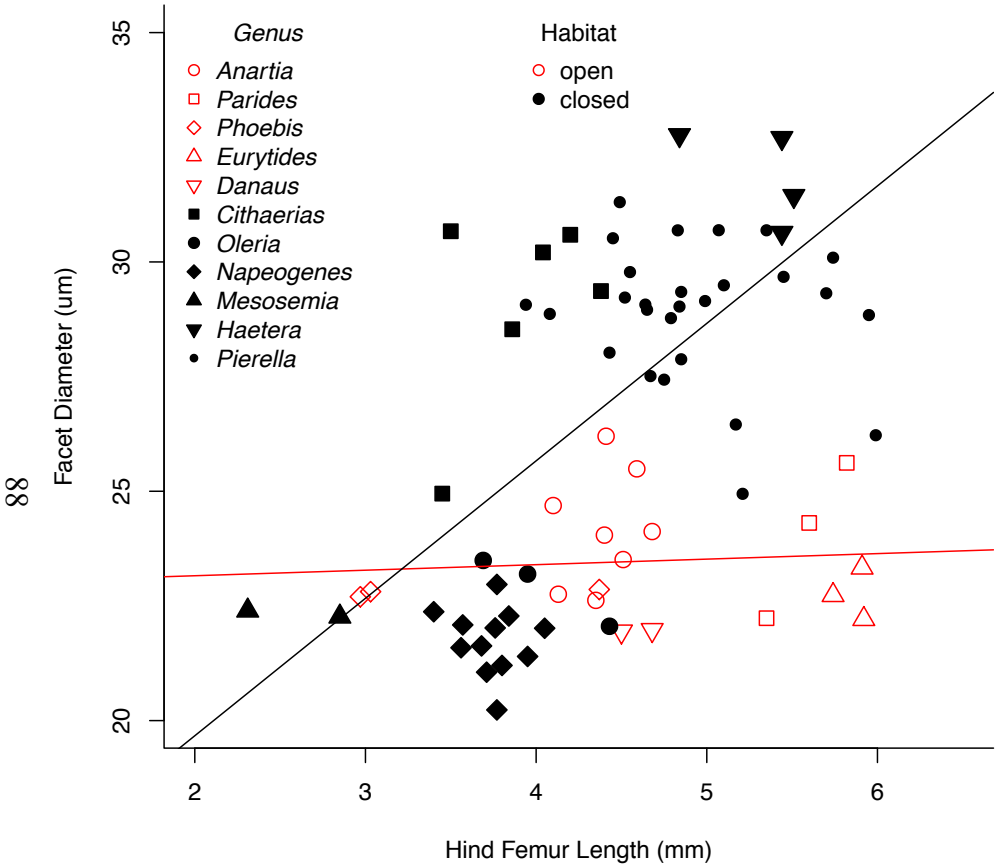

Figure 24. Average facet diameters relative to body size (hind femur length) for butterflies from open (open shapes) and closed habitats (filled shapes). There was an interaction between facet diameter and habitat with large butterflies from closed habitat having much larger facet diameters than similarly sized butterflies from open habitat, but small butterflies from closed habitat had smaller facet diameters than similarly sized open habitat butterflies. Furthermore, the open habitat butterflies have similar sized facet diameters regardless of body size.

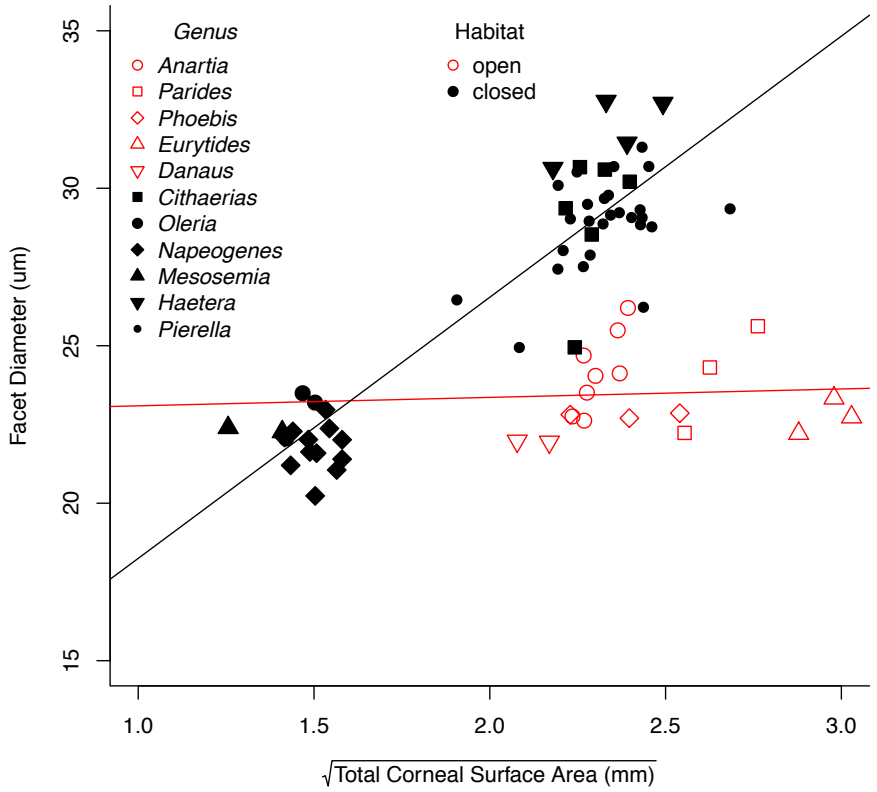

Figure 25. Average facet diameter relative to the square root of total corneal surface area. This figure demonstrates that butterflies from the two habitats differ in the relationship of facet diameter to corneal surface area with the open habitat butterflies having similar sized facet diameters regardless of corneal size, whereas the closed habitat butterflies have much larger facets as corneal area increases.

REFERENCES

Ackery, P. R., R. de Jong, and R. I. Vane-Wright. 1999. The butterflies: Hedyloidea, Hesperioidea, and Papilionoidea. Lepidoptera: Moths and Butterflies. 1. Evolution, Systematics, and Biogeography. Handbook of Zoology Vol. IV, Part 35. N. P. Kristensen, ed. De Gruyter, Berlin and New York.

Ali, M. A., and W. R. A. Muntz. 1975. Vision in Fishes: New Approaches in Research. In M. A. Ali, ed., (pp. 159–167). Springer US, Boston, MA.

Angehr, G. R., and R. Dean. 2010. The Birds of Panama: A Field Guide. Cornell University Press, Ithaca, NY.

Arenas, L. M., D. Walter, and M. Stevens. 2015. Signal honesty and predation risk among a closely related group of aposematic species. Scientific Reports 5:11021.

Beldade, P., and P. M. Brakefield. 2002. The genetics and evo-devo of butterfly wing patterns. Nature reviews. Genetics 3:442–452.

Bergman, M., N. Lessios, B. M. Seymoure, and R. L. Rutowski. 2015. Mate detection in a territorial butterfly--the effect of background and luminance contrast. Behavioral Ecology 00:1–10.

Bergman, M., and C. Wiklund. 2009. Visual mate detection and mate flight pursuit in relation to sunspot size in a woodland territorial butterfly. Animal Behaviour 78:17–23.

Brill, R., C. Magel, M. Davis, R. Hannah, and P. Rankin. 2008. Effects of rapid decompression and exposure to bright light on visual function in black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepis*). Fishery Bulletin 106:427–437.

Briscoe, A. D., S. M. Bybee, G. D. Bernard, F. Yuan, M. P. Sison-Mangus, R. D. Reed, A. D. Warren, et al. 2010. Positive selection of a duplicated UV-sensitive visual pigment coincides with wing pigment evolution in Heliconius butterflies. Proceedings of the National Academy of Sciences of the United States of America 107:3628–33.

Brower, L. P. 1969. Ecological chemistry. Scientific American 220:22–29.

Brown, K. S. J. 1981. The Biology of *Heliconius* and Related Genera. Annual review of entomology 26:427–56.

Brown, K. T. 1968. The electroretinogram: Its components and their origins. Vision research 8:633–677.

Bybee, S. M., F. Yuan, M. D. Ramstetter, J. Llorente-Bousquets, R. D. Reed, D. Osorio, and A. D. Briscoe. 2012. UV photoreceptors and UV-yellow wing pigments in Heliconius butterflies allow a color signal to serve both mimicry and intraspecific communication. The American naturalist 179:38–51.

Camara, M. D. 1997. Predator responses to sequestered plant toxins in buckeye caterpillars: Are tritrophic interactions locally variable? Journal of Chemical Ecology 23:2093–2106.

Cardoso, M. Z., and L. E. Gilbert. 2013a. Pollen feeding, resource allocation and the evolution of chemical defence in passion vine butterflies. Journal of Evolutionary Biology 26:1254–1260.

Cardoso, M. Z., and L. E. Gilbert. 2013b. Pollen feeding, resource allocation and the evolution of chemical defence in passion vine butterflies. Journal of evolutionary biology 1–7.

Carroll, J., and T. N. Sherratt. 2013. A direct comparison of the effectiveness of two anti-predator strategies under field conditions. Journal of Zoology 291:279–285.

Chai, P. 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. Biological Journal of the Linnean Society 29:161–189.

Chai, P. 1996. Butterfly visual characteristics and ontogeny of responses to butterflies by a specialized tropical bird. Biological Journal of the Linnean Society 59:37–67.

Chai, P., and R. B. Srygley. 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. American Naturalist 135:748–765.

Chittka, L., M. Vorobyev, A. Shmida, and R. Menzel. 1993. Bee colour vision - the optimal system for the discrimination of flower colours with three spectral photoreceptor types.pdf. Sensory Systems of Arthropods 211–218.

Cole, G. L., and J. A. Endler. 2015. Variable Environmental Effects on a Multicomponent Sexually Selected Trait. American Naturalist 185:452–468.

Cott, H. B. 1940. Adaptive coloration in animals. Methuen, London.

Cronin, T. W., S. Johnsen, N. J. Marshall, and E. J. Warrant. 2014. Visual Ecology. Princeton University Press, Princeton, NJ.

Cuthill, I. C., M. Stevens, J. Sheppard, T. Maddocks, C. A. Párraga, and T. S. Troscianko. 2005. Disruptive coloration and background pattern matching. Nature 434:72–74.

Dalrymple, R. L., F. K. C. Hui, H. Flores-moreno, D. J. Kemp, and A. T. Moles. 2014. Roses are red , violets are blue – so how much replication should you do ? An assessment of variation in the colour of flowers and birds.

Darst, C. R., and M. E. Cummings. 2006. Predator learning favours mimicry of a less-toxic model in poison frogs. Nature 440:208–11.

de Moura, P. A., S.-P. Quek, M. Z. Cardoso, and M. R. Kronforst. 2011. Comparative population genetics of mimetic Heliconius butterflies in an endangered habitat; Brazil's Atlantic Forest. BMC genetics 12:9.

Devries, P. J. 1987. The Butterflies of Costa rica and Their Natural History, Volume I: Papilionidae, Pieridae, Nymphalidae. Princeton University Press.

Devries, P. J., D. Murray, and R. Lande. 1997. Species diversity in vertical , horizontal , and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. Biological Invasions 62:343–364.

Douglas, J. M. 2013. *Ambient Light Environment and the Evolution of Brigthness, Chroma, and Perceived Chromaticity in the Warning Signals of Butterflies*. Arizona State University.

Dressler, R. L. 1979. Eulaema bombiformis , E . meriana , and Mullerian Mimicry in Related Species (Hymenoptera : Apidea). Biotropica 11:144–151.

Edmunds, M. 1974. Defence in animals. Prentice Hall Press.

Endler, J. 1998. Sensory ecology, receiver biases and sexual selection. Trends in Ecology & Evolution 13:415–420.

Endler, J. a, and J. J. D. Greenwood. 1988. Frequency-Dependent Predation, Crypsis and Aposematic Coloration [and Discussion]. Philosophical Transactions of the Royal Society of London Series B 319:505–523.

Endler, J. a, and P. W. J. Mielke. 2005. Comparing color patterns as birds see them. Biol. J. Linn. Soc. 86:405–431.

Endler, J. A. 1978. A predator's view of animal color patterns. Evolutionary Biology 319–364.

Endler, J. A. 1981. An overview of the relationships between mimicry and crypsis. Biological Journal of the Linnean Society 16:25–31.

Endler, J. A. 1984. Progressive background in moths , and a quantitative measure of crypsis. Biological Journal of the Linnean Society 22:187–231.

Endler, J. a. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41:315–352.

Endler, J. A. 1992. Signal conditions and the direction of evolution. The American Naturalist 139:S125–S153.

Endler, J. A. 1993. The color of light in forests and its implications. Ecological Monographs 63:1–27.

Endler, J. A., and M. Thery. 1996. Interacting effects of lek placement, Display behavior, Ambient light, and Color patterns in three neotropical forest-dwelling birds. The american Naturalist 148:421–452.

Endler, J. A., D. A. Westcott, J. R. Madden, and T. Robson. 2005. Animal visual systems and the evolution of color patterns: sensory processing illuminates signal evolution. Evolution 59:1795–1818.

Estrada, C., and C. D. Jiggins. 2002. Patterns of pollen feeding and habitat preference among Heliconius species. Ecological Entomology 27:448–456.

Finkbeiner, S. D., A. D. Briscoe, and R. D. Reed. 2012. The benefit of being a social butterfly: communal roosting deters predation. Proceedings. Biological sciences / The Royal Society 279:2769–76.

Finkbeiner, S. D., A. D. Briscoe, and R. D. Reed. 2014. Warning signals are seductive: Relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. Evolution 68:3410–3420.

Flanagan, N. S., A. Tobler, A. Davison, O. G. Pybus, D. D. Kapan, S. Planas, M. Linares, et al. 2004. Historical demography of Mullerian mimicry in the neotropical Heliconius butterflies. Proceedings of the National Academy of Sciences of the United States of America 101:9704–9.

Fleishman, L. J., M. Leal, and J. Sheehan. 2006. Illumination geometry, detector position and the objective determination of animal signal colours in natural light. Animal Behaviour 71:463–474.

Fleishman, L., E. Loew, and M. Leal. 1993. Ultraviolet vision in lizards. Nature 365:397.

Frazer, G. W., C. . Canham, and K. P. Lertzman. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Users Manual and program Documentation.

Frazer, G. W., R. A. Fournier, J. A. Trofymow, and R. J. Hall. 2001. A comparison of digital and film fisheye photography for analysis of forest canopy structure and gap light transmission. Agricultural and Forest Meteorology 109:249–263.

Frederiksen, R., and E. J. Warrant. 2008. Visual sensitivity in the crepuscular owl butterfly Caligo memnon and the diurnal blue morpho Morpho peleides: a clue to explain the evolution of nocturnal apposition eyes? The Journal of experimental biology 211:844–851.

Gomez, D., and M. Théry. 2004. Influence of ambient light on the evolution of colour signals: comparative analysis of a Neotropical rainforest bird community. Ecology Letters 7:279–284.

Guilford, T. 1990. The Evolution of Aposematism (pp. 23–62).

Guilford, T., and M. S. Dawkins. 1993. Are Warning Colors Handicaps? Evolution 47:400–416.

