Title
Sensory Ecology of Ithomiine Butterflies: signal quality, strategy and relative importance (Ithomiini spp.)

Permalink
https://escholarship.org/uc/item/7110x1d7

Author
Gonzalez-Karlsson, Adrea Susan

Publication Date
2016

Peer reviewed|Thesis/dissertation
Sensory Ecology of Ithomiine Butterflies:
signal quality, strategy and relative importance (*Ithomiini* spp.)

A dissertation submitted in partial satisfaction of the

Requirements for the degree Doctor of Philosophy

in Biology

by

Adrea Gonzalez-Karlsson

2016
ABSTRACT OF THE DISSERTATION

Sensory Ecology of Ithomiine Butterflies: signal quality, strategy and relative importance (Ithomiini spp.)

by

Adrea Gonzalez-Karlsson

Doctor of Philosophy in Biology

University of California, Los Angeles, 2016

Professor Gregory F. Grether, Chair

Ithomiine butterflies form large multispecies aggregations, the formation of which is mediated by pheromones. In ithomiine butterflies, males require secondary plant metabolites to produce pheromones but those same compounds reduce longevity. Males transfer pyrrolizidine alkaloids (PAs), exogenous plant compounds, to females during copulation. Male Greta morgane butterflies that feed longer on alkaloid-containing plants are preferred by females. Both male Mechanitis polymnia and Greta morgane butterflies fed a diet containing PAs had a shorter lifespan than males fed a diet without PAs indicating a trade-off between survival and reproduction. Despite the importance of chemical cues to mate choice, within multispecies aggregations, ithomines use visual cues initially in conspecific discrimination. However, ithomiines do rely more heavily on chemical cues in discriminating between conspecifics and heterospecific co-mimics. Although chemical cues are important in discriminating between highly visually similar mimics, not all ithomiines are equally faithful mimics. Large, aposematic species of ithomiines are less mimetically faithful while small, cryptic species are highly mimetically faithful.
The dissertation of Adrea Gonzalez-Karlsson is approved.

David B. Green

Richard Kent Zimmer

Peter Nicholas Nonacs

Gregory F. Grether, Committee Chair

University of California, Los Angeles

2016
# TABLE OF CONTENTS

Abstract of the Dissertation .............................................................................................................iii

Acknowledgements..........................................................................................................................vi

Vita...................................................................................................................................................vii

Chapter 1: Mate choice and longevity mediated by plant use in Ithomiine butterflies ........1

Chapter 1 Bibliography.....................................................................................................................11

Chapter 2: The use of chemical and visual cues for conspecific identification in co mimic and hetero-mimic Ithomiini (Lepidoptera: Nymphalidae)...........................................17

Chapter 2 Bibliography.....................................................................................................................46

Chapter 3: Signaling strategy, community diversity, body size and mimetic fidelity in Ithomiine Butterflies.................................................................................................................................51

Chapter 3 Bibliography.....................................................................................................................69

Chapter 3 Supplement.......................................................................................................................73
ACKNOWLEDGEMENTS

Above all, I want to acknowledge Dr. Greg Grether for his support, guidance and patience throughout my PhD. I have learned much about curiosity, science and writing under his tutelage.

I’ve received help in data collection and processing from many people and would like to thank Ricardo Murillo, Jim Cordoba-Alfaro, Jonathan Chang, Talavai Denipah-Cook, Deseree Povijua, Jeromalyn Santos, Alexis Meltel, Katherine Wilkinson, Dr. Barbara Dugelby, Juan Moriera, Aaron Stevenson, Tyler Hoppenfield and Christian Martinez.

I’m also grateful for the feedback and guidance I have received from my labmates, Dr. Kathryn Peiman, Dr. Jonathan Drury, Robert Cooper and Rachel Chock, and from my committee members. Specifically, I want to thank Dr. David Green for expanding my knowledge of Chemistry and providing valuable and prompt feedback on many drafts. Thanks to Dr. Richard K. Zimmer for being an inspiration with constant input of classic and new work in the field of Chemical Ecology through journal club meetings and for helping me connect with collaborators at home and abroad. Thanks to Dr. Peter Nonacs for always reminding me to see the big picture and for help with data analysis and editing.

Thanks to all the people who facilitated my dissertation. Thanks to Jocelyn Yamadera and all the staff of Las Cruces Biological Station, especially Dr. Zak Zahawi and to Glenn Baines of the Butterfly Conservatory of Costa Rica and Ricardo Murillo of the University of Costa Rica. Thanks to Dr. Shannon Olsson and the Neuroethology department of the Max Planck for
Chemical Ecology for hosting me for coursework and for data collection. Thanks to Dr. Jeff Riffell of the University of Washington in Seattle for giving me my first steps in insect physiology.

During my PhD I was supported by the Cota Robles Fellowship, GAANN Fellowship, and the NSF GK-12 program, which has been unfortunately discontinued.
VITA

Education

- 2013-2014, visiting Graduate Student, Max Planck for Chemical Ecology, Jena, Germany
- 2014, Methods in Chemical Ecology, SLU Alnarp, Sweden
- 2013, Insect Chemical Ecology, Max Planck for Chemical Ecology, Jena, Germany
- 2009-present, Graduate Student, Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, CA
- 2004-2008, Double Major in Spanish Literature and Integrative Biology, University of California at Berkeley
- 2006-2007, Universidad de Granada, Granada, Spain: year abroad studying Spanish Literature

Conference publications


Honors and Awards

• GAANN Fellowship, $30,000 plus tuition--Fall 2014 to Fall 2015
• OTS fellowship--$3250--Spring 2013
• Betty Franklin Research Grant, $1000--Spring 2013
• Carl Storm Gordon Conference Award, $600--Fall 2012
• Cota Robles Fellowship, $26,000 plus tuition--Fall 2013 to Spring 2014
• Graduate Research travel Award, $1000--Fall 2012
• Departmental research grant, $700--fall 2012
• NSF GK-12 Fellowship, $30,000 and tuition--Fall 2012 to Spring 2013
• Departmental Research Grant, $500--Spring 2012
• Latin American Institute Summer Research Grant, $2500--summer 2011
• Sigma Xi Research grant, $800--summer 2010
• GAANN Fellowship, $30,000 plus tuition--Fall 2011 to spring 2012
• NSF Competitive Edge Scholarship, $3500--Summer 2009
Chapter 1: Mate choice and longevity mediated by plant use in Ithomiine butterflies

Abstract

There is a cost to signaling and reproduction; sexiness is costly. In ithomiine butterflies, males require secondary plant metabolites to produce pheromones but those same compounds reduce longevity. Males transfer pyrrolizidine alkaloids (PAs), exogenous plant compounds, to females during copulation. Male *Greta morgane* butterflies that feed longer on alkaloid-containing plants are preferred by females. Despite female preference, males have multiple feeding strategies: either focusing on acquiring alkaloid-laden nectar or nectar alone. Both male *Mechanitis polymnia* and *Greta morgane* butterflies fed a diet containing PAs had a shorter lifespan than males fed a diet without PAs indicating a trade-off between survival and reproduction.

Introduction

Live fast, die young is not just a strategy limited to young men on motorcycles. In general, sexiness is costly (Zahavi 1975; Hunt et al. 2004). Trade-offs between reproduction and survival are found across the tree of life (Kodric-Brown and J.H. Brown 1984). Theoretically small shifts in survival outcomes equilibrate quickly with immediate successes in reproduction in fitness calculations; therefore, reductions in long term survival for short term reproductive success are favored (Alonzo and Warner 2000). Multiple male mating strategies are often compensatory for the energy or survival costs of mating; these shifts in mating strategy can be either condition or frequency dependent in which either the male’s condition determines his strategy or in which the more common male strategy is less successful (Gross 1996; Alonzo and Warner 2000). Female preference, however, is not the only determining factor; courtship and mating behaviors which
are less energetically costly compared to others can be maintained in the population regardless of female preference if the survival trade off balances lifespan with rate of mating success (Lucas et al. 1996; Alonzo and Warner 2000).

Ithomiine butterflies use pyrrolizidine alkaloids (PAs) sequestered from plant compounds, either as larvae in the case of basal ithomiines or as adults in the derived ithomiines, as pheromone precursors and chemical defense (Pliske 1975; Masters 1990; Schulz et al. 2004). The switch from larval acquired to adult acquired exogenous pheromone components correlates with host plant radiation (Fardyce 2010). Trigo and Brown (1990) compared the quantity of pyrrolizidine alkaloids in basal larval-acquiring ithomiines and the more derived adult-acquiring ithomiines and found that in basal ithomiines females consistently have higher levels of PAs than males. Adult-acquiring ithomiines are also found to have higher variance in PA levels than the larval-acquiring species, with males having a larger range and higher variance than females (Trigo and K.S. Brown Jr 1990).