Halpin, C. G., J. Skelhorn, and C. Rowe. 2008. Being conspicuous and defended: Selective benefits for the individual. Behavioral Ecology 19:1012–1017.

Hart, N. S. 2001. The visual ecology of avian photoreceptors. Progress in Retinal and Eye Research 20:675–703.

Hart, N. S., and D. M. Hunt. 2007. Avian visual pigments: characteristics, spectral tuning, and evolution. The American naturalist 169 Suppl :S7–26.

Hegna, R. H., R. a. Saporito, and M. a. Donnelly. 2012. Not all colors are equal: predation and color polytypism in the aposematic poison frog Oophaga pumilio. Evolutionary Ecology.

Heiling, A. M., L. Chittka, K. Cheng, and M. E. Herberstein. 2005. Colouration in crab spiders: substrate choice and prey attraction. The Journal of experimental biology 208:1785–92.

Hines, H. M., and P. H. Williams. 2012. Mimetic colour pattern evolution in the highly polymorphic Bombus trifasciatus (Hymenoptera: Apidae) species complex and its comimics. Zoological Journal of the Linnean Society 166:805–826.

Horodysky, A. Z., R. W. Brill, E. J. Warrant, J. a Musick, and R. J. Latour. 2008. Comparative visual function in five sciaenid fishes inhabiting Chesapeake Bay. The Journal of experimental biology 211:3601–3612.

Hoyo, J. D., A. Elliot, and D. Christie. 2004. Handbook of the birds of the world. Lynx Edicions.

Hutton, P., R. a Ligon, K. J. McGraw, B. M. Seymoure, and R. K. Simpson. 2015. Dynamic color communication. Current Opinion in Behavioral Sciences 6:41–49.

Jiggins, C. D., C. Estrada, and a Rodrigues. 2004. Mimicry and the evolution of premating isolation in Heliconius melpomene Linnaeus. Journal of evolutionary biology 17:680–91.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302–305.

Johnsen, S. 2012. The Optics of Life. Princeton University Press, Prince.

Joron, M. 2005. Polymorphic mimicry, microhabitat use, and sex-specific behaviour. Journal of evolutionary biology 18:547–56.

Joron, M., and J. Mallet. 1999. Diversity in mimicry 14:5347.

Joron, M., and J. L. B. Mallet. 1998. Diversity in mimicry: Paradox or paradigm? Trends in Ecology and Evolution 13:461–466.

Joron, M., I. A. N. R. Wynne, G. Lamas, and J. Mallet. 2001. Variable selection and the coexistence of multiple mimetic forms of the butter ¯ y Heliconius numata. Evolutionary Ecology 13:721–754.

Kapan, D. D. 2001. Three-butterfly system provides a field test of mullerian mimicry. Nature 409:18–20.

Kikuchi, D. W., and D. W. Pfennig. 2013. Imperfect mimicry and the limits of natural selection. The Quarterly review of biology 88:297–315.

Kikuchi, D. W., B. M. Seymoure, and D. W. Pfennig. 2014. Mimicry's palette: Widespread use of conserved pigments in the aposematic signals of snakes. Evolution and Development 16:61–67.

Kingsolver, J. G. 1985. Butterfly thermoregulation: organismic mechanisms and population consequences. Journal of Research on the Lepidoptera.

Kleckova, I., M. Konvicka, and J. Klecka. 2014. Thermoregulation and microhabitat use in mountain butterflies of the genus Erebia: Importance of fine-scale habitat heterogeneity. Journal of Thermal Biology 41:50–58.

Kozak, K. M., N. Wahlberg, A. Neild, K. K. Dasmahapatra, J. Mallet, and C. D. Jiggins. 2015. Multilocus Species Trees Show the Recent Adaptive Radiation of the Mimetic Heliconius Butterflies. Systematic biology 0:1–20.

Kricher, J. 2011. Tropical Ecology. Princeton University Press, Princeton,.

Land, M. F., and D. E. Nilsson. 2012. Animal Eyes (2nd Editio.). Oxford University Press, Oxford.

Langham, G. M. 2004. Specialized avian predators repeatedly attack novel color morphs of Heliconius butterflies. Evolution; international journal of organic evolution 58:2783–7.

Langham, G. M. 2005. Rufous-tailed jacamars and aposematic butterflies: do older birds attack novel prey? Behavioral Ecology 17:285–290.

Leal, M., and L. Fleishman. 2004. Differences in visual signal design and detectability between allopatric populations of anolis lizards. The American naturalist 163:26–39.

Leal, M., and L. J. Fleishman. 2001. Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. Proceedings. Biological sciences / The Royal Society 269:351–9.

Leal, M., and L. J. Fleishman. 2013. Differences in Visual Signal Design and Detectability between Allopatric Populations of Anolis Lizards. The American naturalist 163:26–39.

Legrand, D., V. M. Stevens, and M. Baguette. 2011. Selection on the wing in Heliconius butterflies. BMC genetics 12:31.

Llaurens, V., M. Joron, and M. Théry. 2014. Cryptic differences in colour among Müllerian mimics: How can the visual capacities of predators and prey shape the evolution of wing colours? Journal of Evolutionary Biology 27:531–540.

Long, K. D., and G. Rosenqvist. 1998. Changes in male guppy courting distance in response light environment to a fluctuating. Behavioral Ecology and Sociobiology 44:77–83.

Maan, M. E., and O. Seehausen. 2011. Ecology, sexual selection and speciation. Ecology letters 14:591–602.

Maia, R., C. M. Eliason, P. P. Bitton, S. M. Doucet, and M. D. Shawkey. 2013. pavo: An R package for the analysis, visualization and organization of spectral data. Methods in Ecology and Evolution 4:906–913.

Mallet, J., and N. H. Barton. 1989. Strong Natural Selection in a Warning-Color Hybrid Zone. Evolution 43:421–431.

Mallet, J., and L. E. Gilbert. 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in Heliconius butterflies. Biological Journal of the Linnean Society 55:159–180.

Mappes, J., N. Marples, and J. a Endler. 2005. The complex business of survival by aposematism. Trends in ecology & evolution 20:598–603.

Marek, P. E., and J. E. Bond. 2009. A Müllerian mimicry ring in Appalachian millipedes. Proceedings of the National Academy of Sciences of the United States of America 106:9755–60.

Meadows, M. G., N. I. Morehouse, R. L. Rutowski, J. M. Douglas, and K. J. McGraw. 2011. Quantifying iridescent coloration in animals: A method for improving repeatability. Behavioral Ecology and Sociobiology 65:1317–1327.

Merrill, R. M., K. K. Dasmahapatra, J. W. Davey, D. D. Dell'Aglio, J. J. Hanly, B. Huber, C. D. Jiggins, et al. 2015. The diversification of Heliconius butterflies: What have we learned in 150 years? Journal of Evolutionary Biology 28:1417–1438.

Merrill, R. M., Z. Gompert, L. M. Dembeck, M. R. Kronforst, W. O. McMillan, and C. D. Jiggins. 2011. Mate preference across the speciation continuum in a clade of mimetic butterflies. Evolution; international journal of organic evolution 65:1489–500.

Merrill, R. M., R. W. R. Wallbank, V. Bull, P. C. a Salazar, J. Mallet, M. Stevens, and C. D. Jiggins. 2012. Disruptive ecological selection on a mating cue. Proceedings. Biological sciences / The Royal Society.

Montgomerie, R. 2006. Analyzing colors. Bird Coloration. Volume 1 Mechanisms and measurements. (pp. 90–147). Harvard University Press, Cambridge, Massachusetts.

Müller, F. 1878. Ituna and Thyridia: a remarkable case of mimicry in butterflies. Proceedings of the Entomological Society of London 20–29.

Nokelainen, O., J. Valkonen, C. Lindstedt, and J. Mappes. 2014. Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. Journal of Animal Ecology.

Noonan, B. P., and K. P. Wray. 2006. Neotropical diversification: The effects of a complex history on diversity within the poison frog genus Dendrobates. Journal of Biogeography 33:1007–1020.

Odeen, A., and O. Hastad. 2003. Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. Molecular biology and evolution 20:855–61.

Papageorgis, C. 1975. Mimicry in Neotropical Butterflies: Why are there so many different wing-coloration complexes in one place? American Scientist 63:522–532.

Pegram, K. V., and R. L. Rutowski. 2014. Relative effectiveness of blue and orange warning colours in the contexts of innate avoidance, learning and generalization. Animal Behaviour 92:1–8.

Pegram, K. V, H. a Han, and R. L. Rutowski. 2015. Warning Signal Efficacy: Assessing the Effects of Color, Iridescence, and Time of Day in the Field. Ethology 121:1–13.

Pinheiro, C. E. G. 1996. Palatability and escaping ability in neotropical butterflies: Tests with wild kingbirds (Tyrannus melancholicus, Tyrannidae). Biological Journal of the Linnean Society 59:351–365.

Pinheiro, C. E. G. 2007. Asynchrony in daily activity patterns of butterfly models and mimics. Journal of Tropical Ecology 23:119.

Pinheiro, C. E. G. 2011. On the evolution of warning coloration, Batesian and Müllerian mimicry in Neotropical butterflies: the role of jacamars (Galbulidae) and tyrant-flycatchers (Tyrannidae). Journal of Avian Biology 42:277–281.

Poulton, E. b. 1890. The colour of animals. Their meaning and use. Especially considered in the case of insects.

Pyke, C. R., R. Condit, S. Aguilar, S. Lao, and R. Christopher. 2001. Floristic Composition across a Climatic Gradient in a Neotropical Lowland Forest. Journal of Vegetation Science 12:553–566.

Randel, N., and G. Jékely. 2016. Phototaxis and the origin of visual eyes. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150042.

Reed, R. D., R. Papa, A. Martin, H. M. Hines, B. a Counterman, C. Pardo-Diaz, C. D. Jiggins, et al. 2011. Optix Drives the Repeated Convergent Evolution of Butterfly Wing Pattern Mimicry. Science (New York, N.Y.) 333:1137–41.

Renoult, J. P., A. Kelber, and H. M. Schaefer. 2015. Colour spaces in ecology and evolutionary biology. Biological Reviews.

Rich, P. M. 1990. Characterizing plant canopies with hemispherical photographs. Remote Sensing Reviews 5:13–29.

Rojas, B. 2014. Differential detectability under varying light environments: an alternative explanation for the maintenance of polymorphic warning signals? Behavioural Processes 109:164–172.

Rojas, B., J. Devillechabrolle, and J. a. Endler. 2014. Paradox lost: variable colour-pattern geometry is associated with differences in movement in aposematic frogs. Biology letters 10:20140193.

Rowland, H. M., E. Wiley, G. D. Ruxton, J. Mappes, and M. P. Speed. 2010. When more is less: the fitness consequences of predators attacking more unpalatable prey when more are presented. Biology letters 6:732–5.

Rutowski, R. L., A. C. Nahm, and J. M. Macedonia. 2010a. Iridescent hindwing patches in the Pipevine Swallowtail : differences in dorsal and ventral surfaces relate to signal function and context. Functional Ecology.

Rutowski, R. L., A. C. Nahm, and J. M. Macedonia. 2010b. Iridescent hindwing patches in the Pipevine Swallowtail: differences in dorsal and ventral surfaces relate to signal function and context. Functional Ecology 24:767–775.

Rutowski, R. L., K. V. Pegram, and M. J. Lillo. 2013. Iridescent blue and orange components contribute to the recognition of a multicomponent warning signal. Behaviour 1–16.

Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2004. Avoiding attack: the evolutionary ecology of crypsis, warning signals & mimicry.

Salcedo, C. 2011. Pollen preference for Psychotria sp. is not learned in the passion flower butterfly, Heliconius erato. Journal of insect science (Online) 11:25.

Santiago, L. S., K. Kitajima, J. Wright, and S. S. Mulkey. 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. Oecologia 139:495–502.

Saporito, R. A., R. Zuercher, M. Roberts, K. G. Gerow, and M. A. Donnelly. 2007. Experimental Evidence for Aposematism in the Dendrobatid Poison Frog Oophaga pumilio. Copeia 2007:1006–1011.

Seehausen, O. 2015. Beauty varies with the light. Nature 521:34–35.

Seehausen, O., J. J. M. Van Alphen, and F. Witte. 2012. Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection 1808.

Seehausen, O., J. van Alphen, and F. Witte. 1997. Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. Science 277:1808–1811.

Seymoure, B. M., and A. Aiello. 2015. Keeping the Band Together: Evidence for False Boundary Disruptive Coloration in a Butterfly. Journal of Evolutionary Biology n/a–n/a.

Seymoure, B. M., W. O. McMillan, and R. L. Rutowski. 2015. Peripheral eye dimensions in Longwing (Heliconius) butterflies vary with body size and sex but not light environment nor mimicry ring. The Journal of Research on the Lepidoptera 48:83–92.

Sherratt, T. N. 2008. The evolution of Müllerian mimicry. Die Naturwissenschaften 95:681–95.

Siddiqi, A., T. W. Cronin, E. R. Loew, M. Vorobyev, and K. Summers. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog Dendrobates pumilio. The Journal of experimental biology 207:2471–85.

Silberglied, R. E., A. Aiello, and G. Lamas. 1979. Neotropical Butterflies of the Genus Anartia: Systematics, Life Histories and General Biology (Lepidoptera: Nymphalidae). Psyche: A Journal of Entomology 86:219–260.

Sillen-Tullberg, B. 1985. Higher survival of an aposematic than of a cryptic form of a distasteful bug. Oecologia 67:411–415.

Skelhorn, J., and C. Rowe. 2009. Distastefulness as an antipredator defence strategy. Animal Behaviour 78:761–766.

Skutch, A. F. 1968. The Nesting of Some Venezuelan Birds. Condor 70:66–82.

Speed, M. 2000. Warning signals, receiver psychology and predator memory. Animal behaviour 60:269–278.

Speed, M. P., M. a Brockhurst, and G. D. Ruxton. 2010. The dual benefits of aposematism: predator avoidance and enhanced resource collection. Evolution 64:1622–33.

Srygley, R. B., and C. P. Ellington. 1999. Discrimination of flying mimetic, passion-vine butterflies Heliconius. Proceedings of the Royal Society B: Biological Sciences 266:2137–2140.

Stevens, M. 2013. Sensory Ecology. Oxford University Press.

Stevens, M., I. C. Cuthill, A. M. M. Windsor, and H. J. Walker. 2006. Disruptive contrast in animal camouflage. Proceedings. Biological sciences / The Royal Society 273:2433–8.

Stevens, M., and S. Merilaita. 2009. Animal camouflage: current issues and new perspectives. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 364:423–427.

Stevens, M., and S. Merilaita. 2011. Animal Camouflage: mechanisms and unction. Cambridge University Press.

Stuart, Y. E., N. Dappen, and N. Losin. 2012. Inferring predator behavior from attack rates on prey-replicas that differ in conspicuousness. PloS one 7:e48497.

Summers, K., M. P. Speed, J. D. Blount, and a. M. M. Stuckert. 2015. Are aposematic signals honest? A review. Journal of Evolutionary Biology 28:1583–1599.

Team, R. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austra.

Théry, M., and S. L. Vehrencamp. 1995. Light Patterns as Cues for Mate Choice in the Lekking White-Throated Manakin (Corapipo gutturalis). The Auk 112:133–145.

Thurman, T., and B. M. Seymoure. 2016. A Bird's Eye View of Two Mimetic Tropical Butterflies: Coloration Matches Predator's Sensitivity. Journal of Zoology 298:159–168.

Twomey, E., J. Yeager, J. L. Brown, V. Morales, M. Cummings, and K. Summers. 2013. Phenotypic and Genetic Divergence among Poison Frog Populations in a Mimetic Radiation. PloS one 8:e55443.

Uy, J. a. C., and J. A. Endler. 2004. Modification of the visual background increases the conspicuousness of golden-collared manakin displays. Behavioral Ecology 15:1003–1010.

Vorobyev, M., and D. Osorio. 1998. Receptor noise as a determinant of colour thresholds. Proceedings. Biological sciences / The Royal Society 265:351–358.

Wallace, a R. 1867. Mimicry and other protective resemblances among animals. Westminster and Foreign Quarterly Review 32:1–43.

Warrant, E. 2004. Vision in the dimmest habitats on Earth. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 190:765–789.

Warrant, E. J. 1999. Seeing better at night: Life style, eye design and the optimum strategy of spatial and temporal summation. Vision Research 39:1611–1630.

Warrant, E. J. 2015. Visual tracking in the dead of night. Science 348:1212–1213.

Warrant, E. J., and S. Johnsen. 2013. Vision and the light environment. Current Biology 23:R990–R994.

Warrant, E. J., and D. E. Nilsson. 2006. Invertebrate Vision. CAMBRIDGE UNIV PRESS, Cambridge.

Wiklund, C., and T. Järvi. 1982. Survival of Distasteful Insects After Being Attacked by Naive Birds: A Reappraisal of the Theory of Aposematic Coloration Evolving Through Individual Selection. Evolution 36:998–1002.

Wilson, J. S., J. P. Jahner, M. L. Forister, E. S. Sheehan, K. A. Williams, and J. P. Pitts. 2015. North American velvet ants form one of the world's largest known Müllerian mimicry complexes. Current Biology 25:R704–R706.

Woodruff, B. W. 1972. Natural Selection for Mullerian Mimicry in Heliconius erato in Costa Rica. Science 176:936–939.

APPENDIX A

PERIPHERAL EYE DIMENSIONS IN LONGWING (*HELICONIUS*) BUTTERFLIES VARY WITH BODY SIZE AND SEX BUT NOT LIGHT ENVIRONMENT NOR MIMICRY RING

LRF JRL
The Journal of Research on the Lepidoptera
Volume 48: 83-92
THE LEPIDOPTERA RESEARCH FOUNDATION, 27 November 2015
ISSN 0022-4324 (print)
ISSN 2156-5457 (online)

Peripheral eye dimensions in Longwing (*Heliconius*) butterflies vary with body size and sex but not light environment nor mimicry ring

Brett M. Seymoure[1,2], W. Owen McMillan[2], Ronald Rutowski[1]
[1] School of Life Sciences, Arizona State University, Tempe 85287, United States
[2] Tupper, Smithsonian Tropical Research Institute, Ciudad de Panama, Panama
brett.seymoure@asu.edu, mcmillano@si.edu, r.rutowski@asu.edu

Abstract. This study tests if tropical forest butterflies occupying similar light environments converge on eye morphology to meet shared demands of visual sensitivity. Total corneal surface area and facet diameters were measured and adjusted to body size for four species of *Heliconius* (Lepidoptera: Nymphalidae) butterflies that belong to two mimicry rings that frequent different light environments. Total corneal surface area and facet diameter differed among species, but not between mimicry rings and light environment. *Heliconius cydno* had the largest corneal surface areas, *H. erato* had the second largest, while *H. sapho* and *H. melpomene* did not differ from each other. *Heliconius cydno* and *H. erato* had larger facets than *H. cydno* and *H. melpomene*. Facet diameter was not linked to either mimicry ring or clade. Males had larger corneas relative to body size than females, but facet diameter did not differ by sex. As predicted, facet diameter differed by region of the eye. Lastly, we found that larger eyes had more facets. While the eyes of *Heliconius* generally seem to be larger than those of similarly sized butterflies, the hypothesis that light environment affects eye morphology was not supported and the finding that neither mimicry ring nor phylogeny explains facet diameter is perplexing, but suggests that adaptation to contrasting light environments might be instead found in the physiology of the visual system.

Keywords: Cornea, Eye size, Facet counts, Facet diameter, Mimicry.

Introduction

Many animals use vision to gather information about their surroundings (Lythgoe, 1979; Land & Nilsson, 2012). Their success in doing this depends on the match between their eye structure and the light available for visual processing. Irradiance, a measure of light available, is nine orders of magnitude greater on sunny days than on starlit nights (Johnsen, 2011). As expected, terrestrial species that live at the extremes of this continuum display very different eye structures with nocturnal animals showing features that enhance photon capture at the photoreceptors (Warrant, 2006; Frederiksen & Warrant, 2008; Johnsen, 2011; Land & Nilsson, 2012). These features include larger eyes and facets than found in their diurnal relatives (Greiner *et al.*, 2004; Greiner, 2005; Warrant *et al.*, 2006; Somanathan *et al.*, 2008; Frederiksen & Warrant, 2008). Moreover, nocturnal and crepuscular species typically have superposition eyes in which a rhabdom (the microvilli component of the ommatidium's photoreceptors) is illuminated by light from several facet lenses enhancing sensitivity at the expense of resolution (Swihart, 1969; Horridge *et al.*, 1972; Warrant, 1999; Warrant *et al.*, 2004; Kelber, 2006). In contrast, diurnal insects (e.g. all non-skipper butterflies) often have apposition eyes in which the rhabdom in an ommatidium is illuminated only by light from the facet lens at the distal end of that ommatidium. Apposition eyes are much less sensitive than superposition eyes because photons from only one facet are caught by the individual photoreceptors.

Light environments that differ by several orders of magnitude in overall brightness can clearly lead to differences in eye morphology (i.e. night versus day), but how different are the eye features of diurnal animals that occupy habitats with smaller differences in available light (e.g. deep shaded forest vs. open field)? In this study, we test if eye morphology differs

Received: 7 July 2015
Accepted: 3 August 2015

among four related species of diurnal *Heliconius* (Kluk) (Lepidoptera; Nymphalidae) butterflies that occur in light environments that can differ in brightness by one order of magnitude (Papageorgis, 1975; Endler, 1993; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). This difference in brightness is relatively smaller than are differences in brightness encompassed by previous studies. For example, Frederiksen & Warrant (2008) compared the eyes of butterflies that fly at dusk to those that fly at midday when there is 100 times more light.

The four unpalatable species of *Heliconius* we studied include representatives of two different mimicry rings that occur in central Panama, the postman ring (*H. erato* and *H. melpomene*) and the blue-white ring (*H. cydno* and *H. sapho*. Brown, 1981; Chai, 1986). These two rings of Müllerian mimics occur in different microhabitats that present different light conditions (Gilbert, 1991; Mallet & Gilbert, 1995; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). *Heliconius erato* and *H. melpomene* occur in more disturbed and open habitats, while *H. sapho* and *H. cydno* occur in established forest with full canopy cover (DeVries, 1987; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). Endler (1993) quantified the differences in brightness (quantum flux) of forest understory and large open gaps in tropical forest in Panama. Large gaps, where *H. melpomene* and *H. erato* occur, are an order of magnitude brighter and are richer in long wavelengths than forest understory, where *H. cydno* and *H. sapho* occur (Endler, 1993; Estrada & Jiggins, 2002; B. Seymoure, unpublished data).

Do co-mimics share eye morphology that is adapted to shared environment and similar behaviors? Here, the results presented test the predictions that mimetic *Heliconius* butterflies that occur in darker environments (*H. sapho* and *H. cydno*) will have larger eyes and larger facets to improve sensitivity, while postman butterflies which live in more open environments will have smaller eyes and facets (Warrant, 2006). Note that the mimicry rings do not reflect phylogenetic relationships among these species (Brown, 1981; Kozak *et al.*, 2015; Figure 1). *Heliconius cydno* and *H. melpomene* are more closely related than *H. sapho* and *H. erato*. Hence, if recent common ancestry is an important determinant of eye morphology, it is predicted that eye morphology will be more similar within these pairs than among mimetic pairs.

Several patterns of variation in eye size and facet diameter in butterflies are known from previous studies (Ziemba & Rutowski, 2000; Rutowski, 2000; Merry *et al.*, 2006; Rutowski *et al.*, 2009). Eye size and facet diameter increase with body size, males typically have larger eyes than females, and facets in the frontal region of the eye tend to be larger than in other eye regions (Land, 1997; Rutowski, 2009). Hence, our analysis took into consideration both size and sex of all sampled individuals and included measurements from several eye regions.

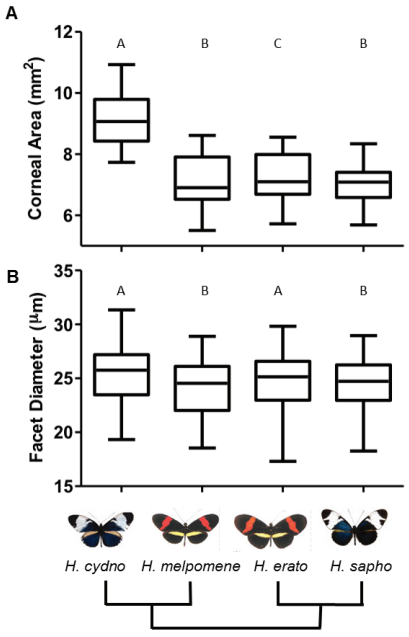

Figure 1. Interspecific differences in unadjusted eye morphology for the four *Heliconius* species studied. **A)** Absolute total corneal surface area. **B)** Mean absolute facet diameter. Letters (A, B, C) within each graph represent significantly different groups when controlling for body size. The data plotted here are not adjusted for body size unlike the statistical tests. Plots for each data set show the maximum and minimum values (upper and lower whisker, respectively), 1st and 3rd quartiles (top and bottom of box, respectively), and the mean (horizontal line within box). Phylogenetic relationships among these species are shown at the bottom (Brown 1981; Kozak *et al.* 2015). Note that *H. melpomene* and *H. erato* are found in brighter environments than *H. cydno* and *H. sapho*.

Material and methods

Specimen collection

Ninety-two adult *Heliconius* butterflies were collected for measurements in Parque de Nacional Soberanía in Panama from February to May 2013

Table 1. Sample sizes, body area measurements, and total corneal surface area for the *Heliconius* species studied. Means are given with standard deviations

Species	Sex	N	Forewing (mm)	Femur (mm)	PC1	Cornea (mm^2)
H. cydno	M	12	39.6±1.76	4.57±0.34	-1.17±0.87	9.45±0.90
	F	10	39.7±2.21	4.46±0.41	-0.41±1.84	8.77±0.78
H. melpomene	M	12	34.8±3.19	4.26±0.37	0.32±1.07	7.32±1.03
	F	12	35.2±1.44	4.04±0.31	0.67±0.95	6.79±0.56
H. sapho	M	11	36.8±3.32	4.06±0.44	-0.77±0.77	7.31±0.66
	F	12	38.8±1.51	4.34±0.27	0.67±.66	6.76±0.43
H. erato	M	12	32.5±2.26	3.61±0.30	1.37±1.09	7.27±0.93
	F	11	34.7±2.28	3.74±0.37	0.68±1.19	7.21±0.77

(Table 1). Adults with little wing wear were netted and then stored in glassine envelopes for transportation to lab facilities in Gamboa, Panama, where the butterflies were euthanized by freezing.

Body size covariate

As measures of body size we used hind femur length and forewing length of each individual measured with digital calipers to the nearest 0.01 mm (Rutowski, 2000; Rutowski *et al.*, 2009). Principal component analysis on these two measures revealed a first principal component that explained 90% of variation (hind femur length factor loading = -0.707; forewing length loading = -0.707). This component was used as a covariate representing body size in our analyses.

Cornea preparation

The head of each individual was severed from the thorax and the antennae, proboscis, and labial palps were removed. Following the methods of Ziemba and Rutowski (2000), the heads were soaked in 20% NaOH for 18 to 24 h to loosen the tissues behind the cuticular cornea. Once the soft tissues were removed, the cornea was cut along the dorsal-ventral axis and then laid flat on a microscope slide. A coverslip was placed over the cornea and then preserved and sealed with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI). These prepared slides were air dried for 24 h before being photographed.

Total corneal surface area measurements

Corneal squashes were photographed at approximately 20x magnification with a microscope (model MZM1, Askania Mikroskop Technik Rathenow, Germany) fitted with an OptixCam (Summit Series, The Microscope Store, Roanoke, VA) run with OCView Software (The Microscope Store, Roanoke, VA). A photograph taken of a micrometer scale was used to calibrate measurements made from other images. Total corneal surface area was measured by one observer in ImageJ with the lasso tool (Rasband, 2012); repeatability of these measurements was very high (intraclass correlation coefficient = 0.998).

Facet diameter measurements

Diameter of facets was measured in each of six regions of the eye: posterior, dorsal, anterior, anterioventral, ventral, and lateral (Figure 2). For these measurements, mounted corneas were photographed with the OptixCam attached to a compound microscope (Spencer Phase Star, American Optical, Hicksville, NY) at 100x magnification. The photographs were calibrated with a slide micrometer and all measurements were made within ImageJ. Within each region of each eye, distance was measured across ten facets in a row in two separate locations at least ten facets apart. The distance for each location was divided by ten to get an average facet diameter for each location. Then the two locations in each region were averaged to provide an average facet diameter for each region. As with total corneal

surface area measurements, one observer measured facet diameters and again repeatability was very high (intraclass correlation coefficient = 0.984).

Facet counts

To further understand the eye morphology of *Heliconius* butterflies, the number of facets were counted for two individuals for each sex and species. Utilizing the total corneal surface area photographs, the cell counter plugin in ImageJ was used for counting the number of facets. We selected photos where all facets were easily countable.

Statistical analyses

Body size principal components were calculated in R (R Development Core Team, 2008). All other tests were run in SPSS version 19 (IBM, Armonk, NY). Total corneal surface area was analyzed using a three-way nested analysis of covariance (ANCOVA). The covariate was PC1 of body size, the between factors were sex, mimicry ring, clade membership, and species. Species was nested both within mimicry ring and clade membership. Facet diameter was analyzed using repeated-measures ANCOVA. The facet diameters for each region of the eye were the within factor, and PC1 of body size served as the covariate. Sex, mimicry ring, and clade membership were the between factors, and again, species was nested within mimicry ring and clade membership. For both tests, post-hoc Helmert contrasts were implemented to determine differences among groups. All statistical inferences were made at the 0.05 level of significance.