Seminal gifts are known throughout Lepidoptera and consist of nutrients and defensive compounds that increase female survival and fecundity (Gwynne 2008; Dussourd et al. 2009). The contents of a seminal gift often directly benefit the female, and indirectly benefit both male and female by increasing number and fitness of offspring. In the arctiid moth *Utethesia ornatrix*, both females and males sequester alkaloids and females choose males offering nuptial gifts with larger quantities of PAs (Conner et al. 2000). In ithomiines, where the females do not directly acquire PAs, male PA quantity seems likely to play a role in mate choice.
Ithomiine butterflies are known to convert and store PAs as the n-oxide which is not toxic but it is not known whether there is reduced lifespan due to autotoxicity or cost of sequestration (Trigo et al. 1994; Schulz et al. 2004). In the moth, *U. ornatrix*, PAs carry a cost in lifespan but not a reduction in fecundity because of the concomitant increased rate of oviposition (Conner et al. 2000). In ithomiines, females do not possess alkaloids before copulation, and so perhaps the cost to longevity for females is reduced due to transference of previously converted n-oxides (Pliske et al. 1976). However, selection on females to choose males with higher levels of alkaloids should be greater in adult-deriving than larval-deriving species due to male sourcing of the alkaloids.

Female costs of reproduction have been extensively documented but the male side of reproduction has often been disregarded since males are thought to produce cheap sperm, however thoughts on male costs of reproduction are changing (Wedell and Karlsson 2003). In the moth *U. ornatrix*, a PA using species of arctiid moth, higher levels of alkaloids carry a physiological cost for both females and males (Conner et al. 2000). In other species of butterflies, nuptial gifts have been shown to be costly for the males that produce them (Karlsson 1998; Ferkau and Fischer 2006). In adult-acquiring ithomiine butterflies, males alone suffer the cost of acquisition and conversion of PAs in addition to costs of courtship.

Because of their combination of pheromone synthesis from exogenous plant sources, aggregation and courtship behavior ithomiines are a system in which male investment in reproduction is variable and female choice behaviorally regulates levels of investment. This makes the group useful in studying trade-offs in reproduction and male investment in reproduction. Ithomiines
are Neotropical butterflies, ranging from southern Mexico to Brazil and the tribe is composed of 350 species (Beccaloni and Gaston 1995; Beccaloni 1997). Ithomiini is the major taxonomic group in many Neotropical mimicry rings (Beccaloni 1997). Ithomiines form six mimicry patterns, ranging from opaque orange, black and yellow tiger patterns to clear winged butterflies (Beccaloni 1997; Jiggins et al. 2006). Although color pattern sharing is common among closely related species and genera, butterflies can also share color patterns with distantly related ithomiines (Haber 1978; Beccaloni and Gaston 1995; Beccaloni 1997; DeVries and Lande 1999; Brower et al. 2014) Most ithomiines participate in multispecies aggregations, a rare behavior within Lepidoptera (Haber 1978; Mallet 1986; Srygley and Penz 1999). Ithomiine aggregations likely serve the dual purpose of lek and to chemically defend sites against predators (Haber 1978; Ross 1995). Plant derived secondary compounds, pyrrolizidine alkaloids (PAs), are necessary for the production of pheromones in ithomiines and make ithomiines unpalatable to predators (Pliske et al. 1976; K.S. Brown Jr 1984). All ithomiines use PAs as precursors for pheromone production but PA derivatives vary between species (Schulz et al. 2004). Ithomiine aggregations likely also serve as a lek because, unlike in moths, in butterflies males secrete pheromones (DeVries 1987)

In this study, I tested male feeding patterns, the impact of pyrrolidizine alkaloids on male survival and female mate choice based on pyrrolizidine alkaloid levels. Males were provided with both PA containing and non-PA containing nectar sources and preference for and use of these resources was monitored to evaluate male use of toxic and non-toxic nectar resources. Mating pairs were recorded to evaluate the relationship between mating success and nectar use.
Methods

The plant preference and mating portion of this study was conducted in a large enclosure at the Butterfly Conservatory in El Castillo, Costa Rica using *Greta morgane* in 2011. The enclosures are approximately 5m by 10m by 10m and are made of black mesh. The conservatory rears butterflies, refreshing captive populations from the wild, and upon emergence, butterflies are transferred to the enclosures. I gave each butterfly an individual number and noted sex and date of emergence. To test male use of alkaloid plants, four vases of flower were set up in the enclosure at a distance of at least two meters from one another. Two vases contained PA-containing *Ageratum conyzoides* and the other two contained *Tithonia diversifolia*. Each vase was watched for 10-minute periods and vases were rotated on a daily basis to eliminate preference due to light or other differences between vase locations. On an hourly basis, the enclosure was searched for mating pairs; mating in ithomiines lasts for upwards of four hours (mean= 3.44, SD=1.13, n=22) and it is reasonable to presume that all mating pairs were located. Mating males and females and non-mating males and females were measured to examine size differences between mating and non-mating individuals.

Preliminary data visualization of plant use by males indicated that males specialized in the usage of particular plants, either *Ageratum conyzoides* (PA containing) or *Tithorea diversifolia*. Data on male plant use was randomized and redistributed to see whether males were specialized in use of toxic and non-toxic nectar sources. Existing male plant visit counts were redistributed across plant types 10000 times and a chi-squared test applied to the redistributed data. The observed chi-square values were compared to the distribution of chi-square values generated by randomization.
To test the impact of PAs on longevity, freshly eclosed male *Mechanitis polymnia* and *Greta morgane* were individually syringe-fed artificial nectar containing either no PAs or 3 µg/mL concentrations of heliotrine (Laotaxan, France). Heliotrine was chosen both on the basis of the use of ithomiine butterflies of *Heliotropium* in nature and its use in previous studies (Pliske et al. 1976; Masters 1990). The level of 3 µg/mL was chosen because it is within the range naturally found in nectar of PA-containing plants (Smith and Culvenor 1981). Both PA and PA-free artificial nectar contained 30% sucrose and a few drops of soy sauce. All butterflies were syringe-fed 10 mL per day. A previous study found a rate of 20% rate of storage over the long term, and 80% in the short term of PAs in males, which equates to an average of 70 µg body load in my experimental butterflies which is within the range found in nature (Trigo et al. 1996; Brückmann et al. 2000). Butterflies were kept in small mesh enclosures, 15cm by 15 cm by 30 cm, with three males per enclosure. Butterflies were kept in groups of three individuals per cage and individuals were labeled with individual numeric labels in sharpie and checked daily to monitor lifespan.

Results

Mated males spent more time feeding on *Ageratum conyzoides* relative to unmated males (Wilcoxon, mated=8, unmated=34, \( p=0.01 \), Fig 1) but males who have higher rates of *Tithonia diversifolia* use are not more likely to mate (Wilcoxon, mated=8, unmated=34, \( p=0.11 \)) nor are males who have higher overall rates of plant use more likely to mate (Wilcoxon, mated=8, unmated=34, \( p=0.11 \)). Overall, without taking into account mating status, males use *Ageratum conyzoides* more than *Tithorea harmonia* while females use *Tithorea harmonia* more than
Ageratum conyzoides (Wilcoxon, n=57, p=.04; Wilcoxon, n=41, p<.00001). However, contrary to documented plant use (Pliske 1975), I observed females utilizing PA-containing plants. Females successful at mating did not have higher rates of use of either plant (Wilcoxon, n=42, p=0.13; Wilcoxon, mated=6, unmated=35, p=.57). Males who successfully mated were not larger than males who were unsuccessful (Wilcoxon, mated=6, unmated=35, p=0.67). Mated males were not older than unmated males (Wilcoxon mated=8, unmated=34, p=0.07).

![Time on Ageratum conyzoides by mating status](image)

Figure 1. Mating males have a higher overall use and rate of use of PA containing plants than males who are not mating.

Male Mechanitis polymnia fed on high levels of PAs had a reduced life span in comparison to those fed on a PA-free diet (Fig 2, Wilcoxon, PA free=32, PA diet=23, p=.01). Male Greta morgana fed on high levels of PAs also had a reduced lifespan in comparison to those fed on a no PA diet (Fig 2, Wilcoxon, PA free=39, PA diet=13, p=.02).
Figure 2. Male ithomiines have a longer life span on a PA-free diet. On the left, Mechanitis polymnia and on the right, Greta morgane.