Results

Total corneal surface area

As in other species of butterflies, total corneal surface area scaled positively with body size (ANCOVA, $F_{1,97}$=48.515, p<0.001; Figure 3) and males had larger eyes than females independent of body size ($F_{1,97}$=20.42, p<0.001; Figure 4). However, further Helmert analysis revealed that there was a significant difference between the sexes for total corneal surface area for *H. sapho* (p=0.004), and *H. cydno* (p=0.038), but corneal surface area did not differ by sex for *H. melpomene* (p=0.067) and for *H. erato* (p=0.332). Within each sex of each species there was a strong negative allometry in the relationship between eye size and body size, which means small individuals had relatively larger eyes compared to their larger counterparts (Figure 3).

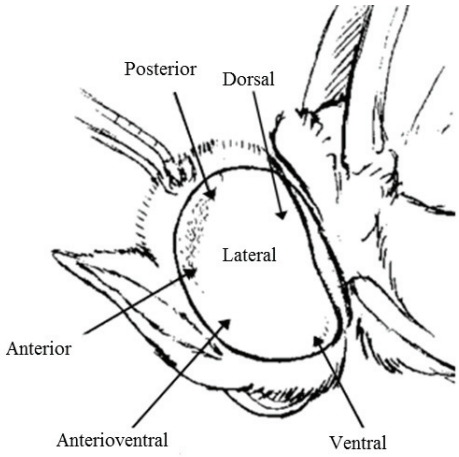

Figure 2. Eye regions in which facet diameter was measured. Figure modified from Rutowski (2000) and Merry *et al.* (2006).

Body-size-adjusted corneal surface area of *H. cydno* and *H. erato* were significantly different from each other and the other two species ($F_{3,47}$=46.365, p<0.001). Specifically, Helmert contrasts revealed that *H. cydno* had the largest eyes (p<0.001) while *H. erato* had the second largest (p<0.001; Figure 1). *H. sapho* and *H. melpomene* did not differ from one another and had the smallest eyes (p=0.064; Figure 1). Contrary to our prediction, there was no difference in total corneal surface area between the two mimicry rings ($F_{1,97}$=0.510, p=0.477) but the effect of clade was significant ($F_{1,97}$=40.394, p<0.001).

Facet diameter

As expected from studies of other butterflies, facet diameters differed among eye regions (ANCOVA with Greenhouse-Geisser correction, $F_{3,95}$=210.39, p<0.001; Figure 5). Lateral facets were the largest, anterior and anterioventral facets were next largest in diameter; then facets became smaller from posterior to ventral to dorsal. Body size positively predicted facet diameter (ANCOVA, $F_{1,97}$=11.295, p=0.001; Figure 6), but facet size did not differ by sex ($F_{1,97}$=0.829, p=0.365), mimicry ring ($F_{1,97}$=0.001, p=0.970), or phylogeny ($F_{1,97}$=0.775, p=0.381). Facet size differed among species ($F_{3,47}$=7.438, p=0.001; Figure 1B). As with total corneal surface size, *H. sapho* and *H. melpomene* had similarly smaller facets (p=0.472) than *H. cydno* and

Table 2. Facet diameter by region of the eye as a function of species and sex. Means are given with standard deviations.

			Facet Diameter (μm)					
Species	**Sex**	**N**	**Posterior**	**Ventral**	**Dorsal**	**Anterior**	**Lateral**	**Anterioventral**
H. cydno	M	12	24.9±1.43	23.9±0.90	21.2±1.09	26.4±1.14	27.2±0.98	27.0±1.44
	F	10	24.6±1.48	25.3±1.51	21.1±1.18	26.9±0.78	27.5±2.05	27.7±0.64
H. melpomene	M	12	23.8±1.17	23.5±1.80	21.0±1.03	26.2±1.96	26.0±2.03	26.5±1.53
	F	12	23.9±1.29	22.3±1.50	20.9±1.75	24.9±1.79	25.9±1.23	24.3±2.34
H. sapho	M	11	24.6±1.04	23.9±1.25	20.8±1.69	25.8±0.93	26.2±1.23	25.5±1.93
	F	12	24.3±2.02	23.3±1.43	21.4±1.50	26.1±1.13	26.2±1.52	26.0±1.81
H. erato	M	12	23.4±1.50	22.8±2.15	21.5±1.79	26.7±1.18	27.1±1.42	26.3±1.98
	F	11	24.3±1.93	23.6±1.84	21.7±2.29	26.2±1.09	26.7±1.47	25.1±1.48

H. erato, which had the largest facet diameters and did not differ from one another (p=0.639). The data were suggestive of a three-way interaction of region by sex by species (ANCOVA, $F_{15,243}$=1.71, p=0.051). And as with total corneal surface area and body size, there was a strong negative allometry in the relationship between facet diameter and body size (Figure 6).

Facet counts

Facet number was highly positively correlated with total corneal surface area (R^2=0.92 for males and R^2=0.73 for females; Figure 7). The largest corneas had the most facets and the smallest corneas had the fewest facets (Table 2). Males have absolutely larger eyes than females and therefore have more facets.

Discussion

Eye size varies with body size

Previous research has shown that eye size in Lepidoptera increases with body size (Yagi & Koyama, 1963; Rutowski, 2000; Rutowski *et al.*, 2009) and the *Heliconius* species examined here are no different. Here we found that larger *Heliconius* individuals have larger total corneal surface area and larger facets. However, we found the rate with which eye size changes with body size is much lower in *Heliconius* than reported for other butterflies (Rutowski, 2000; Figures 3 & 6). The very negatively allometric relationships between body size and eye size are unexpected and suggest selective pressures on *Heliconius* that favor development of large eyes regardless of body size. Regardless of the degree of allometry, eye performance is related to body size and depends on eye shape, facet number and facet size (Land, 1989; Land, 1997; Zollikofer *et al.*, 1995). Therefore, larger *Heliconius* butterflies should have increased sensitivity, acuity, larger visual fields or a combination of these characteristics (Rutowski, 2000; Frederikson & Warrant, 2008).

Interestingly, all of the *Heliconius* species we examined have a higher corneal surface area to body size ratio than that reported for other butterflies (Rutowski, 2000; Rutowski *et al.*, 2009). Rutowski (2000) found that the corneal surface area to body size ratio is close to 1:1 for 16 different species of butterflies with lower ratios of 1:2 and higher ratios of 11:10. Here we found corneal surface area to body size ratios greater than 2:1, indicating that *Heliconius* have the largest eyes relative to body size of butterflies studied thus far.

Larger total corneal surface areas could have several effects on vision including a larger visual field (ommatidia pointing in a larger number of directions), more acute and sensitive vision, or both. Visual field dimensions of butterflies are generally huge and do not change much with body size (Rutowski *et al.*, 2009). There is no reason to think this will not also be true for *Heliconius*. However, in *Heliconius* the number and diameter of facets do increase with body size. So, given no change in visual field dimensions, the increase in cornea size and in facet number should mean overall

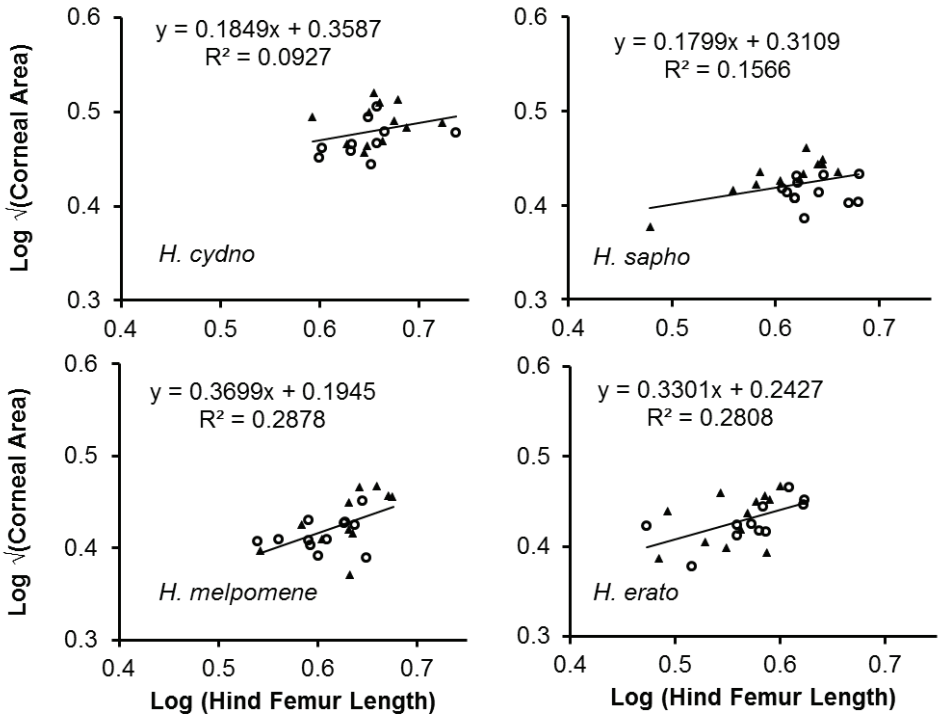

Figure 3. The relationship between eye size and body size as measured by hind femur length for each sex of each species (triangles, males; open circles, females). The y-axis represents the log of the square root of the total corneal surface area and the x-axis represents the log of hind femur length. The double-logarithmic plot is used to determine if the relationship between total corneal surface area and hind femur length is allometric. A slope of 1 would indicate an isometric relationship between body size (hind femur length) and eye size (total corneal surface area). However, the slopes here indicate that eye size has a very negative allometric relationship with body size.

lower inter-ommatidial angles in larger eyes. Similarly, the increase in facet diameter with body size will mean a higher photon catch per ommatidium such that larger eyes should be more sensitive. Hence, *Heliconius* should have better low light vision than most other butterflies in the same body size range. What selective pressures might have driven this divergence is not clear. Perhaps it is that they frequent forest shade (i.e. low light) environments which makes visual detection and recognition tasks more demanding than those of butterflies in environments with higher light levels. Interestingly in Rutowski *et al.* (2009) the species examined in the *Heliconius* size range, *P. sylvia*, with its relatively smaller facets frequents open environments with high light levels.

Blue-white males have larger eyes than females

Previous studies showed that male Lepidoptera have larger corneas and facets than conspecific females (Yagi & Koyama, 1963; Ziemba & Rutowski ,2000; Rutowski, 2000; Lund *et al*., 2001). Blue-white males had larger eyes than females when controlled for body size, but postman individuals did not differ in eye size between species. Why only blue-white individuals would have an intraspecific difference is intriguing because other studies hypothesize that male Lepidoptera have generally larger eyes as a result of the visual demands of finding mates (Yagi & Koyama, 1963; Rutowski, 2000).

Facet diameter varies by region

The largest facets in butterflies are in the anterior regions of the eyes for maintaining flight and for locating and recognizing food resources, mates, and larval host plants (Land, 1997, Rutowski & Warrant, 2002; Rutowski, 2003; Rutowski *et al.*, 2009). We observed a similar pattern in the *Heliconius* species studied here but with large facets also in the anterioventral and lateral eye regions. Unlike in previous studies (Rutowski & Warrant, 2002; Rutowski *et al.*, 2009), there were no differences in facet diameters among the sexes or mimicry rings. This again supports the notion that vision may function similarly in males and females of *Heliconius* butterflies.

The lateral facets, located in the center of the cornea, are the largest for all four species, which contrasts with previous reports that largest facets in butterflies are found anteriorly and anterioventrally, most likely for locating and recognizing both host plants and mates (Merry *et al.*, 2006; Rutowski *et al.*, 2009). Large lateral facets may enhance processing of optic flow in flight, the pattern of apparent motion of elements in the visual scene as the observer moves (Srinivasan *et al.*, 2000). The greatest angular velocity of objects in the visual scene of a flying butterfly will be in the lateral regions and thus the lateral optical flow is most likely to suffer from visual blur which will be minimized when photon flux and signal to noise ratios are high. These conditions will happen when facets are large, such as they are in the lateral regions of the eye. Of course, this explanation warrants testing and further comparative research on compound eyes and optic flow is needed.

Larger eyes have more but not larger facets

Very little is known about the relationship between eye size and facet number for the Lepidoptera. Ziemba & Rutowski (2000) found that although eye size differs between males and females in the butterfly *Asterocampa leilia*, the number of facets per eye was the same in males and females. Males of *A. leilia* have larger facets than females, which leads to a larger eye size without more facets. Unlike *A. leilia*, in *Heliconius* the sexes differ in the number of facets per eye. Furthermore, eye size correlates with facet number with similar negative allometry to body size as was found with corneal surface area and body size. Again, this negative allometry is likely due to selection for very large facets regardless of body size and because larger eyes have more facets instead of larger facets, a very negative allometric relationship would be predicted. This finding is comparable to what has been found in eusocial hymenoptera in which the larger the eye, the greater number of facets (Jander & Jander, 2002; Streinzer *et al.*, 2013).

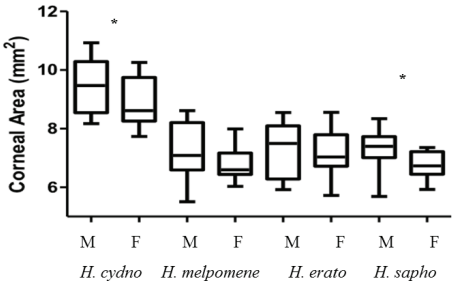

Figure 4. Absolute total corneal surface area for each sex of each species. See legend in Figure 1 for further details of the box-and-whisker plots. The asterisks mark intraspecific sexual differences that were significant at the 0.05 level.

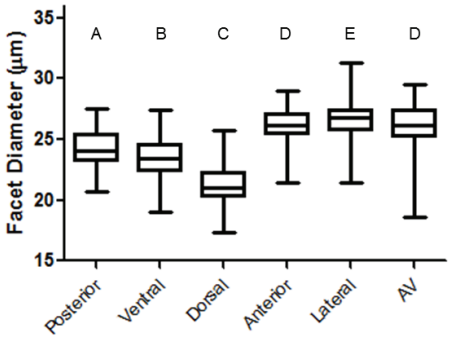

Figure 5. Mean facet diameter across different regions of the eye for all individuals of all species (n=92). Letters represent significantly different groups when body size is a covariate. Only anterior and anterioventral regions are not statistically different from one another.

Eye morphology, mimicry ring and light environment

The predictions about the relationship between mimicry rings, which correspond to light environment, and eye features were not supported. One possible reason for this result is that differences in light

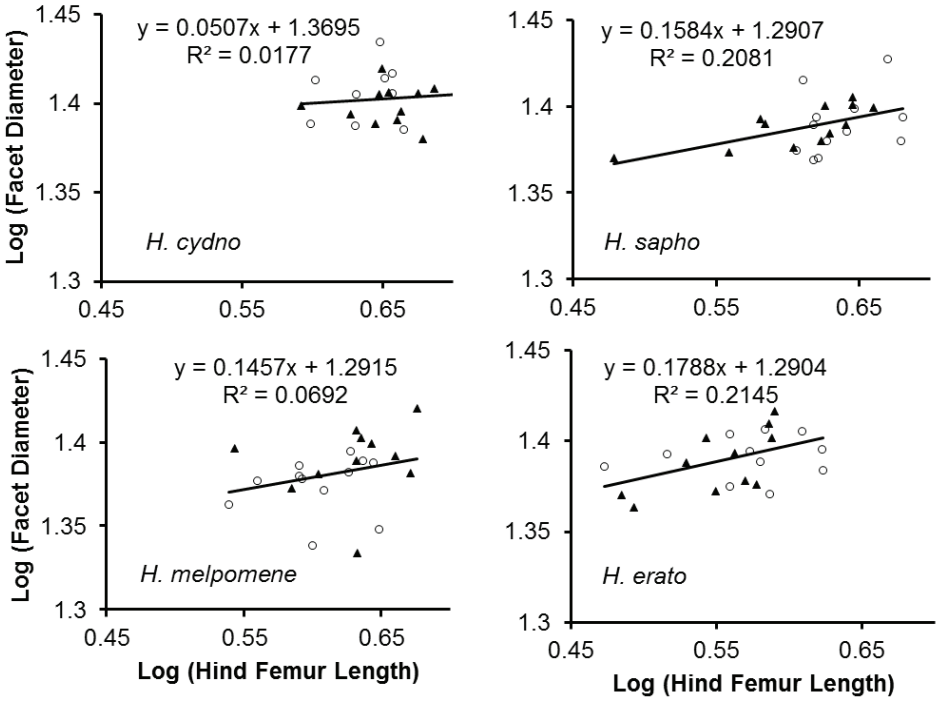

Figure 6. The relationship between facet diameter and body size as measured by hind femur length for each sex of each species (triangles, males; open circles, females). See figure 3 for explanation of the double-logarithmic plots.

intensity where these species typically occur are too small to have shaped the peripheral features of eye morphology that we examined. Preliminary results from electroretinograms of these butterflies reveal that the blue-white butterflies that live in forest shade environments have greater absolute sensitivity (i.e. can see in darker environments) than the postman butterflies which live in very open environments (B. Seymoure *et al.*, unpublished). Because these two groups did not differ in the measures of eye structure reported here, physiological differences in eye performance between animals that live in different light environments are expected to be the result of differences in eye structure other than those measured here.

Apposition compound eyes can be rendered more sensitive through a pupil mechanism, by lengthening and/or widening the rhabdoms or through spatial and/or temporal summation of responses to dim light signals (Jonson *et al.*, 1998; Warrant *et al.*, 2004; Greiner *et al.*, 2005; Warrant, 2006; Land & Nilsson, 2012). In fact, Jonson *et al.* (1998) revealed that butterflies that occur in different light environments vary in pupil response with dim habitat species having a pupil mechanism that restricts photons entering the rhabdom in much dimmer environments than bright habitat species. Furthermore, Frederiksen & Warrant (2008) found that the crepuscular Owl butterfly (*Caligo memnon*) has four times the sensitivity of a similar sized diurnal butterfly that stems from not only increased facet diameters, but also wider rhabdoms and neural summation. Perhaps *Heliconius* individuals in darker environments have similar features that increase sensitivity. This is currently under investigation in our lab (B. Seymoure *et al.*, unpublished).

This work reveals several potentially fruitful research directions into the visual ecology and behavior of *Heliconius* butterflies. This study only investigated the eye morphology of four of the 44

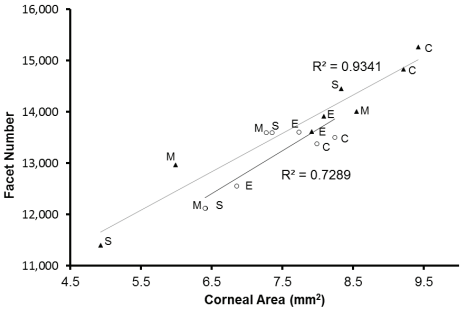

Figure 7. Relationship between facet number and total corneal surface area (mm²) for selected *Heliconius* males and females. Letters near data points represent species: S = *H. sapho*, M = *H. melpomene*, E = *H. erato*, C = *H. cydno*. Lines represent least squares regression for males (closed triangles) and females (open circles).

Heliconius species and further *Heliconius* research is needed to understand why these species differ drastically from other butterflies and the role of ancestry in eye morphology. Furthermore, to understand how light environment has affected compound eye morphology, compelling studies could include phylogenetically-controlled comparisons of eye structure of diurnal species that differ in the light environments where they tend to occur. Such studies might also include a larger array of eye features including inter-ommatidial angles, visual field dimensions, pupillary responses, rhabdom lengths as well as physiological recordings such as electroretinograms or intracellular recordings. Such studies are currently underway in our lab and will shed light on the nature and tuning of visual adaptations in insects that occur in diverse light environments.

Acknowledgements

This work was supported by Arizona State University and the Smithsonian Tropical Research Institute (STRI). B. Seymoure was funded by a STRI Short Term Fellowship, Grants in Aid of Research from Sigma Xi and the Society for Integrative and Comparative Biology, and the Arizona State University and Smithsonian Tropical Research Institute Partnership. A travel grant from the Lepidoptera Research Foundation enabled B. Seymoure to present preliminary findings of this work and receive valuable feedback. Andrew Raymundo and Ben Rice provided crucial assistance in the field. Rachel Olzer, Kaci Fankhauser, Britney Southers and Jennifer Armstrong were helpful with data collection. We appreciate logistical support from R. Urriola, S. Van Bael, A. Tapia, A. Bilgray, B. van Schooten and comments on the project and manuscript from K. McGraw, B. Jones, R. Ligon, K. Pegram, N. Lessios, and from the Rutowski and McGraw labs at ASU. For the collecting permit (SE/A-7-13), we thank Autoridad Nacional del Ambienta (ANAM) of the Republic of Panama.

Disclosure

The authors have no conflicts of interest, including specific financial interests, relationships and affiliations relevant to the subject of this manuscript.

Literature cited

Benson, W.W., K.S. Brown & L.E. Gilbert. 1975. Coevolution of plants and herbivores: Passion flower butterflies. Evolution 29: 659-680.

Brown, K.S.J. 1981. The biology of *Heliconius* and related genera. Annual Review of Entomology 26: 427–456.

Chai, P. 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. Biological Journal of the Linnean Society 29: 161–189.

DeVries, P.J. 1987. Butterflies of Costa Rica and their natural history. Princeton University Press, New Jersey.

Endler, J.A. 1993. The color of light in forests and its implications. Ecological Monographs 63: 1-27.

Estrada, C. & C. Jiggins. 2002. Patterns of pollen feeding and habitat preference among *Heliconius* species. Ecological Entomology 27: 448-456.

Frederiksen, R. & E.J. Warrant. 2008. Visual sensitivity in the crepuscular owl butterfly, *Caligo memnon* and the diurnal blue morpho, *Morpho peleides*: a clue to explain the evolution of nocturnal apposition eyes? The Journal of Experimental Biology 211: 844–851.

Gilbert, L.E. 1991. Biodiversity of Central American *Heliconius* community: Pattern, process and problems. *In*: Price, P.W., Lewinsohn, T.M., Fernandes, G.W., and Benson, W.W. (Eds), Plant-animal interaction: Evolutionary ecology in tropical and temperate regions. John Wiley and Sons, London, pp. 403-427.

Greiner, B. 2005. Visual adaptations in the night active wasp *Apoica pallens*. Journal of Comparative Neurology 495: 255-262.

Greiner, B., W.A. Ribi, & E.J. Warrant. 2004. Retinal and optical adaptations for nocturnal vision in the halictid bee *Megalopta genalis*. Cell Tissue Research 316: 377-390.

Greiner, B., W.A. Ribi & E.J. Warrant. 2005. A neural network to improve dimlight vision? Dendritic fields of the first-order interneurons in the nocturnal bee *Megalopta genalis*. Cell Tissue Research 322: 313-320.

Horridge, G.A., C. Giddings, & G. Stange. 1972. The superposition eye of skipper butterflies. Proceedings of the Royal Society London Biological Society 182: 457-495.

Jander, U. & R. Jander. 2002. Allometry and resolution of bee eyes (Apoidea). Arthropod Structure and Development 30: 179-193.

Johnsen, S. 2011. Optics of life. Princeton University Press, Princeton.

Jonson, A.C.J., M.F. Land, D.C. Osorio, & D.-E Nilsson. 1998. Relationships between pupil working range and habitat luminance in flies and butterflies. Journal of Comparative Physiology A 182: 1-9.

Kelber, A. 2006. Invertebrate colour vision. Invertebrate Vision. Cambridge University Press.

Kozak, K.M., N. Wahlberg, A.F.E. Neild, K.K. Dasmahapatra, J. Mallet & C. Jiggins. 2015. Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius* species. Systematic Biology 64: 505-524.

Land, M.F. 1989. Variations in the structure and design of compound eyes. Facets of Vision. Berlin; Springer.

Land, M.F. 1997. Visual acuity in insects. Annual Reviews of Entomology 42: 147-177.

Land, M.F. & D.E. Nilsson. 2012. Animal eyes. Oxford University Press.

Lund, N., E. Cwengros, & R.L. Rutowski. 2001. Sexual dimorphism in eye morphology in *Eucheira socialis* (Pieridae). Journal of the Lepidopterists' Society 55: 74-77.

Lythgoe, J.N. 1979. The ecology of vision. Clarendon Press, Oxford.

Mallet, J. & L.E. Gilbert. 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. Biological Journal of the Linnean Society 55: 159–180.

Merry, J.W., N.I. Morehouse, K. Yturralde, & R.L. Rutowski. 2006. The eyes of a patrolling butterfly: Visual field and eye structure in the Orange Sulphur, *Colias eurytheme* (Lepidoptera, Pieridae). Journal of Insect Physiology 52: 240-248.

Papageorgis, C. 1975. Mimicry in Neotropical butterflies. American Scientist 63: 522-532.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Rasband, W.S. 1997-2012. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA. Available at http://imagej.nih.gov/ij/.

Rutowski, R.L. 2000. Variation of eye size in butterflies: inter- and intraspecific patterns. Journal of Zoology 252: 187–195.

Rutowski, R.L. 2003. Visual ecology of adult butterflies. *In*: Boggs, C., Watt, W., Ehrlich, P.R. (Eds.), Butterflies: Ecology and evolution taking flight. University of Chicago Press, Chicago, pp. 9-25.

Rutowski, R. L. & E. Warrant. 2002. Visual field structure in the Empress Leilia, *Asterocampa leilia*: dimensions and regional variation in acuity. Journal of Comparative Physiology A 188: 1-12.

Rutowski, R.L., L. Gislén, & E.J. Warrant. 2009. Visual acuity and sensitivity increase allometrically with body size in butterflies. Arthropod Structure & Development 38: 91–100.

Somanathan, H., R. Borges, E. Warrant, & A. Kelber. 2008. Visual ecology of Indian carpenter bees I: Light intensities and flight cavity. Journal of Comparative Physiology A 194: 97-107.

Srinivasan, M.V., S.W. Zhang, M. Altwein & J. Tautz. 2000. Honeybee navigation: nature and calibration of the "odometer." Science 287: 851-853.

Streinzer, M., A. Brockmann, N. Nagaraja, & J. Spaethe. 2013. Sex and caste-specific variation in compound eye morphology of five honeybee species. PLoS ONE 8:e57702.

Swihart, S.L. 1969. Colour vision and the physiology of the superposition eye of a butterfly (Hesperiidae). Journal of Insect Physiology 15: 1347-1365.

Warrant, E.J. 1999. Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. Vision Research 39: 1611–30.

Warrant, E.J. 2006. Invertebrate vision in dim light. Invertebrate Vision. Cambridge University Press.

Warrant, E.J., Kelber, A., Gislén, A., Greiner, B., Ribi, W. and Wcislo, W.T. 2004. Nocturnal vision and landmark orientation in a tropical halictid bee. Current Biology 14: 1309-1318.

Yagi, N. & N. Koyama. 1963. The compound eye of Lepidoptera: Approach from organic evolution. Shinkyo Press, Tokyo.

Ziemba, K.S. & R.L. Rutowski. 2000. Sexual dimorphism in eye morphology in a butterfly (*Asterocampa leilia*). Psyche 103: 25-36.

Zollikofer, C., R. Wehner & T. Fukushi. 1995. Optical scaling in conspecific Cataglyphis ants. Journal of Experimental Biology 198: 1637-1646

APPENDIX B

A BIRD'S EYE VIEW OF TWO MIMETIC TROPICAL BUTTERFLIES; COLORATION MATCHES PREDATOR'S SENSITIVITY

Journal of Zoology

ZSL LET'S WORK FOR WILDLIFE

Journal of Zoology. Print ISSN 0952-8369

A bird's eye view of two mimetic tropical butterflies: coloration matches predator's sensitivity

T. J. Thurman[1,3] & B. M. Seymoure[2,3]

1 Redpath Museum and Department of Biology, McGill University, Montreal, QC,
2 School of Life Sciences, Arizona State University, Tempe, AZ, USA
3 The Smithsonian Tropical Research Institute, Panamá, Panama

Keywords
avian visual models; Batesian mimicry; coloration; *Mimoides*; *Heliconius*; JNDs; aposematism; UV vision.

Correspondence
Brett Seymoure, School of Life Sciences, Arizona State University, Tempe, AZ 85287. Tel: 269 501 8761
Email: brett.seymoure@gmail.com

Both authors contributed equally to this manuscript.

Editor: Robert Knell

Received 22 June 2015; accepted 30 September 2015

doi:10.1111/jzo.12305

Abstract

Unprofitable prey with conspicuous warning signals are often mimicked by other species, which then gain protection from predators. How closely two mimetic species resemble one another depends upon the visual perception of the signal receiver. However, most studies of mimetic coloration have been conducted using only the human visual system, which differs greatly from that of most animals. To better understand mimicry, we should study mimetic visual signals through the eyes of the intended receiver. Here, we use avian visual models to test predictions of putative Batesian mimicry in two Amazonian butterflies, *Mimoides pausanias* and *Heliconius sara*. We calculated Just Noticeable Differences (JNDs) and tetrahedral color volumes for 11 different patches: iridescent blue, yellow bars, red spots and black background. Several color patches were not visually discriminable for both avian visual systems (UV/VIS and V/VIS), and visual discrimination (i.e. degree of mimicry) of color patches depended upon the avian visual system. These two butterfly species are more mimetic when viewed by their likely avian predators, which have V/VIS vision. Therefore, this mimetic assemblage may have evolved to be more spectrally accurate in the non-UV wavelengths which their avian predators are able to see. However, while many color patches of the two species were modeled to be difficult to discriminate, most color patches were not perfect matches regardless of visual system, and several patches were very poor mimics. Through this study we demonstrate the importance of testing putative mimetic assemblages using known predator perceptual models and lay a foundation for behavioral studies to further test mimicry in *H. sara* and *M. pausanius*.

Introduction

The three players of Batesian mimicry are involved in an evolutionary arms race: the palatable mimic is under selection to resemble the model to avoid predator recognition, the unpalatable model is under selection to appear different from the mimic, and the signal receiver (i.e. the predator) is under selection to improve discrimination between the model and mimic (Bates, 1862; Dawkins & Krebs, 1979). Mimetic resemblance is dependent upon the sensory ecology and physiology of the signal receiver (Stevens, 2013). Much previous research on mimicry has relied on our human perception and not the perception of the ecologically relevant signal receiver (e.g. Lindström, Alatalo & Mappes, 1997). Colorful mimetic signals have evolved in the context of visually guided predators, and these predators may differ greatly in their visual capabilities. Are individuals that appear similar to humans also mimetic in the eyes of their predators, and do predators differ in their ability to discriminate between mimics?