Males were significantly more specialized than predicted by a chance distribution of plant use (Fig 3, Chi-squared=10.89, n=36, p<.001). However, males who mated were not always specialized although they spent more time on Ageratum conyzoides than did non-mating males. When mating males were specialized it was on Ageratum conyzoides, the PA-containing plant. However, many used both Tithonia diversifolia, the nectar plant and Ageratum conyzoides the PA-containing plant. Males who specialized on Tithonia diversifolia were not found to mate, however an equal number of males were specialized on Ageratum conyzoides and Tithonia diversifolia (Fig 3; Chi-squared=0.2727, n=36, p=0.602).
Figure 3. Male use of nectar and PA containing plants by species, use time for *Tithonia diversifolia* on the y-axis and *Ageratum conyzoides* on the x-axis. *Greta morgane* is on the right and *Oleria paula* is on the left.

**Discussion**

Male ithomiine butterflies trade longevity for sex appeal. Age and size did not determine investment in PA sequestration; this implies that female perception of PA levels is important per se and not a signal of another desirable trait and also that size and age are not accurate indicators of PA levels for choosing females. However, investment in sequestration may ultimately be condition dependent in a way not measured by this study and may relate to larval growth rate or parasite load.

I found a trade-off and multiple male strategies for resource acquisition and prioritization in ithomiine butterflies. This is especially interesting in the context of ithomiine natural history; ithomiines are unusual among butterflies in having multi-species aggregations. These
aggregations likely serve as a protected space, buffering both palatable females and low-alkaloid males against predation (Haber 1978). Additionally, interspecies copulations have been observed in dense aggregations with conspecifics and heterospecifics present and therefore one can imagine that sneaker males less devoted to gathering alkaloids may benefit from the pheromonal signals of conspecific males (Vasconcellos-Neto and K.S. Brown Jr 1982).

Alternatively, lifetime analyses of fitness of males prioritizing nutrient acquisition and alkaloid acquisition may be equivalent in nature if the low levels of alkaloids contained by nutrient specializing males is adequate to deter predation or if the aggregation space is sufficient to deter predation on low-PA individuals. Although I did not find any difference between mating and non-mating males in other qualities, it is possible that PA quantity represents some other measure of quality not measured here and therefore alkaloid sequestration patterns are condition dependent either over the lifetime of a male or dependent on energy reserves of the male. Strategies may be more viable at different times, for example, seasonally.

Since male lifespans are shown to be reduced by PAs as was shown for both males and females in Utethesia ornatrix, a larval sequestering arctiid moth (Conner et al. 2000), it seems likely that the resource acquisition by males and nuptial gift in Ithomiines is an adaptation that increases female lifespan (Pliske et al. 1976; Conner et al. 2000). Feeding studies with ithomiines indicate that mated females are unpalatable while unmated females are palatable and freshly emerged males are palatable until they have fed on a PA source (Masters 1990). This indicates that the seminal gift provides a direct benefit to the female. However, in related species some defensive compounds are excreted during oviposition and so eggs and freshly emerged larvae
may also be protected (Dussourd et al. 2009). Both sexes would likely benefit from the increased longevity of the females and offspring.

Based on lifespan and previously recorded measures of alkaloid content in adult butterflies, rates of ingestion must be higher than even the high rate recorded in this study (Brückmann et al. 2000). However, the levels of alkaloids in ithomiines fluctuate seasonally and males fluctuate in PA content between acquisition, display and mating (Trigo et al. 1996). A study in which alkaloids were fed to ithomiines to chart the biosynthetic pathway of pheromone production found a maximum survival of only three days (Trigo et al. 1994) Since this study was conducted within enclosures protecting butterflies against predation, it is possible that while this experiment demonstrates the physiological costs of alkaloid sequestration, lifespan in nature would be curtailed by predation if alkaloid levels were too low. Previous experiments indicate that butterflies released into open environments without chemical defenses do not survive for long (Schneider et al. 1975). Perhaps this is part of the defensive function of ithomiine aggregations--to protect recently emerged individuals, or individuals in the low part of the PA fluctuation.

Further research on the mating system of these butterflies incorporating multiple generations and looking at further nutrition costs would be interesting both to better understand this group and the parameters of reproduction strategies and trade-offs overall. Ultimately, sex appeal and survival must find a balance with potentially multiple fitness maxima.

References


Beccaloni GW, Gaston KJ. 1995. Predicting the species richness of neotropical forest butterflies: Ithomiinae (Lepidoptera:Nymphalidae) as indicators. Biological Conservation 71:77–86.


speciation and wing pattern change in neotropical *Ithomia* butterflies (Lepidoptera: Nymphalidae). Evolution 60:1454.


Chapter 2: The use of chemical and visual cues for conspecific identification in co-mimic and hetero-mimic Ithomiini (Lepidoptera: Nymphalidae)

Abstract
Multimodal communication in mimetic species enables convergence within individual sensory modalities. Because ithomiine butterflies form multispecies aggregations with many individuals of multiple species, they are an interesting test case within which to examine modality use in conspecific discrimination. We found that social interactions within natural ithomiine aggregations are largely intraspecies interactions between males and females. Using two-way choice tests, we showed that ithomiines are more strongly attracted to the pheromones of their own species than to the pheromones of other ithomiine species. Using artificial models of butterflies, we found that ithomiines preferentially approach models of their own versus other mimetic color patterns. In other lepidopterans, reliance on visual and chemical cues is equal. However, visually mimetic butterflies are hypothesized to rely more heavily on chemical than visual cues. To test this, we performed four-way choice tests with mimetic and non-mimetic pairs of ithomiine species, *Greta morgane* (clear), *Ithomia patilla* (clear) and *I. heraldica* (amber). The butterflies chose between pairs of cues; either conspecific or heterospecific wing patterns paired with conspecific or heterospecific hairpencils (pheromone distributing organ). We found that the mimetic species *G. morgane* (clear) and *I. patilla* (clear) rely more heavily on chemical cues than on visual cues for discriminating conspecifics from heterospecifics while the non-mimetic species *G. morgane* (clear) and *I. heraldica* (amber) rely to the same degree on chemical and visual cues. This is the first experimental evidence that visual mimicry is associated with reliance on chemical cues in butterflies.
Key words: Ithomiini, Lepidoptera, sensory ecology, multimodal, mimicry, interspecific communication, conspecific discrimination, chemical communication

Introduction

In Lepidopterans, both visual and chemical cues play important roles in mate choice. Visual cues are thought to play a larger role in discrimination between species while pheromonal cues are important for choice between individuals of the same species (Scott 1973; Silberglied 1984; Costanzo and Monteiro 2007). Mistaking visual co-mimics (i.e., members of the same mimicry group) for conspecifics is costly due to energy spent in courtship or aggressive interactions (Lorenz 1962; Candolina 2003; Merrill and Jiggins 2009). Co-mimics could avoid species identity confusion by diverging in non-mimetic modalities and relying more heavily on another sense to discriminate conspecifics from heterospecifics; for example, being visually similar but chemically distinct. In support of this hypothesis, the diversity of the mimetic visual pattern is low while the variation in pheromone chemistry is high suggesting this strategy for conspecific discrimination between heterospecific mimics in *Amauris* butterflies (Vane-Wright and Boppre 1993). However, previous studies have shown that multimodal signals are responded to, and learned more quickly than unimodal signals (Uetz et al. 2009; Balkenius and Dacke 2013; Riffell and Alarcón 2013). Since heterospecific mimics lose a modality within which to discriminate there may be a trade-off between mimicry and conspecific identification (Kikuchi and Pfennig 2013).

In assessing cues, there is also a tradeoff between accuracy and speed, and this may influence predator generalization across mimicry groups, as well as conspecific discrimination when the
mimicry groups contain multiple species (Chittka and Osorio 2007). Context can be important in the use of multimodal versus unimodal cues. Insects can perceive multimodal stimuli as fused or unfused stimuli in which either only the multimodal cue is regarded and unimodal components are ignored or in which both multimodal and unimodal cues elicit responses. *Cataglyphis fortis*, desert ants, for example, can be trained to use multimodal olfactory and visual landmarks as well as unimodal olfactory or visual landmarks, but they learn the unimodal cues more slowly and once acquainted with the multimodal cue subsequently ignore unimodal cues (Steck et al. 2011). Hawkmoths, however, view floral stimuli as unfused stimuli, perhaps because of the need to be flexible with regards to food type (Balkenius and Dacke 2013).

Ithomiines are Neotropical butterflies, ranging from southern Mexico to Brazil, and the tribe is composed of 350 species (Beccaloni and Gaston 1995; Beccaloni 1997). Ithomiini is the major taxonomic group in many Neotropical mimicry rings (Beccaloni 1997). Ithomiines form six mimicry patterns, ranging from opaque orange, black and yellow tiger patterns to clear winged butterflies (Beccaloni 1997; Jiggins et al. 2006). Although color pattern sharing is common among closely related species and genera, butterflies can also share color patterns with distantly related ithomiines (Haber 1978; Beccaloni and Gaston 1995; Beccaloni 1997; DeVries and Lande 1999; A.V.Z. Brower et al. 2014). Most ithomiines participate in multispecies aggregations, a rare behavior within Lepidoptera (Haber 1978; Mallet 1986; Srygley and Penz 1999). Ithomiine aggregations likely serve the dual purpose of leks and chemical defense against predators (Haber, 1978; Ross, 1995). Plant derived secondary compounds, pyrrolizidine alkaloids (PAs), are necessary for the production of pheromones in ithomiines and make ithomiines unpalatable to predators (Brown, 1984; Pliske, Edgar, & Culvenor, 1976). All
Ithomiines use PAs as precursors for pheromone production but PA derivatives vary between species (Schulz et al., 2004). Ithomiine aggregations likely also serve as leks because, unlike in moths, in butterflies males secrete pheromones (DeVries, 1987).


Since ithomiine butterflies are visually mimetic, we tested their reliance on chemical and visual cues to discriminate between conspecifics and mimetic heterospecifics. Ithomiines are ideal for testing the modality shift hypothesis of conspecific discrimination in mimetic species because they form aggregations of individuals from multiple species across several mimicry groups.
Chemical or visual cues alone could be sufficient for conspecific discrimination or ithomiines could rely on multimodal cues to recognize conspecifics. Butterflies are highly visual insects with well-developed visual systems attuned to perception of wing pigments of conspecifics (Briscoe 2010). Therefore, ithomiines likely also use visual cues, at least initially, in assessing the identity of conspecifics. Since multimodal combinations of cues have been shown to be important in conspecific recognition, we used a four-way choice test to test the relative importance of chemical and visual cues for ithomiines. In the four-way choice test, we presented butterflies with choices between pairs of conspecific and heterospecific chemical and visual stimuli. It has long been hypothesized that visually mimetic butterflies might rely more heavily on chemical than visual cues (Vane-Wright 1993, Montiero and Constanza 2007), but this is the first experimental test of this hypothesis.
Methods

Study System

Ithomiine butterflies are mimetic, with multiple species represented within each mimicry group (see Table 1).

<table>
<thead>
<tr>
<th>Mimicry group</th>
<th>Example</th>
<th>Species used</th>
</tr>
</thead>
</table>
| Amber         | ![Butterfly](image) | *Ceratinia tutia*  
*Dircenna chiriquensis*  
*Dircenna klugii*  
*Godyris zygia*  
female |
| Clearwing                      | Godyris zygia male  
Ithomia heraldica  
Ithomia xenos  
Napeogenes cranto  
Pteroymia agalla  
Pteronymia donata  
Pteronymia notilla |
|-------------------------------|----------------------|
| Orange and Black              | Episcada salvinia  
Greta anette  
Greta andromica  
Greta polisenna  
Hypoleria cassotis  
Heterosais edessa  
Oleria vicina  
Pteronymia artena  
Pteronymia simplex |
| Small dark transparent        | Callithomia hezia  
Hyposcada virginiana  
Napeogenes tolosa  
Tithorea harmonia  
Thyridia psdii  
Tithorea tarricina |
| Tiger                         | Greta andromica  
Greta morgane  
Greta nero  
Ithomia patilla  
Ithomia terra  
Oleria paula  
Oleria rubescens  
Pseudoscada utillia |
|                               | Hypothyris lacaste  
Mechanitis lysimnia  
Mechanitis menapis  
Mechanitis polymnia |

Table 1. Species by mimicry group with a representative example
Study sites and permits

Experiments one and four were conducted in the University of Costa Rica’s Biological Reserve Bosquecito Lionel Oveido (9°56'14.9"N 84°02'58.0"W) with permission from the School of Biology. Butterflies sacrificed for use as visual and chemical stimuli were collected from outside of the reserve in Parque del Este (9°56'33.1"N 84°00'39.3"W) to reduce the impact on the reserve’s population. Experiments two and three were conducted in the Las Cruces Biological Station and Reserve (8°47'7" N, 82°57'32" W). Experiments were conducted only during clement weather (not raining or windy with temperatures above 20 degrees C) between the hours of 09:00 and 15:00 based on the aggregation activity of wild ithomiines. All collections and experiments were in accordance with local laws and permitting.

Experiment 1: Importance of color in first-pass discrimination within aggregations. To test the use of visual cues in conspecific discrimination within aggregations we used plastic models of butterflies from two separate mimicry groups as well as a control non-aggregating Heliconius erato butterfly. We observed free flying butterflies entering the aggregations and quantified approaches to the plastic models. We used the clearwing species Ithomia patilla, a colored wing congener, Ithomia heraldica, and Heliconius erato a butterfly outside of ithomiini that is not part of an ithomiine mimicry group (Fig 2). Ithomia patilla (clearwing) and Ithomia heraldica (amber butterfly) are common congeners, of different mimicry groups. Heliconious erato is a common butterfly within the Biological Reserve that is outside of the tribe Ithomiini and does not participate in ithomiine aggregations.
We made models of the three species by printing wing patterns on transparencies using an ICON 650 printer. The models were purely visual stimuli with no chemical signal paired with the model. Chemical components of the ink or projector paper are unlikely to be attractive to insects and were consistent across models. We presented models to free-flying butterflies within a portion of an aggregation site. Models were placed within a 2 square meter area in pairs to simulate an aggregation in randomized order. Models were posed with wings open to 180 degrees to simulate the display pose assumed by perching ithomiine males. Free-flying ithomiines entering the aggregation were scored as approaching if they flew within 10 cm of the models and color pattern of approaching individual ws recorded. Each trial lasted for half an hour and experiments were conducted in July and August, 2010 between 10:00 and 14:00 with 60 trials per model arrangement. Differences between plastic and real *Ithomia patilla* and *Ithomia heraldica* were tested by measuring wing reflectance spectra using an Ocean Optics spectrometer (USB 2000) equipped with a reflectance probe (Ocean Optics R200 7-UV-VIS) and a pulsed xenon light source (Ocean Optics PX-2), with reference to a Labsphere certified reflectance standard using Ocean Optics’ OOIBase32 software. We placed the reflectance standard behind the wings when taking readings, and the light path was oriented at 90 degrees relative to the wing surface. The resulting measurements include both light reflected off the wings and light transmitted through the wings. We took three repeat measurements at four positions: forewing apex, forewing discal cell, hindwing apex, hindwing discal cell (Fig 3). Differences were calculated from a PCA of 10 nanometer bins. PC scores were used if the eigenvalue was greater than one and represented more than one component. Generally, only the first two PC scores were greater than one. The first loading in a spectrophotometric PCA corresponds to brightness while the second corresponds to the shape of the spectra. PC 3’s
meaning is harder to interpret but we included it if the eigenvalue was greater than one. Points measured on the wing were forewing apical, forewing discal, hindwing apical and hindwing discal following DeVries (1987).

Figure 3. A diagram of butterfly wing morphology showing the locations measured: forewing apex, forewing discal cell, hindwing apex, hindwing discal cell. From DeVries (1987).

Experiment 2: Two-way hair pencil choice test

Ithomiine butterflies were collected in the Las Cruces Biological Research Station in Costa Rica in July 2014. A total of 56 bioassays were conducted with 12 species of ithomiines. Individuals were tested with conspecific and heterospecific pheromones. The level of phylogenetic relatedness was determined using cophenetic distances from Brower (2014).

Wild-caught male ithomiine hair pencils (pheromone distributing organ located between the forewing and the hindwing) were extracted and the pheromones used in bioassay tests. To extract pheromones, hair pencils were removed from the hindwing and placed in 2 ml of hexane.
Pheromone bioassays were conducted using 2ml vials with a septum with a 5 cm piece of untreated cotton string inserted to act as a wick for the evaporating extract, such that the string reaches the bottom and protrudes through the septum by 1 cm. Hexane and pentane are widely used as solvents in bioassays because they are nonpolar and evaporate at a constant rate that roughly matches the release rate of signaling insects lepidopterans although the precise release rates in ithomiine butterflies are unknown (El-Sayed et al. 2002; Carde et al. 2004). Although Ithomiines were not tested for attraction to solvent only, quantities of solvent and surface area of the wick were standardized across treatments.