Recently there have been several tests of mimicry involving predator perception. Through an exhaustive study of reef fish mimicry, Cheney & Marshall (2009) found that individuals with a greater number of photoreceptors were better able to discriminate between mimics. Further work on mimicry in salamanders (Kraemer & Adams, 2014), orchids (Papadopulos *et al.*, 2013), avian brood parasites (Langmore *et al.*, 2011; Stoddard, 2012) and butterflies (Bybee *et al.*, 2012; Stoddard, 2012; Llaurens, Joron & Théry, 2014) has shown the importance and specificity of predator perception in the evolution of mimetic assemblages. Collectively, these studies demonstrate that the effectiveness of mimicry is dependent upon the visual system of the predator. However, most studies have only used one predator (Langmore *et al.*, 2011; Stoddard, 2012; Papadopulos *et al.*, 2013) or predators with very different visual systems (Kraemer & Adams, 2014). Cheney & Marshall (2009) examined how mimetic individuals are perceived by different predators with similar visual systems, but in a marine setting, making comparisons to terrestrial systems difficult (Lythgoe,

1979). Few studies have tested how differences in disparate predator perception affect mimicry signals.

In terrestrial ecosystems, avian predators are an important selective pressure on visual mimicry complexes due to birds' sensitive color vision and high visual acuity (Stoddard & Stevens, 2010). Birds are tetrachromatic, possessing four different photoreceptors. The three photoreceptors tuned to the visible spectrum (VIS) are conserved across bird species, and bird visual systems are classified by the sensitivity of the fourth photoreceptor. There are two categories: UV/VIS (ultraviolet sensitive) and V/VIS (violet sensitive) (Vorobyev & Osorio, 1998; Hart *et al.*, 2000; Hart & Hunt, 2007). The UV/VIS system is common in non-flycatcher and non-corvid Passeriformes, while the V/VIS system is found in flycatchers and most non-passerines (Hart *et al.*, 2000). Birds with different visual systems will likely differ in their ability to distinguish between species in a mimetic pair, especially if the species' coloration has an ultraviolet component; therefore, it is important to test mimicry with the appropriate avian visual system.

Neotropical butterflies (Lepidoptera) are an excellent system for studying mimicry. Indeed, the biologists who first described defensive mimicry, H. W. Bates and F. Müller, derived their hypotheses from observations of butterflies in South America (Bates, 1862; Müller, 1879). The Neotropics are known for diverse and complex mimicry systems of Lepidoptera, which primarily focus on unpalatable species in the subfamilies Heliconiinae, Ithomiinae and Danainae (DeVries, 1987; Mallet & Gilbert, 1995). Here, we study a sexually dimorphic butterfly, *Mimoides pausanias*, in which females are similarly sized and colored to *Heliconius sara*; in eastern Ecuador both species are black, yellow and blue (Fig. 1). The aposematic *H. sara* has cyanogenic glycosides, and birds will avoid attacking *H. sara* in aviaries (Chai, 1986). There are no explicit tests of the palatability of *M. pausanias*, but there are no known unpalatable species of *Mimoides* and the putatively mimetic color is restricted to females, most likely rendering this a Batesian relationship (De'Abrera, 1981). Furthermore, both species occur in the same gap habitats and fly at the same height to collect nectar from similar flowers (*Lantana spp*, *Salvia* spp; pers. obs), again rendering them likely to be mimetic to predators.

The main avian predators of *Heliconius* butterflies are tyrant flycatchers (Tyrannidae) and jacamars (Galbulidae), neither of which have ultraviolet sensitivity (Pinheiro, 1996, 2013; Hart, 2001). Observations of predation on *Heliconius* are rare and we are unaware of instances in which birds with the UV/VIS system have attacked *Heliconius*. Therefore, to be effective mimics *M. pausanias* and *H. sara* may not need to match in UV coloration. Here, we first test the hypothesis that *H. sara* and *M. pausanias* are mimetic by measuring the coloration of each species with spectrometry and then using visual models of UV/VIS and V/VIS birds to determine whether these colors are distinguishable to birds. We further hypothesize that these two species of butterflies will be more mimetic to their avian predators, which have the V/VIS visual system, than to avian species with the UV/VIS, which are not likely predators of these tropical butterflies. This work not only tests if there is a *H. sara* mimetic assemblage, but also if mimetic assemblages have been selected to match the visual sensitivities of their predators.

Materials and methods

Specimen collection and preparation

In June 2014, we collected four female *H. sara* and four female *M. pausanias* individuals near Tena, Ecuador (1°06′28″ S, 77°45′45″W). Four *M. pausanias* female individuals were

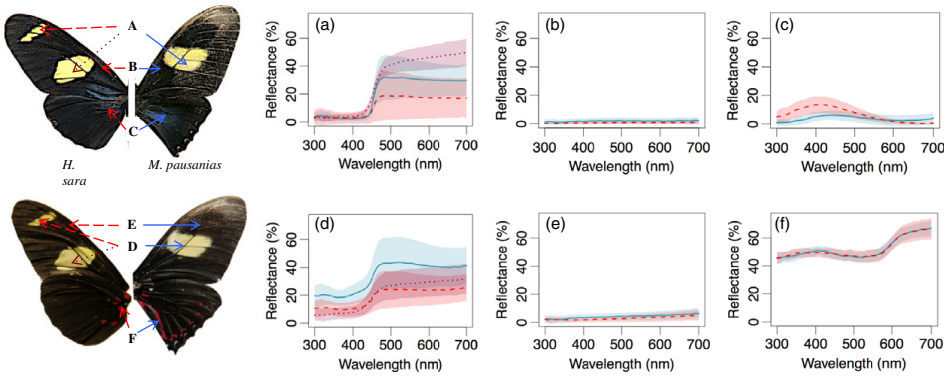

Figure 1 Dorsal and ventral wing patch reflectance for females of *H. sara* and *M. pausanias* for select patches. The left wings represent *H. sara*, whereas the right wings represent *M. pausanias*. Panels (a) and (d) are the dorsal and ventral yellow coloration, respectively, (b) and (e) are dorsal and ventral black, (c) is the iridescent blue on the dorsal hindwing and (f) is the red on the ventral hindwing. The blue line represents the average spectrum for *M. pausanias*, whereas the dashed red line represents the average for *H. sara*. The colored shading shows the 95 percent confidence interval for each species. In panels (a) and (d), the dotted red lines represent the proximal yellow patch of *H. sara*.

caught due to logistical constraints, to reduce population disturbances, and in certain locales it is suspected that only female *M. pausanias* mimic *H. sara* (DeVries, 1987). A recent study shows that four individuals are sufficient to test for the differences in spectral reflectance between species if each individual patch is measured repeatedly (Dalrymple *et al.*, 2015). Butterflies were collected with aerial nets and transported to the lab in glassine envelopes. Individuals were then euthanized by freezing, and each individual's wings were mounted for measurement on black cardstock with Scotch Photo Mount (3M, St. Paul, MN, USA).

Reflectance measurements

Once butterfly wings were mounted, we measured the spectral reflectance of each differently colored patch on both the dorsal and ventral surface of each wing, including the yellow patches of the forewing, black on both the forewing and hindwing, and the iridescent blue patches on the forewing and hindwing (Fig. 1, Table 1). All patches were measured at three separate points where wing wear was minimal (see Supporting Information for photographs of wings). Dorsal measures were taken from the right wing and ventral measures were taken from the left. Except for the iridescent blue patches, all patches were diffusely reflecting, enabling us to use a bifurcated reflectance probe connected to an Ocean Optics USB 2000 spectroradiometer (Dunedin, FL, USA). We first standardized the reflectance measurements with a white standard (Spectralon standard, Ocean Optics, Dunedin, FL, USA) and dark standard in which we occluded any light reaching the spectroradiometer. The reflectance probe was then held perpendicular to the wing surface and reflectance spectra were gathered with SpectraSuite software (Ocean Optics, Dunedin, FL, USA).

Hue and brightness of iridescent coloration depends upon the angle of illumination and observation (Meadows *et al.*, 2011). Therefore, iridescent reflectance must be measured under settings that control both illumination and viewing angle. We placed the mounted wing on the stage of a light table, set illumination angle and viewing angle to 60°, and then adjusted the viewing angle until the iridescent patch was maximally reflected (Meadows *et al.*, 2011).

Light environment measurements

The light environment in which a color is viewed can affect a viewer's perception of that color (Endler, 1990; Stevens, 2013). We were unable to collect light environment measurements from the habitats in Ecuador in which we collected these animals. Previous research on tropical light environments has demonstrated that they do not differ drastically between different rainforests (Endler, 1993). Thus, we measured irradiance and background spectra during mid-day in May 2014 in

Table 1 *P*-values for Just Noticeable Difference (JND) comparisons for chromatic contrasts between *H. sara* and *M. pausanias*

Patch	Visual model	JND	# JNDs > 1	*P*, mean JND > 1	# JNDs > 3	*P*, mean JND > 3	*W*, peafowl JND < blue tit JND	*P*, peafowl JND < blue tit JND
DFW-Blue	Blue Tit (UV)	14.59 (5.47)	16	0.00017	16	0.00017	115	1
	Peafowl (V)	11.70 (5.56)	16	0.00017	15	0.00285		
DFW-Black	Blue Tit (UV)	8.12 (5.39)	16	0.00017	12	**0.42247**	90	0.87786
	Peafowl (V)	4.66 (3.29)	14	0.02299	10	**1**		
DFW-Distal Yellow	Blue Tit (UV)	6.92 (3.23)	15	0.00285	14	0.02299	30	**0.00048**
	Peafowl (V)	2.80 (1.23)	15	0.00285	5	**1**		
DFW-Proximal Yellow	Blue Tit (UV)	3.55 (2.40)	15	0.00285	7	**1**	100	1
	Peafowl (V)	2.15 (1.03)	13	**0.11699**	3	**1**		
DHW-Black	Blue Tit (UV)	12.23 (6.42)	16	0.00017	15	0.00285	123	1
	Peafowl (V)	9.95 (6.53)	16	0.00017	14	0.02299		
DHW-Blue	Blue Tit (UV)	21.31 (11.06)	16	0.00017	16	0.00017	120	1
	Peafowl (V)	16.96 (9.66)	16	0.00017	16	0.00017		
VFW-Black	Blue Tit (UV)	3.98 (2.05)	15	0.00285	9	**1**	115	1
	Peafowl (V)	3.30 (1.72)	14	0.02299	7	**1**		
VFW-Distal Yellow	Blue Tit (UV)	5.21 (3.21)	15	0.00285	11	**1**	63	0.07459
	Peafowl (V)	2.42 (1.30)	14	0.02299	4	**1**		
VFW-Proximal Yellow	Blue Tit (UV)	6.40 (2.81)	16	0.00017	14	0.02299	44	**0.00583**
	Peafowl (V)	3.11 (1.16)	15	0.00285	9	**1**		
VHW-Black	Blue Tit (UV)	11.50 (4.78)	16	0.00017	15	0.00285	47	**0.00922**
	Peafowl (V)	5.72 (2.70)	16	0.00017	14	0.02299		
VHW-Red	Blue Tit (UV)	0.71 (0.43)	4	**1**	0	**1**	140	1
	Peafowl (V)	0.66 (0.39)	3	**1**	0	**1**		

Mean JNDs are given for each patch under each visual system, with standard deviations in parentheses. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. The number of JNDs greater than 1 and 3 are shown with Bonferroni-corrected *P*-values for sign tests examining whether the mean JND is significantly greater than 1 or 3. Bolded values indicate that the JND for that patch are not significantly different from 1 or 3. The final columns present the test statistic, *W*, and Bonferroni-corrected *P*-values for one-tailed Mann–Whitney tests of whether the mean JND under the peafowl model is less than the mean JND under the blue tit model, with significant *P*-values in bold.

lowland tropical rainforest in Soberania National Park, Panama (9.1167°N, 79.7000°W), which is similar to other rainforest irradiance (Endler, 1993).

Heliconius sara and *M. pausanias* both occur in disturbed rainforest and are frequently found in bright, open forest gaps (DeVries, 1987). We therefore measured the light environment of large gaps, which are characterized by no or little vegetative cover. We measured irradiance using a cosine-corrected irradiance probe, a USB 2000 Ocean Optics spectroradiometer and SpectraSuite software (Ocean Optics, Dunedin, FL, USA). We also characterized the spectral properties of the background against which these butterflies occur by measuring background radiance. Each radiance spectra were collected under optimal integration time using SpectraSuite software and a collimating radiance lens connected to an Ocean Optics USB 2000 spectroradiometer via an optic fiber (Ocean Optics, Dunedin, FL, USA).

Data processing and visual models

All data processing and analysis was performed using the *pavo* package version 0.5-1 (Maia *et al.*, 2013) implemented in R version 3.1.2 (R Core Team, 2013). To determine how well avian predators might discriminate the wing colors of *M. pausanias* and *H. sara*, we calculated the Just Noticeable Differences (JNDs) of each of the eleven wing patches we measured. JNDs quantify the discriminability of two colors, with JNDs less than one being physiologically indistinguishable by the viewer due to the large signal to noise ratio within the photoreceptor (Vorobyev & Osorio, 1998; Osorio & Vorobyev, 2005). Two colors with a JND above one will be seen as different colors of stationary objects when side by side in bright light. In more natural settings, two colors with a JND of three or less are unlikely to be seen as different (Siddiqi *et al.*, 2004; Langmore *et al.*, 2011). Furthermore, coloration is perceived by both chromatic differences (e.g. short wavelengths vs. long wavelengths) and by achromatic differences (e.g. gray vs. black). Therefore, we performed both chromatic and achromatic JND comparisons.