Bioassays were conducted in a 2x1x2 m mesh enclosure with extracted pheromones. Butterflies were presented with pheromone bottles containing conspecific and heterospecific pheromones (extracted from one individual each) placed on opposite sides of the enclosure. Wild caught male and female butterflies were released individually in the middle of the enclosure and observed for 10 minutes after a five-minute acclimation period. The observer consistently observed from the same position beside the enclosure. Tested butterflies were identified to species and marked to prevent retesting. Pheromones were rotated between sides of the enclosure to allow time for scent from previous trials to dissipate and experiments were rotated between two enclosures to reduce biases due to light environment or pheromones from a prior assay. We recorded butterfly approaches within 10cm or landing on pheromone extracts.

**Experiment 3: Frequency of interactions in ithomiine aggregations**

To measure frequency of interspecific interactions in ithomiine aggregations, ithomiine interactions within the aggregation were observed and interacting individuals were caught, individually labeled and gender, species and color pattern were recorded. Color pattern
designation was in accordance with the discrete mimicry groups established by Jiggins (2006) and Beccaloni (1994). The ithomiine aggregation site covered 50 m$^2$ and included several species of ithomiines (Table 1). Abundance and diversity of ithomiines within aggregations were estimated periodically by catching and releasing all individuals within the aggregation to estimate expected frequencies of interactions. Observations were taken from June 23, 2014 to August 1, 2014 from 10:00-14:00.

Experiment 4: Four-way choice test of relative importance of modalities

To test the relative importance of chemical versus visual cues, a four-way test with mimetic and non-mimetic species pairs was used (Fig 4). Butterflies were presented with combinations of conspecific and heterospecific visual and chemical cues and approaches were quantified. The species *Greta morgane*, *Ithomia patilla* and *Ithomia heraldica* were used. Both *Greta morgane* and *Ithomia patilla* are part of the clearwing complex as defined in (Beccaloni 1997). *Ithomia heraldica* is a colored wing butterfly as defined in (Jiggins et al. 2006). These species are all abundant members of their mimicry complex in the Central Valley of Costa Rica (see Mimetic Fidelity chapter). Species abundance in a mimicry complex varies both temporally between years and spatially between sites (see Mimetic Fidelity chapter). *Greta morgane* and the *Ithomia* species are not close phylogenetically within Ithomiini with a cophenetic distance of 0.20 (A.
Preserved male butterflies were used to create models of males in display position. Butterflies were collected and dried in an entomological oven at 50° C for one week to preserve and fix the specimen in display position as well as to remove residual chemical cues; cues persist for only a few hours post death (Edgar et al. 1976; Haber 1978). Three sets of models were created and rotated between trials. Butterfly models were paired with a conspecific or heterospecific hair pencil at the time of the model presentation. Hair pencils were excised from live males caught the same day as experiments and were used for no longer than three hours based on prior experiments on hair pencil emissions with all hair pencils being excised within minutes of one another (Pliske et al. 1976). Models were presented to individual *Greta morgane* and *Ithomia*
"patilla" in a four-way choice design with four separate models: *Greta morgane* wings with *Greta morgane* hair pencils, *Greta morgane* wings with *Ithomia patilla* hair pencils, *Ithomia patilla* with *Greta morgane* hair pencils and *Ithomia patilla* with *Ithomia patilla* hair pencils. These pairs represent two conspecific cues, one cue being conspecific and one cue being heterospecific and both cues being heterospecific. *Greta morgane* were additionally presented with four separate models: *Greta morgane* wings with *Greta morgane* hair pencils, *Greta morgane* wings with *Ithomia heraldica* hair pencils, *Ithomia heraldica* wings with *Greta morgane* hair pencils and *Ithomia heraldica* wings with *Ithomia heraldica* hair pencils. These pairs represent the same combinations of stimuli but in butterflies of two different mimicry groups. One of each type of model was presented in situ in a two square meter portion of an aggregation site spaced at distances of 0.5 m from one another. Hairpencils were rotated between conspecific and heterospecific models to remove bias of more attractive models and models and hair pencils were rotated clockwise within the 2 m area between trials to remove bias of direction or ambient light. All individuals tested were individually labeled to ensure that each butterfly was tested only once. Individuals presented with the four way choice were acclimatized in a 30.5cm² Bioquip cube for five minutes prior to the start of the experiment. The cages are made of white polyester/nylon netting; mesh size is 330 holes per square inch. Butterflies were monitored for 10 minutes; all individuals left the cube during that time. Individuals were scored as approaching the model if they came within 10 cm of the model. Experiments were conducted in August 2011 on *Greta morgane* choice and in July 2012 on *Ithomia patilla* choice.

Results

*Experiment 1*
In the choice test between plastic models of *Ithomia patilla* (clearwing), *Ithomia heraldica* (amber winged) and *Heliconius erato* (black and red non-ithomiine), free flying ithomiine butterflies of the clearwing and amber wing pattern entering the aggregation were significantly more attracted to models of their own color pattern than to models of the other color pattern or the control model (Fig 5; Chi-squared test, $x^2=37.3$, df=96, $p<0.0001$). Both amber and clearwing butterflies visited the control, *Heliconius erato*, more often than butterflies of the other ithomiine mimicry group. Significantly more visits by live clearwing butterflies to plastic models of the control butterfly were recorded than to the amber winged butterfly model (Chi-squared test, $x^2=244.1$, df=81, $p<0.001$). However, this difference was not significant for the visits of amber winged butterflies to the control versus the heterospecific model as there were...
only two visits to the control models and none to the clearwinged models (Chi-squared test, 
χ²=2.00, p=0.16). Latency of approaches of conspecific, mixed and heterospecific models did 
not vary significantly among categories (ANOVA, df=3, p=0.25).

Plastic models were significantly different in reflectance from real ithomiine butterflies and 
across species of model (Kruskall Wallis, df=3, p=0.004). In *Ithomia patilla*, models were 
different from real butterflies in the forewing apex (FWA), and forewing discal cell (FWD) but 
equivalent in the hindwing locations ,based on Wilcoxon tests (see Table 2). Although the plastic 
models did differ from the butterfly wings, since models were generally more similar to their 
equivalent real butterflies than to one another, their use is still justified (Table 1, Table 3 and Fig 
6). The plastic models of *Ithomia patilla* were more different from their model butterfly than 
were the *Ithomia heraldica* models, perhaps due to the plastic models being more reflective than 
wing tissue, an effect which was ameliorated by pigment in the non-transparent models.

<table>
<thead>
<tr>
<th>Location</th>
<th>IHR</th>
<th>IHR-</th>
<th>IHP</th>
<th>IPR-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHP</td>
<td>IPR</td>
<td>-IPP</td>
<td>IPP</td>
</tr>
<tr>
<td>FWA</td>
<td>1.94</td>
<td>4.07</td>
<td>4.13</td>
<td>1.74</td>
</tr>
<tr>
<td>FWD</td>
<td>1.95</td>
<td>1.73</td>
<td>6.00</td>
<td>5.65</td>
</tr>
<tr>
<td>HWA</td>
<td>1.30</td>
<td>2.39</td>
<td>4.17</td>
<td>3.00</td>
</tr>
<tr>
<td>HWD</td>
<td>1.75</td>
<td>2.40</td>
<td>5.71</td>
<td>4.96</td>
</tr>
</tbody>
</table>

Table S1. Euclidian distances in principal components space between the reflectance spectra of 
plastic and real *Ithomia patilla* and *Ithomia heraldica* (IHR=real *Ithomia heraldica*, IHP=plastic 
*Ithomia heraldica*, IPR=real *Ithomia patilla*, IPP=plastic *Ithomia patilla*).
Figure 6. Graphs by wing location of PC1 (brightness) and PC2 (hue) of the spectrophotometer data. Blue=plastic butterflies and orange=real butterflies.
<table>
<thead>
<tr>
<th>Location</th>
<th>PC</th>
<th>Eigenvalue</th>
<th>Percentage</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWA</td>
<td>PC1</td>
<td>35.8795</td>
<td>0.88</td>
<td>33</td>
<td>0.5161</td>
</tr>
<tr>
<td>FWA</td>
<td>PC2</td>
<td>2.87869</td>
<td>0.0702</td>
<td>33</td>
<td>0.6400</td>
</tr>
<tr>
<td>FWA</td>
<td>PC3</td>
<td>1.78505</td>
<td>0.0435</td>
<td>33</td>
<td>0.1238</td>
</tr>
<tr>
<td>FWD</td>
<td>PC1</td>
<td>39.0862</td>
<td>0.9533</td>
<td>33</td>
<td><strong>0.0358</strong></td>
</tr>
<tr>
<td>FWD</td>
<td>PC2</td>
<td>1.02708</td>
<td>0.0251</td>
<td>33</td>
<td><strong>0.0058</strong></td>
</tr>
<tr>
<td>HWA</td>
<td>PC1</td>
<td>38.2287</td>
<td>0.9324</td>
<td>33</td>
<td>0.3333</td>
</tr>
<tr>
<td>HWA</td>
<td>PC2</td>
<td>2.02219</td>
<td>0.0493</td>
<td>33</td>
<td>0.9375</td>
</tr>
<tr>
<td>HWD</td>
<td>PC1</td>
<td>39.0862</td>
<td>0.9533</td>
<td>33</td>
<td><strong>0.0092</strong></td>
</tr>
<tr>
<td>HWD</td>
<td>PC2</td>
<td>1.02708</td>
<td>0.0251</td>
<td>33</td>
<td><strong>0.0092</strong></td>
</tr>
</tbody>
</table>