Within each color patch, the three reflectance measures were averaged and smoothed using *pavo* (Maia *et al.*, 2013). We then generated quantum catches of these colors with the von Kries transformation (Vorobyev & Osorio, 1998). Using the environmental measurements from Panama, we included tropical irradiance and tropical background vegetation in the visual models. Finally, we calculated chromatic and achromatic JNDs under two different models of bird vision (Vorobyev & Osorio, 1998; Hart, 2001). We used the visual system of the blue tit *Parus caerulus* as a model for UV/VIS (ultraviolet sensitive) vision (Hart *et al.*, 2000), and that of the peafowl *Pavo cristatus* as a model for V/VIS (non-UV sensitive) vision (Hart, 2002). Therefore, the lambda max values for the spectral sensitivities were 371, 448, 503 and 563 for the UV/VIS (blue tit) visual system and 421, 457, 505 and 563 for the V/VIS (peafowl) visual system (Hart, 2001). For the achromatic visual models the double cones were used with lambda max of 503

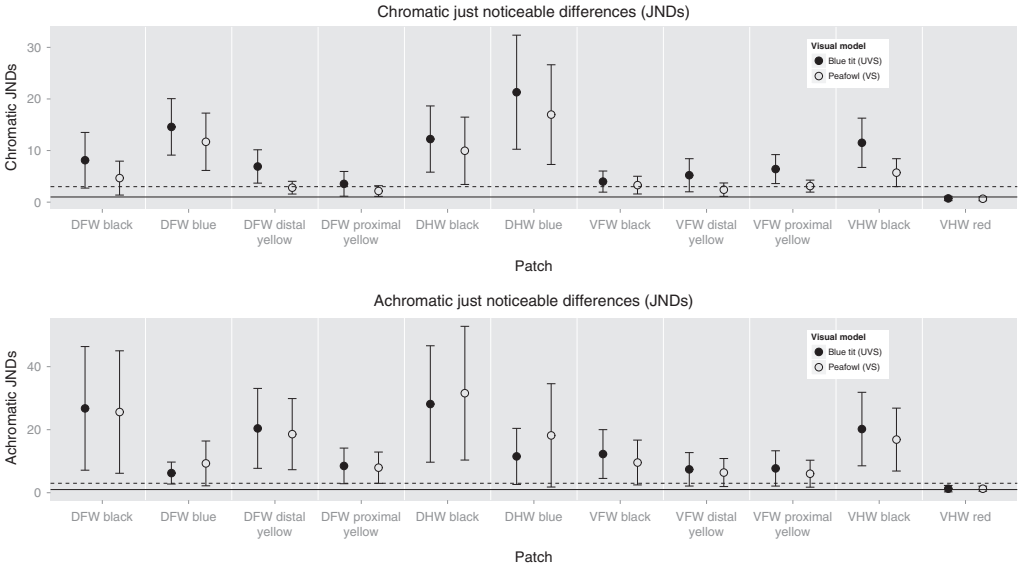

Figure 2 Chromatic and achromatic Just Noticeable Differences (JNDs) between *H. sara* and *M. pausanias* for both avian visual systems at 11 different color patches. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. Circles mark the mean JND for each patch, and the error bars show the standard deviation for each mean.

for the blue tit and 504 for the peafowl (Hart, 2001). The cone abundances were set to 1:1.9:2.68:2.7 for the blue tit model and 1:1.9:2.2:2.1 for the pea fowl (Hart, 2001, 2002. We only included neural noise, not quantum noise. For further details of the models, see the R code in Supporting Information.

Although the two species we used as the visual models do not occur in the tropics, avian visual systems are conserved and both models are reliable approximations of the visual sensitivities of Neotropical UV/VIS and V/VIS birds (Hart, 2001). We calculated all 16 possible pairwise JNDs between the four individuals of each species, and then found the mean JND for each color patch. The JNDs for each patch were idiosyncratically distributed, often highly skewed, and not normal. For these reasons, we used nonparametric sign tests. Because JNDs are threshold measures, differences are only biologically relevant when they are greater than the chosen threshold. Therefore, we used one-tailed tests to determine whether the mean JND was greater than 1 or 3. We also hypothesized that the blue tit visual model would be better able to distinguish between color patches, as *Heliconius* color patterns can have a UV component. To test this, we used one-tailed Mann–Whitney tests to determine whether the mean JND under the blue tit model was greater than the mean JND under the peafowl model. For all tests we examined 11 patches and used Bonferroni correction to adjust *P*-values to account for multiple testing.

We further tested the color match for each analogous patch between these species by comparing color volumes within avian tetrahedral color space. Color volumes encompass the variation in the color patch within avian perceptual color space (Stoddard & Prum, 2011). If two volumes are near and/or overlap, they are very similar if not identical as seen by the receiver (Stoddard & Prum, 2011). For this analysis, we did not average reflectance spectra within a patch, and instead used all 12 measures per species for each patch (3 measures × 4 individuals) to characterize the full color space occupied by each patch. We used *pavo* functions to plot convex hulls of the color space for each species and calculate the volume of the overlap between these hulls (see Supporting Information for R code).

Results

Model-mimic color similarity

The discriminability of analogous color patches of *H. sara* and *M. pausanias* varied greatly. The models suggest that several of the color patches would not be easily discriminable between the two species both chromatically and achromatically when seen by both avian visual systems. The chromatic JNDs were not significantly greater than one for the ventral hindwing red patch and the dorsal forewing proximal yellow patch for the V/VIS system (Sign test, *P*-value = 1 for red, Sign test, *P*-value = .011 for yellow) and not greater than one for the ventral hindwing red patch for the UV/VIS system (sign test, *P*-value = 1; Table 1). The achromatic JNDs were not significantly greater than one for only the ventral hindwing red patch for both visual systems (sign test, *P*-value = 1 for BT; sign test, *P*-value = 1 for PF; Table 1; Fig. 2).

Many patches had mean chromatic JNDs not significantly greater than 3, and thus would be difficult for birds to distinguish in natural lighting conditions. The UV/VIS system would have difficulties discriminating between the two species for the proximal yellow and black patches on the dorsal forewing (sign test, *P*-value = 1; sign test, *P*-value = 0.422; respectively; Table 1; Fig. 2), and the distal black and yellow on the ventral forewing (sign test, *P*-value = 1; sign test, *P*-value = 1; respectively; Table 1; Fig. 2). The V/VIS system would be unlikely to discriminate between all patches on the dorsal forewing except for the iridescent blue patch (see Table 1 for *P*-values). The V/VIS system would also be unlikely to differentiate between the two species for all patches on the ventral forewing. Seven of the 11 patches would be difficult for the V/VIS to distinguish, while only five of the 11 patches would be difficult for the UV/VIS (Fig. 2).

These difficulties in distinguishing color patches also extended to the achromatic component of bird vision, as many patches had mean achromatic JNDs not significantly greater than 3. The UV/VIS system would struggle to distinguish between the two species for the yellow patches on the dorsal forewing and ventral forewing (sign test, $P = 0.12$; sign test, $P = 0.42$; respectively, Fig. 2; Table 2). The V/VIS system would have even more difficulties distinguishing achromatic differences under non-ideal lighting for both iridescent patches, all yellow patches, and the black patch on the ventral forewing (see Table 2 for *P*-values; Fig. 2). The V/VIS would have difficulty discerning between seven of the 11 patches, whereas the UV/VIS would have difficulty with three of the 11 patches (Fig. 2). Furthermore, JND analysis of within-in species comparisons reveals great variation (Supporting Information Table S1), showing that some individuals of the same species are more discriminable than two individuals from the two different species.

Differences between visual systems

The UV/VIS and V/VIS visual systems were quite similar in their ability to distinguish achromatic differences in wing color between the mimetic pair: there were no color patches for which the mean achromatic JND of UV/VIS system was significantly greater than the V/VIS system (one-tailed Mann–Whitney test, see Table 2 for *P*-values; Fig. 2). However, the UV/VIS system was better able to distinguish between the species for three color patches: the dorsal forewing distal yellow (Mann–Whitney, *P*-value < 0.001); the ventral forewing proximal yellow (Mann–Whitney, *P*-value = 0.006); and the ventral hindwing black (Mann–Whitney, *P*-value = 0.009; see table 1 for all patches). These color patches had more variation in their UV spectra, such that UV-sensitive birds could distinguish between the species more readily than birds without UV vision.

Differences in color space volume

The color volumes of each patch comprised a very small area within tetrahedral color space and several patches overlapped in tetrahedral color space for the two species under both visual

Table 2 *P*-values for Just Noticeable Difference (JND) comparisons for achromatic contrasts between *H. sara* and *M. pausanias*

Patch	Visual model	JND	# JNDs > 1	*P*, mean JND > 1	# JNDs > 3	*P*, mean JND > 3	*W*, peafowl JND < blue tit JND	*P*, peafowl JND < blue tit JND
DFW-Blue	Blue Tit (UV)	6.24 (3.49)	16	0.00017	16	0.00017	168	1
	Peafowl (V)	9.30 (7.11)	15	0.00285	12	**0.42247**		
DFW-Black	Blue Tit (UV)	26.76 (19.61)	16	0.00017	16	0.00017	148	1
	Peafowl (V)	25.60 (19.43)	16	0.00017	16	0.00017		
DFW-Distal Yellow	Blue Tit (UV)	20.42 (12.68)	16	0.00017	14	0.02299	149	1
	Peafowl (V)	18.59 (11.28)	14	0.02299	14	0.02299		
DFW-Proximal Yellow	Blue Tit (UV)	8.51 (5.65)	14	0.02299	13	**0.11699**	148	1
	Peafowl (V)	7.94 (4.96)	16	0.00017	12	**0.42247**		
DHW-Black	Blue Tit (UV)	28.16 (18.47)	16	0.00017	16	0.00017	172	1
	Peafowl (V)	31.58 (21.22)	16	0.00017	16	0.00017		
DHW-Blue	Blue Tit (UV)	11.52 (8.90)	16	0.00017	15	0.00285	169	1
	Peafowl (V)	18.19 (16.39)	15	0.00285	13	**0.11699**		
VFW-Black	Blue Tit (UV)	12.28 (7.74)	16	0.00017	16	0.00017	122	1
	Peafowl (V)	9.59 (7.11)	15	0.00285	13	**0.11699**		
VFW-Distal Yellow	Blue Tit (UV)	7.43 (5.30)	14	0.02299	12	**0.42247**	136	1
	Peafowl (V)	6.40 (4.45)	16	0.00017	12	**0.42247**		
VFW-Proximal Yellow	Blue Tit (UV)	7.71 (5.60)	14	0.02299	14	0.02299	125	1
	Peafowl (V)	6.04 (4.28)	15	0.00285	11	**1**		
VHW- Black	Blue Tit (UV)	20.20 (11.66)	15	0.00285	14	0.02299	133	1
	Peafowl (V)	16.86 (9.98)	15	0.00285	15	0.00285		
VHW-Red	Blue Tit (UV)	1.29 (1.02)	8	**1**	1	**1**	155	1
	Peafowl (V)	1.28 (0.80)	9	**1**	1	**1**		

Mean JNDs are given for each patch under each visual system, with standard deviations in parentheses. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. The number of JNDs greater than 1 and 3 are shown with Bonferroni-corrected *P*-values for sign tests examining whether the mean JND is significantly greater than 1 or 3. Bolded values indicate that the JND for that patch are not significantly different from 1 or 3. The final columns present the test statistic, *W*, and Bonferroni-corrected *P*-values for one-tailed Mann–Whitney tests of whether the mean JND under the peafowl model is less than the mean JND under the blue tit model, with significant *P*-values in bold.

models (Table 3, Fig. 3). The dorsal and ventral forewing yellow patches had high percentage overlap as did the dorsal hindwing black (Table 3, Fig. 3.). Several color patches did not have any overlap between the two species, including the red patches, which had a JND of less than one. However, the non-overlapping color patches were close to one another in color space (see Fig. 3 for select patches).

Discussion

Differences between avian visual systems

The perception and classification of visual mimics is crucial to understanding mimicry and predator avoidance strategies. We used visual models to test several predictions of a mimicry assemblage from different predators' perspectives. The discriminability of the colors of these two species varied greatly between color patches and was dependent upon the visual system of the bird species viewing them. Furthermore, these species were more similar when viewed by the V/VIS system of their presumptive predators and were more discriminable by birds with UV vision.

Female *M. pausanias* have likely evolved to mimic only the non-UV reflectance of the unpalatable *Heliconius* model because the avian predators with which it has evolved only see the visible spectrum, rendering mimicry in the UV spectrum unnecessary. The findings here support previous research on *Heliconius* mimicry in which the V/VIS system is poor at discriminating between mimics (Bybee *et al.*, 2012; Llaurens *et al.*, 2014). Bybee *et al.* (2012) investigated the perceptual differences in the yellow patch in *Heliconius* butterflies and closely related genera to find that *Heliconius* butterflies were the best at distinguishing between yellow patches, while birds were inept. Llaurens *et al.* (2014) tested the mimetic resemblance of tiger patterned *Heliconius* butterflies to *Melinaea* species and found that the V/VIS system was the least likely to discriminate between mimetic species, whereas *Heliconius* individuals were able to discriminate between mimics.

The fact that the greatest difference between these two mimetic species was in the UV spectrum is intriguing in the context of conspecific communication between butterflies. Several recent studies have found that butterflies mate assortatively and that UV reflectance may be crucial in this process (Jiggins, Estrada & Rodrigues, 2004; Finkbeiner, Briscoe & Reed, 2014). Furthermore, *Heliconius* species have two different UV-sensitive photoreceptors (Briscoe *et al.*, 2010), suggesting that ultraviolet patterns are important for *Heliconius*. It is likely that individuals within this mimetic complex use UV reflectance for conspecific interactions.

Table 3 Patch color volume overlap for the two mimetic species

Color patch	Visual model	*M. pausanias* volume	*H. sara* volume	Overlap volume	% Overlap
DFW-Blue	Blue Tit (UV)	0.00586	0.00068	0	0
	Peafowl (V)	0.00286	0.00028	0	0
DFW-Black	Blue Tit (UV)	0.00271	0.01210	0.00008	3.06%
	Peafowl (V)	0.00137	0.01598	1.480×10^{-06}	0.11%
DFW-Yellows	Blue Tit (UV)	0.00022	0.00148	0.00004	19.22%
	Peafowl (V)	0.00010	0.00145	0.00001	12.37%
DHW-Black	Blue Tit (UV)	0.01882	0.09849	0.00352	18.68%
	Peafowl (V)	0.01837	0.08868	0.00530	28.86%
DHW-Blue	Blue Tit (UV)	0.02026	0.00278	0	0
	Peafowl (V)	0.01020	0.00136	0	0
VFW-Black	Blue Tit (UV)	0.00076	0.00069	0	0
	Peafowl (V)	0.00009	0.00024	0.00001	11.20%
VFW-Yellows	Blue Tit (UV)	0.00042	0.00098	0.00003	6.36%
	Peafowl (V)	0.00015	0.00093	0.00004	27.87%
VHW-Black	Blue Tit (UV)	0.00109	0.00221	0	0
	Peafowl (V)	0.00031	0.00110	0	0
VHW-Red	Blue Tit (UV)	0.00001	0.00001	2.461×10^{-10}	0.003%
	Peafowl (V)	0.00001	0.00001	0	0

The values for each patch for *M. pausanias*, *H. sara* and the overlap volume are represented as a percentage of total tetrahedral color space. The patch names are represented by the location (D for dorsal, V for ventral, FW for forewing, and HW for hindwing) and the color. Percentage overlap is the quotient of the overlap volume divided by the smaller of the two volumes. Each patch volume is a very small area within tetrahedral color space. There are nine overlaps listed because the two yellow patches of H. sara were combined.

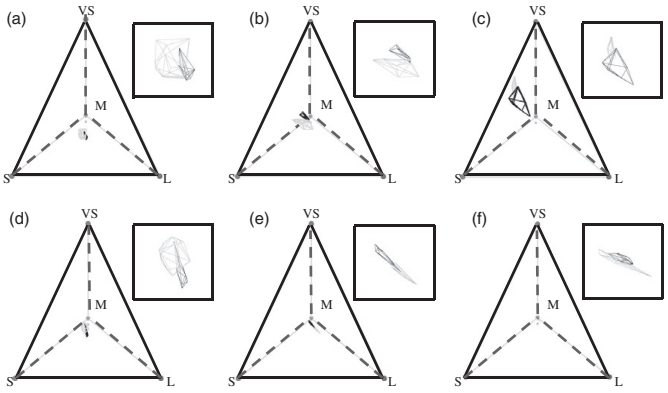

Figure 3 Avian tetrahedral color spaces and color volumes for the six patches in Fig. 1. All colorspaces are for peafowl (V/VIS) vision. The inlays are magnified images of the color volumes to show overlap between the two species. Light gray volumes are *H. sara* and black volumes are *M. pausanias*. (a) Dorsal yellow patch with both the proximal and distal yellow patches of *H. sara* being incorporated. (b) Dorsal black patch. (c) Dorsal hindwing iridescence. (d) Ventral yellow patches with both proximal and distal yellow patches of *H. sara* being incorporated. (e) Ventral black patch. (f) Ventral hindwing red patch.

Imperfect mimetic coloration

The finding that the coloration of several patches of *H. sara* and *M. pausanias* are difficult for predators with V/VIS visual systems to differentiate is perhaps not surprising, since the species' color resemblance is what prompted us to conduct this research. Our results demonstrate that most of the coloration of *H. sara* and *M. pausanias* is mimetic as seen by V/VIS birds under natural conditions, as many patches had JNDs not significantly greater than 3. These two species of butterfly are sympatric both spatially and temporally. Both species occupy disturbed rainforest habitats and are seen under variable light environments and against different backgrounds (Endler, 1993), rendering their mimetic coloration even more difficult to distinguish (Siddiqi *et al.*, 2004). The JNDs of one and three are estimates of true discriminability and tests with live predators and learning trials are needed to determine how mimetic these two species truly are in nature.

As revealed here, these two species are not perfect mimics. Most patches, while difficult to distinguish under natural lighting conditions, are discriminable by both avian visual systems under ideal conditions. Researchers previously expected that strong natural selection should drive mimics to achieve perfect resemblance (Fisher, 1930), but now there are many examples where mimics do not resemble their models perfectly (e.g. hover flies and bees: Edmunds, 2000; Penney *et al.*, 2012;

snakes: Kikuchi & Pfennig, 2012). This has led to several hypotheses explaining "imperfect mimicry": "eye-of-the-beholder", "jack-of-all-trades" and "relaxed selection" (Pfennig, 2012; Pfennig & Kikuchi, 2012). The eye-of-the-beholder hypothesis asserts that imprecise mimicry is due to artifacts of human perception (Cuthill & Bennett, 1993). We have negated this possibility through the use of visual models of predators, again demonstrating the benefits of testing mimicry by incorporating predator perception. The jack-of-all-trades hypothesis posits imperfect mimics are under selection pressures to resemble more than one unpalatable model. This may explain some of the variation we found in this mimetic pair, as there is anecdotal evidence that three other butterfly species, *Heliconius leucadia*, *Heliconius doris* and *Battus belus*, are involved with this mimetic assemblage (De'Abrera, 1981). The relaxed-selection hypothesis asserts that model species that are particularly abundant and well-defended will increase avoidance behaviors in predators, resulting in weaker selection for a perfect mimetic match. *H. sara* is abundant throughout the Neotropics and is protected by cyanogenic glycosides resulting in strong aversion by predators (Nahrstedt & Davis, 1980; Chai, 1986; Pinheiro, 1996) and perhaps there is weak selection for *M. pausanias* to improve its mimetic resemblance. Another possible explanation could be that *H. sara*, like all models in Batesian pairs, is under selection to "escape" from its mimic by evolving new colors patterns (Edmunds, 2000). Further research into the predation pressures on the mimetic coloration of all species involved with the *H. sara* and *M. pausanias* will enable a better understanding of the imperfect mimicry reported here.

Developmental constraints could also lead to imperfect mimicry. Studies of butterflies and vertebrates have revealed a convergent molecular basis for a variety of color pattern traits (Reed *et al.*, 2011; Kikuchi, Seymoure & Pfennig, 2014). Given this, it is possible that pigments in color patches of *M. pausanias* and *H. sara* that are indistinguishable (e.g. the ventral hindwing red patch) are produced by the same or similar molecular pathways while color patches that are easily distinguishable might be produced by different pathways that are developmentally constrained and unable to produce identical color phenotypes. For example *Heliconius* butterflies use 3-hydroxykynurenine as a yellow pigment, whereas *Mimoides* use papiliochrome pigments for yellow coloration (Nijhout, 1991; Koch *et al.*, 2000; Briscoe *et al.*, 2010). *M. pausanias* may be unable to perfectly mimic the yellow of *H. sara* due to constrained pigment production.

The data here reveal large variation in patch reflectance not just within species, but also within individual patches (see Supporting Information Table S1). This large intra-individual variation may further confuse predators and lead to predators avoiding a range of similar mimetic colors. The proximate mechanisms leading to the variation that we found here could be due to differences in condition dependence of the individual, and/or wing degradation due to age and wear on individual wings (Lehnert, 2010; Pegram, Nahm & Rutowski, 2013). Unfortunately, we had little control over wing wear for these wild-caught insects, although we did take precaution in our measurements to avoid damaged or worn areas of the wing.

Conclusion

Batesian mimicry requires mimics to resemble unprofitable models as perceived by natural predators. Differences between visual systems due to disparate spectral sensitivities are crucial for understanding visual signals. We show that two species of tropical butterflies from different families have mimetic coloration as seen by their predators with V/VIS-sensitive vision, but are more easily discriminable by birds with UV-sensitive vision. This leads us to conclude that *M. pausanias* and *H. sara* have evolved mimetic coloration for predators without UV-sensitive vision.

Acknowledgments

We are grateful to the Environmental Ministry of Ecuador for permission to collect butterflies (14-EXP-C3-FAU-DNB/MA). W.O. McMillan and the Smithsonian Tropical Research Institute were crucial for logistical support. We thank I. Aldas and the McMillan Lab for assistance with butterfly collecting, and R. Maia for help with *pavo*. R.L. Rutowski, K.J. McGraw, R. Simpson, the McGraw lab at Arizona State University, and two anonymous reviewers provided valuable comments on an earlier version of this manuscript.

References

Bates, H.W. (1862). Contributions to an insect fauna of the Amazon Valley (Lepidoptera: Heliconiidae). *Trans. Linn. Soc. Lond.* **23**, 495–556.

Briscoe, A.D., Bybee, S.M., Bernard, G.D., Yuan, F., Sison-Mangus, M.P., Reed, R.D., Warren, A.D., Llorente-Bousquets, J. & Chiao, C.C. (2010). Positive selection of a duplicated UV-sensitive visual pigment coincides with wing pigment evolution in *Heliconius* butterflies. *Proc. Natl Acad. Sci. USA* **107**, 3628–3633.

Bybee, S.M., Yuan, F., Ramstetter, M.D., Llorente-Bousquets, J., Reed, R.D., Osorio, D. & Briscoe, A. (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**, 38–51.

Chai, P. (1986). Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biol. J. Linn. Soc.* **29**, 161–189.

Cheney, K.L. & Marshall, N.J. (2009). Mimicry in coral reef fish: how accurate is this deception in terms of color and luminance? *Behav. Ecol.* arp017.

Cuthill, I.C. & Bennett, A.T.D. (1993). Mimicry and the eye of the beholder. *Proc. R. Soc. Lond. B Biol. Sci.* **253**, 203–204.

Dalrymple, R.L., Hui, F., Flores-Moreno, H., Kemp, D.J. & Moles, A.T. (2015). Roses are red, violets are blue–so how much replication should you do? An assessment of variation in the colour of flowers and birds. *Biol. J. Linn. Soc.* **114**, 69–81.

Dawkins, R. & Krebs, J.R. (1979). Arms races between and within species. *Proc. R. Soc. Lond. B Biol. Sci.* **205**, 489–511.

De'Abrera, B. (1981). *The butterflies of the neotropical region*. Melbourne: Lansdowne Editions.

DeVries, P. (1987). *The Butterflies of Costa Rica and their natural history*. Princeton: Princeton University Press.

Edmunds, M. (2000). Why are there good and poor mimics? *Biol. J. Linn. Soc.* **70**, 459–466.

Endler, J.A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352.

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

Finkbeiner, S.D., Briscoe, A.D. & Reed, R.D. (2014). Warning signals are seductive: relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. *Evolution* **68**, 3410–3420.

Fisher, R.A. (1930). *The genetical theory of natural selection*. Oxford: Clarendon Press.

Hart, N.S. (2001). The visual ecology of avian photoreceptors. *Prog. Retin. Eye Res.* **20**, 675–703.

Hart, N.S. (2002). Vision in the peafowl (Aves: *Pavo cristatus*). *J. Exp. Biol.* **205**, 3925–3935.

Hart, N.S. & Hunt, D.M. (2007). Avian visual pigments: characteristics, spectral tuning, and evolution. *Am. Nat.* **169**, S7–S26.

Hart, N.S., Partridge, J.C., Cuthill, I.C. & Bennett, A.T.D. (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus L.*) and the blackbird (*Turdus merula L.*). *J. Comp. Physiol. A.* **186**, 375–387.

Jiggins, C.D., Estrada, C. & Rodrigues, A. (2004). Mimicry and the evolution of premating isolation in *Heliconius melpomene* Linnaeus. *J. Evol. Biol.* **17**, 680–691.

Kikuchi, D.W. & Pfennig, D.W. (2012). A Batesian mimic and its model share color production mechanisms. *Curr. Zool.* **58**, 4.

Kikuchi, D.W., Seymoure, B.M. & Pfennig, D.W. (2014). Mimicry's palette: widespread use of conserved pigments in the aposematic signals of snakes. *Evol. Dev.* **16**, 61–67.

Koch, P.B., Behnecke, B. & ffrench-Constant, R.A. (2000). The molecular basis of melanism and mimicry in a swallowtail butterfly. *Curr. Biol.* **10**, 591–594.

Kraemer, A.C. & Adams, D.C. (2014). Predator perception of Batesian mimicry and conspicuousness in a salamander. *Evolution* **68**, 1197–1206.

Langmore, N.E., Stevens, M., Maurer, G., Heinsohn, R., Hall, M.L., Peters, A. & Kilner, R.A. (2011). Visual mimicry of host nestlings by cuckoos. *Proc. R. Soc. Lond. B Biol. Sci.* **278**, 2455–2463.

Lehnert, M.S. (2010). New protocol for measuring Lepidoptera wing damage. *J. Lepid. Soc.* **64**, 29–32.

Lindström, L., Alatalo, R.V. & Mappes, J. (1997). Imperfect Batesian mimicry – the effects of the frequency and the distastefulness of the model. *Proc. R. Soc. Lond. B Biol. Sci.* **264**, 149–153.

Llaurens, V., Joron, M. & Théry, M. (2014). Cryptic differences in colour among Müllerian mimics: how can the visual capacities of predators and prey shape the evolution of wing colours? *J. Evol. Biol.* **27**, 531–540.

Lythgoe, J.N. (1979). *Ecology of vision*. Oxford: Clarendon Press; Oxford University Press.

Maia, R., Eliason, C.M., Bitton, P.P., Doucet, S.M. & Shawkey, M.D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.* **4**, 906–913.

Mallet, J. & Gilbert, L.E. (1995). Why are there so many mimicry rings? Correlations between habitat, behavior and mimicry in *Heliconius* butterflies. *Biol. J. Linn. Soc.* **55**, 159–180.

Meadows, M.G., Morehouse, N.M., Rutowski, R.L., Douglas, J.M. & McGraw, K.J. (2011). Quantifying iridescent coloration in animals: a method for improving repeatability. *Behav. Ecol. Sociobiol.* **65**, 1317–1327.

Müller, F.. (1879). Ituna and Thyridia: a remarkable case of mimicry in butterflies. *Proc. Entomol. Soc. Lond.* **1879**, xx–xxiv.

Nahrstedt, A. & Davis, R.H. (1980). The occurrence of the cyanoglucosides linamarin and lotaustralin, in Acraea and Heliconius butterflies. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **68**, 575–577.

Nihjout, F. (1991). *The development and evolution of butterfly wing patterns.* Washington, DC: Smithsonian Institution Press.

Osorio, D. & Vorobyev, M. (2005). Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. Lond. B Biol. Sci.* **272**, 1745–1752.

Papadopulos, A.S., Powell, M.P., Pupulin, F., Warner, J., Hawkins, J.A. & Salamin, N., *et al.* (2013). Convergent evolution of floral signals underlies the success of Neotropical orchids. *Proc. R. Soc. Lond. B Biol. Sci.* **280**, 20130960.

Pegram, K., Nahm, A.C. & Rutowski, R.L. (2013). Warning color changes in response to food deprivation in the Pipevine Swallowtail Butterfly, *Battus philenor*. *J. Insect Sci.* **13**, 110.

Penney, H.D., Hassall, C., Skevington, J.H., Abbott, K.R. & Sherratt, T.N. (2012). A comparative analysis of the evolution of imperfect mimicry. *Nature* **483**, 461–464.

Pfennig, D. (2012). Mimicry: ecology, evolution and development. *Curr. Zool.* **58**, 604–607.

Pfennig, D. & Kikuchi, D. (2012). Life imperfectly imitates life. *Nature* **483**, 410–411.

Pinheiro, C.E.G. (1996). Palatability and escaping ability in Neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biol. J. Linn. Soc.* **59**, 351–365.

Pinheiro, C.E.G. (2013). Jacamars (Aves, Galbulidae) as selective agents of mimicry in neotropical butterflies. *Rev. Bras. Ornitol.* **12**, 3.

R Core Team. (2013). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org/.

Reed, R.D., Papa, R., Martin, A., Hines, H.M., Counterman, B.A. & Pardo-Diaz, C., *et al.* (2011). *Optix* drives the repeated convergent evolution of butterfly wing pattern mimicry. *Science* **333**, 1137–1141.

Siddiqi, A., Cronin, T.W., Loew, E.R., Vorobyev, M. & Summers, K. (2004). Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J. Exp. Biol.* **207**, 2471–2485.

Stevens, M. (2013). *Sensory Ecology, Behaviour, & Evolution*. Oxford: Oxford University Press.

Stoddard, M.C. (2012). Mimicry and masquerade from the avian visual perspective. *Curr. Zool.* **58**, 630–648.

Stoddard, M.C. & Prum, R.O. (2011). How colorful are birds? Evolution of the avian plumage color gamut. *Behav. Ecol.* **22**, 1042–1052.

Stoddard, M.C. & Stevens, M. (2010). Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc. R. Soc. Lond. B Biol. Sci.* rspb20092018.

Vorobyev, M. & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B Biol. Sci.* **265**, 351–358.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figures S1–S8. Photographs of individual butterflies. The first photograph and the last three are the *M. Pausanias* (labeled with B_ or Bat_) individuals and 2–5 are *Heliconius sara* (labeled with *H. sara*).
Table S1. Results of the within-species JND comparisons
Data S1. R scripts: this file contains all data preparation and analysis, as implemented using *pavo*.
Data S2. Background spectra, illumination spectra, and photographs of all specimens used in the analysis.

APPENDIX C

PERMISSION FOR INCLUSION OF PUBLISHED WORKS

All co-authors have granted permission for published work to be included.

Appendix A is attributed to the Journal of Zoology of John Wiley and Sons and included here under the Creative Commons Attribution-NonCommercial-NoDerivs License.

Appendix B is attributed to the Journal of Research on the Lepidoptera and included here under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported Licence.