Table 2. Differences between plastic models and real *Ithomia patilla* by wing location based on Wilcoxon tests comparing PC values for PCs with eigenvalues greater than one.
<table>
<thead>
<tr>
<th>Location</th>
<th>PC</th>
<th>Eigenvalue</th>
<th>Percentage</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWA</td>
<td>PC1</td>
<td>35.1245</td>
<td>0.8567</td>
<td>22</td>
<td>0.2563</td>
</tr>
<tr>
<td>FWA</td>
<td>PC2</td>
<td>4.76571</td>
<td>4.76571</td>
<td>22</td>
<td>0.1386</td>
</tr>
<tr>
<td>FWD</td>
<td>PC1</td>
<td>35.7433</td>
<td>0.8718</td>
<td>22</td>
<td>0.8484</td>
</tr>
<tr>
<td>FWD</td>
<td>PC2</td>
<td>4.41107</td>
<td>0.1076</td>
<td>22</td>
<td>0.0009</td>
</tr>
<tr>
<td>HWA</td>
<td>PC1</td>
<td>33.7211</td>
<td>0.8225</td>
<td>22</td>
<td>0.6050</td>
</tr>
<tr>
<td>HWA</td>
<td>PC2</td>
<td>6.20816</td>
<td>0.1514</td>
<td>22</td>
<td>0.0033</td>
</tr>
<tr>
<td>HWD</td>
<td>PC1</td>
<td>38.6924</td>
<td>0.9437</td>
<td>22</td>
<td>0.3946</td>
</tr>
<tr>
<td>HWD</td>
<td>PC2</td>
<td>2.08903</td>
<td>0.0510</td>
<td>22</td>
<td>0.0488</td>
</tr>
</tbody>
</table>

Table 3. Differences between plastic models and real *Ithomia heraldica* by wing location based on Wilcoxon tests comparing PC values for PCs with eigenvalues greater than one.
Figure 7. Pheromone bioassay across all species, comparison of cophenetic distances between preferred and un-preferred pheromones (N=56, x²=27.25, p< 0.0001).

In the pheromone bioassay, butterflies visited conspecific pheromones 66% of the time across all species tested which is significantly greater than the chance expectation of 50% (Fig 7; Chi-squared test, x²=27.25, df=64, p <0.0001). Species with enough individuals tested to have sufficient power to analyze followed a similar pattern; *Godyris zygia* visited conspecific pheromones 70% of the time (Chi-squared test, x²=4.8, df=29, p=0.03). *Oleria vicina* visited conspecific pheromones 85% of the time (Chi squared test, x²=3.57, df=6, p=0.05). *Hyposcada viriginiana* visited conspecific pheromones 57% of the time which is not a significantly higher rate (Chi-squared test, x²=1, df=6, p=0.705). Other species with fewer than 5 individuals tested were included in the pooled data but were not analyzed individually. The heterospecific
pheromones visited were not from more closely related species than those rejected (Wilcoxon test, df=64, $p=0.205$). Heterospecific co-mimics were not approached significantly more often than non co-mimetic heterospecifics (Chi-squared test, $x^2=0.86$, df=28, $p=0.353$).

**Experiment 3**

![Figure 8](image)

*Figure 8. Frequencies of ithomiine species in the aggregation site.*
Figure 9. Rates of interactions between and within sexes at the aggregation sites

Figure 10. Interactions with co-mimics and hetero-mimics
Within the aggregations, interactions between free-flying conspecifics were significantly more frequent than interactions between heterospecifics in *Godryis zygia*, even after taking into account relative abundance of conspecifics and heterospecifics (Fig. 11, Chi-squared test, $x^2 = 25.13, n=35, p=0.001$). Across species, intersexual interactions were more common than intrasexual interactions (Fig 9, Chi-squared test, $n=49, x^2=6.33, p=0.042$). Females were more common than males in the aggregations, but not significantly so (Binomial test, $n=52, p=0.2$).

The frequency of interactions between co-mimics, excluding conspecifics, was significantly higher than interactions with non-mimetic heterospecifics (Fig 10). This difference was significant for clear-winged butterflies (Chi-squared test, $x^2 = 17.80, n=50, p<0.001$) but not for Tiger butterflies (Chi-squared test, $n=32, x^2 =1.09, p=0.29$).
Figure 12. Visual and chemical cue use, *Greta morgane* choice *Greta morgane x Ithomia patilla*

Figure 13. Visual and chemical cue use, *Ithomia patilla* choice *Greta morgane x Ithomia patilla*
In the four-way test between conspecific and heterospecific paired olfactory and visual cues, all species approached the conspecific paired cues most often. In the case of clearwing species pair (Greta morgane and Ithomia patilla), both species approached models with conspecific visual and chemical cues most often (Figure 12 and Figure 13, G. morgane chi-squared test, x²=41.48 p < 0.001, d.f=82; I. patilla x²= 29.68, p-value<0.001, d.f=46), but when models with mixed cues were approached, it was significantly more often the model with conspecific chemical cues and heterospecific visual cues, not the model with conspecific visual cues and heterospecific chemical cues (Fig 14, test of proportion, G. morgane; z= -4.74, p-value<0.0001, d.f=82; I. patilla z=4.16, p-value<0.001, d.f.=46). Comparing males to females, in Greta morgane, males approached the mismatched paired cue with the conspecific chemical cue more often than did females (test of proportion, z= -3.83, p=0.0001, df=27).

Figure 14. Visual and chemical cue use, Greta morgane choice Ithomia heraldica x Greta morgane
When the four-way choice test included a non-mimetic pair, *Greta morgane* (clearwing) significantly more often approached the model with conspecific cues (Fig 13; chi-squared test, $X= 25.7879$, df=$31$, $p<0.0001$). However, when approaching a conspecific and heterospecific mixed cue pair, *Greta morgane* did not approach either paired cue significantly more often (test of proportion, $z=-0.5057$, df=$31$, $p=0.53$).

### Discussion

Mimetic butterflies are predicted to differ in their modality use from other Lepidoptera, which use visual cues and chemical cues equally in discrimination between conspecifics and heterospecifics (Scott 1974; Vane-Wright and Boppre 1993; Costanzo and Monteiro 2007). This study provides experimental evidence of a selective modality shift in discrimination between conspecific and mimetic heterospecifics versus non-mimetic heterospecifics. Mimetic ithomiine butterflies prioritize non-mimetic modalities to discriminate between conspecifics and co-mimics. When discriminating between conspecifics and mimetic heterospecifics, *Greta morgane* and *Ithomia patilla* are skewed from the equal modality reliance found in non-mimetic butterflies on chemical versus visual cues, closer to 70:30 than 50:50 (Figs. 8 & 9).

However, this is not an absolute modality prioritization because when discriminating between members of different mimicry groups the distribution of misidentification fits with traditional equal modality importance found in non-mimetic Lepidoptera. In the case of *Greta morgane* approaching *Ithomia heraldica*, where the two participants are from different mimicry groups, there is no bias in cue use; mimetic butterflies discriminating between conspecifics and members of other mimicry groups perform like non-mimetic butterflies in cue reliance (Fig 10). Perhaps
initial discrimination of possible conspecifics is done visually and reliance on chemical cues is needed within co-mimetic species. Thus, a trade off between communication with predators and communication with conspecifics may explain the paradox of mimicry discussed by Mallet and Gilbert (1995) where, in theory, Mullerian mimics should converge on a common color pattern to aid predator learning but in reality numerous mimicry groups occur in nature.

Experiments conducted with plastic models lacking chemical cues provide evidence that color patterns are used within the aggregations as a first-pass method of identifying potential conspecifics. Additionally, in scent-only trials, butterflies visited conspecific pheromones more often than heterospecific pheromones. In combination with evidence of plasticity in modality use provided by the four-way choice test, it seems likely that initial visual cues may suppress or amplify responsiveness to chemical cues and possibly vice versa. Lepidopterans and other insects are known to reduce or increase responsiveness to odor cues in the presence of visual cues depending on the combination of stimuli (Balkenius & Dacke, 2013; Riffell & Alarcón, 2013).

Although ithomiines visited models with both the correct visual cue and the correct chemical cue in combination significantly most often, they sometimes visited the mismatched or heterospecific combination of stimuli. Costs of misidentification of conspecifics within aggregations are largely due to the energetic costs of courtship, hybridization and competitor misidentification (Arnott and Ellwood 2009). Since both visual and chemical conspecific cues are attractive to ithomiines, visitation to both mismatched cue pairs were observed although rates of interspecific
interactions in aggregations were quite low. Character shifts in one or more modality may reduce misidentification (Grether et al. 2009).

Comparing the multimodal tests to the unimodal tests, Ithomiines approach unimodal cues more indiscriminately than multimodal cues. Conspecific hair pencil approach rates are at 66% in contrast to 95% accuracy in the four-way choice tests when comparing simply the rate of approach of conspecific cues versus heterospecific cues. The more indiscriminate approach of unimodal chemical stimuli suggests that both visual and chemical cues serve as general attractors for aggregation formation or other behaviors, but without the additional information, the second cue accuracy of discrimination is reduced or eliminated. Relying on multimodal cues is more accurate while unimodal cues require less higher-level processing; modality prioritization may be an optimization strategy in terms of the speed versus accuracy trade off (Kulahci, Dornhaus, & Papaj, 2008).

Within aggregation sites, although the aggregations are multispecific, overall more free-flying individuals interacted with conspecifics than with heterospecifics. Intersexual interactions were more common than male-male or female-female interactions. The higher rate of courtship than male-male interactions indicates that perhaps intrasexual competition is low, or perhaps there is a trade-off between the presence of more males and higher chemical defense of the space. Interestingly, some species of clearwings were more likely to interact with mimetic-heterospecifics than conspecifics; but these species were found in low abundance and perhaps rare species are disproportionately rare in aggregations (see Fig 11). Interactions with co-mimetic heterospecifics were more common than interactions with hetero-mimetic
heterospecifics, either due to mistaken identities. Having other members of the same mimicry group present may be beneficial in terms of predator learning, but may also increase mate search costs (Moller and Pomiankowski 1993).

The neural basis of perception of conspecific and heterospecific visual and chemical cues in ithomiines is unknown; however, it seems likely that it is based on intermodal excitation and/or suppression in which one modality excites or suppresses responsiveness in another modality. Insects can perceive multimodal stimuli as fused or unfused stimuli (Balkenius and Dacke 2013). *Cataglyphis fortis*, desert ants, for example, can be trained to use multimodal olfactory and visual landmarks, but subsequently ignore the landmark when presented with only the visual or olfactory portion (Steck et al. 2011). Because ithomiines approach visual models and hair pencils alone, it seems that ithomiines see conspecifics and heterospecifics as unfused multimodal objects although it is unclear how generally true this is for non-mimetic and non-aggregation forming Lepidopterans (Tang 2004; Neri 2012).

Experiments on *Drosophila melanogaster* show that complex choices requiring binding of stimuli use the mushroom body for multimodal processing (Wu & Guo, 2011). *Heliconius* butterflies, a genus of butterflies that engages in non-display multispecies roosting, have been shown to have huge mushroom bodies, the part of the insect brain that process olfactory stimuli, possibly because of the learning required for sociality; large brains are generally found in social insects (Sivinski, 1989). However, evidence from within Hymenoptera suggests that the large mushroom bodies predate the evolution of sociality, although large mushroom bodies may have
subsequently enabled sociality to evolve (Farris, 2013). Perhaps a similar evolution of higher processing centers has occurred in ithomiines to accommodate their behavioral plasticity.

There remains much to learn about the evolution of complex signal processing within an ecological context in ithomiines and in insects generally. Mimetic systems are of interest as an ecologically relevant system in which one cue becomes less reliable or unusable in conspecific identification. The plasticity of processing of mimetic and non-mimetic heterospecifics in ithomiines provides a valuable example of discrimination and modality reliance in a mimetic system, but leaves the question of cue use and processing and mimicry across taxa open. Further studies of perception within mimetic insects and other organisms would help address whether flexible reliance on non-mimetic cues is a general neuroethological strategy or specific to Ithomiinae.

References


Beccaloni GW, Gaston KJ. 1995. Predicting the species richness of neotropical forest butterflies: Ithomiinae (Lepidoptera:Nymphalidae) as indicators. Biological Conservation 71:77–86.

mitochondrial and two nuclear gene regions. Systematic Entomology 31:288–301.


Chapter 3: Signaling strategy, community diversity, body size and mimetic fidelity in Ithomiine butterflies

Abstract

Mimicry, where one species resembles another due to selective benefits, is studied as a pinnacle of natural selection. Recently cases and causes of imperfect mimicry have become of interest in examining the constraints on natural selection. Some have proposed relaxed selection on small-bodied mimics and on diverse communities as possibly important causes of imperfect mimicry. I investigate the relationship between body size, community diversity and aposematic and cryptic signaling in a group of mimetic Neotropical butterflies, the ithomiines. I did not find a relationship between community diversity in color pattern or species diversity and mimetic fidelity. I found a negative relationship between body size and mimetic fidelity. I propose an additional hypothesis that aposematic signal is related to body size. There are few cases of nonaposematic Müllerian mimics, but clearwinged ithomiines are one such case. I present evidence for increased mimetic fidelity in clearwinged ithomiines in comparison to aposematic ithomiines.

Introduction

The similarity between species that are members of the same Müllerian mimicry group is impressive but imperfect. Kikuchi and Pfennig (2013)’s review of imperfect mimicry proposed a variety of causes for imperfect mimicry, the most supported being the relaxed selection hypothesis where models and mimics have equivalent fitness. Recent papers on imperfect mimicry have come to the separate, but not mutually exclusive, conclusions that larger body size and low community diversity favor mimetic fidelity and increasing resemblance between models and mimics or across a mimicry group (Penney et al. 2012; Wilson et al. 2012; Wilson et al.
Larger organisms are more desirable prey items and are therefore presumed to be under stronger selection pressure to be more mimetically faithful (Penney et al. 2012). Predators encountering a morphologically diverse prey communities ought to generalize less resulting in reduced selection for mimetic fidelity (Wilson et al. 2013). Therefore, I will test the hypotheses that more diverse communities have lower levels of mimetic fidelity and that mimetic fidelity increases with species body size in ithomiine butterflies (Penney et al. 2012; Wilson et al. 2013).

Müllerian mimics share a color pattern to more effectively demonstrate their ubiquitous toxicity to predators (Müller 1879). Although most Müllerian mimic groups consist of aposematic species that have converged on a shared pattern (Symula et al. 2001; Marke and Bond 2009), rare examples of nonaposematic mimics provide the opportunity to separate the effects of coloration from pattern based mimetic warning signals. For example, reduced predation on models of brown vipers with a zigzag pattern compared to those without, showed that inconspicuous mimicry can still be a deterrent (Wuster et al. 2004). However, inconspicuous mimicry might be under reduced or increased pressure relative to aposematic mimicry for increased mimetic fidelity since inconspicuousness will change both perception of prey item and its desirability as well as predator perception of color patterns. Therefore, I will test my hypothesis that nonaposematic mimics and aposematic mimics will have differences in mimetic fidelity, with aposematic mimics having lower levels of mimetic fidelity due to the defensive properties of aposematic signaling.

Study system
Ithomiines are Müllerian Neotropical butterflies, ranging from southern Mexico to Brazil and are the major taxonomic group in many Neotropical mimicry rings (Beccaloni 1997). They are unpalatable due to sequestration of alkaloids from host plants and secondary plants sources (Masters 1990; Pinheiro 1996). Ithomiines have six mimicry patterns, ranging from aposomatic opaque orange, black and yellow tiger patterns to non-aposmatic clear-winged butterflies. Although color pattern sharing is common among closely related species and genera, distantly related ithomiines have also evolved to share color patterns more than once in the taxonomic group (Haber 1978; DeVries 1987; Beccaloni 1997; Jiggins et al. 2006; Brower et al. 2014). Cryptic clearwing Ithomiines are a good example of inconspicuous mimicry; a recent paper showed that the clear scales reflect very little light at most angles of incidence making them cryptic despite their mimetic patterns (Siddique et al. 2015).

Methods

I used 39 human volunteers (college students majoring in biology or related disciplines participating in a field course through the Organization for Tropical Studies) to measure mimetic fidelity in the ithomiine species. None of the participants were minors by US standards and no data relating to the volunteers was gathered, so there was no requirement to anonymize. Human volunteers yield similar results to other methods of analysis and allows more species to be assessed (Penney et al. 2012; Wilson et al. 2013). The volunteers were given a short presentation on mimicry prior to the survey and presented slides showing an individual species compared to all of their mimicry ring in Costa Rica (DeVries 1987). Male dorsal images from Devries (1987) were used as representative of a species typical phenotype. Only one exemplar (e.g, see Fig 1) was used per species and each slide was presented for 20 seconds. Volunteers were directed to rank each butterfly on how well it fit into the mimicry group displayed. Rankings were based on
a scale of 1 (very poor mimic) to 10 (excellent mimic). Volunteers were instructed to complete surveys individually. All butterfly images were presented at magnifications such that they had the same projected body length except for the butterfly being ranked which was presented at a larger magnification. The results of this survey are mimetic fidelity scores based on overall resemblance. The mimetic fidelity of each species was the mean score across participants.

Figure 1. An example slide. Large butterfly on the left is the species being compared to the group on the right. Butterfly presented for comparison on the left is not included in the mimicry group on the right.

For measures of community diversity, data used for butterfly abundance and diversity were collected between July 2011 and August 2014. Butterflies were collected in Costa Rica at Las Cruces Biological Station (8° 47' 7'' N, 82° 57' 32'' W), Reserva Lionel Oveido (9°56'14.9''N 84°02'58.0''W), Parque del Este (9°56'33.1''N 84°00'39.3''W), Bosque de la Hoja (10°03'46.9''N 84°05'41.3''W) and La Universidad para La Paz (9°55'11.3''N 84°16'21.6''W). Butterflies were netted with an aerial net and either preserved or marked and released. Each butterfly’s species, gender and locality data were recorded. Morphological data (length, wing span and weight) were
also collected. Differences in coloration between male and female *Godyris zygia* were also measured using a spectrophotometer (Ocean Optics USB2000), xenon light source (PX2), and reflection probe (R400), in relation to a white standard (WS-1). Differences were calculated from a Principal Components Analysis (PCA) of 10 nanometer bins. Only components with eigenvalues greater than one were used in the analysis and, generally, only the first eigenvalues were greater than one. Following Devries (1987), four points were measured on the butterfly’s wing; forewing apical, forewing discal, hindwing apical and hindwing discal (Fig 2). Comparisons were made by Wilcoxon tests of the principal component scores for components with eigenvalues greater than one.

Figure 2. A diagram of butterfly wing morphology showing the locations measured: forewing apex, forewing discal cell, hindwing apex, hindwing discal cell. From DeVries (1987).

To compare patterns of mimetic fidelity and diversity mimicry groups in ithomiine butterflies, I measured community diversity, color pattern diversity and phylogenetic diversity in order to calculate several diversity indices. Indices were based on field collections data collected between 2011 and 2014 in Costa Rica. Data was then aggregated by location, time and grouped
into species, generic and tribe groups for analysis in statistical Primer (Primer-E Ltd, UK), a software for analyzing ecological and survey data. Primer analyses are non-parametric and are based on Bray-Curtis similarity measures and allows for the analysis of more complex multivariate experimental structures.

Community diversity indices are location-specific Shannon diversity indices as in Wilson et al. (2013), but are calculated from field collections as opposed to range data; I calculated Shannon diversity indices from the sum of the natural log of the proportion of individuals of each species found. This index is a measure of the phylogenetic diversity of the locality. Color pattern diversity indices are Shannon diversity indices; in this case, the sum of the natural log of the proportion of individuals of each color pattern found by locality. This is a measure of the diversity of the appearance of butterflies within each locality. Wilson et al. (2013) used the overlap of range data; however, I used field collections based abundances of species as a more precise measurement. The within-color pattern species diversity indices are Shannon diversity indices: the sum of the natural log of the proportion of individuals of each species found by color pattern. This is a measure of species diversity within the color pattern. Analyses were conducted using Stata, R, and Primer. Primer is used to compare diversity based on the Bray-Curtis similarity matrix which calculates similarity independent of joint exclusion. All Primer analyses were conducted using a 4\textsuperscript{th} root transformation.

Results

Overall, color patterns are significantly different in mimetic fidelity (Fig 2; Kruskal-Wallis, Amber species=11, Clearwing=9, Orange and Black=6, Small Dark Transparent=8, Tiger=4, df=4, \(p<0.000001\)). Community phylogenetic diversity strongly correlates with mimetic fidelity.
in ithomiine butterflies (Spearman rank correlation, $\rho=.85$, $n=6$, $p=0.03$). However, color pattern diversity does not correlate with mimetic fidelity (Fig 2; Spearman rank correlation, $\rho=0.6$, $n=5$, $p=0.208$). Rare species are not significantly different in mimetic fidelity from common species (Wilcoxon test, $z = -0.764$, rare species=24, common species=12, $p = 0.4451$).

Figure 3. Box plot of mimetic fidelity (y-axis) by mimicry group. SDT is Small Dark Transparent.
Figure 4. Plot of the sites by location and year on principal components of generic level diversity across 18 genera with markers for location.

Table 1. Genera used in primer analysis and sample sizes of individuals per genus.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callithomia</td>
<td>5</td>
</tr>
<tr>
<td>Ceratinia</td>
<td>8</td>
</tr>
<tr>
<td>Dirccenna</td>
<td>25</td>
</tr>
<tr>
<td>Episcada</td>
<td>40</td>
</tr>
<tr>
<td>Godyris</td>
<td>76</td>
</tr>
<tr>
<td>Greta</td>
<td>199</td>
</tr>
<tr>
<td>Heterosais</td>
<td>5</td>
</tr>
<tr>
<td>Hypoleria</td>
<td>5</td>
</tr>
<tr>
<td>Hyposcada</td>
<td>40</td>
</tr>
<tr>
<td>Ithomia</td>
<td>282</td>
</tr>
</tbody>
</table>
Differences are observed in species diversity between the regions surveyed. San Vito and the Central Valley are significantly different in species composition (Fig 4 and Table 1, PERMANOVA, df=5, \( p = 0.0165 \)). San Vito and the Central Valley (La Paz, Lionel Oveido and Parque del Este) differed significantly in mimetic fidelity with mean mimetic fidelity higher in the Central Valley (weighted K sample equality of medians test, \( n=930, p<0.001 \)). See supplementary information for further details of species abundances in the different regions. Separating butterflies by aposematism of color pattern into aposematic, warning colored ithomiine patterns (a combination of Orange and Black, Amber and Tiger) and cryptic, transparent ithomiine patterns (a combination of Clearwings and Small Dark Transparents), yields two groups of mimetic fidelity. Cryptic species are significantly more mimetically faithful than aposematic species (Fig 5, Wilcoxon, \( n=32, p<0.0001 \)). Average ratings of mimetic fidelity were not significantly different between rare species and common species (Paired Wilcoxon, \( n=39, p=0.4546 \)). Larger species of butterflies are significantly less mimetically faithful (Spearman rank correlation, \( r = -0.38, p=0.04, n=32 \)). Also, aposematically colored butterflies are less mimetically faithful, regardless of size (Fig 6, Wilcoxon, \( p<0.0001, n=32 \))
Figure 5. Mimetic fidelity by aposematism. Aposematic species are less mimetically faithful than cryptic species ($p<0.00001$).
Looking within one genus, *Ithomia* is inferred to have a clear ancestor, but has abundant congener species that are from the Small Dark Transparent and Amber color patterns (Fig 7). Overall, species of *Ithomia* are significantly different in mimetic fidelity from one another (Friedman, group n=39 observations per species, \( p<0.00001 \)). The Amber congeners, *Ithomia heraldica* and *Ithomia xenos* are not significantly different in mimetic fidelity (Paired Wilcoxon, \( n=39, p=0.8 \)). However, *Ithomia patilla*, clearwing, is a significantly better mimic than either *Ithomia heraldica* (Paired Wilcoxon, \( n=39, p=0.0004 \)) or *Ithomia xenos* (Paired Wilcoxon, \( n=39, p=0.0003 \)). *Ithomia patilla* is an equivalently good mimic as *Ithomia terra* (Paired Wilcoxon, \( n=39, p=0.2462 \)). *Ithomia terra*, a clearwing, is rated as equally mimetically faithful when rated in comparison to its whole mimicry group as when rated only with members with overlapping ranges (Paired Wilcoxon, \( n=39, p=0.2 \)).
Figure 7. Species level phylogeny for *Ithomia* based on CO-I and II and wingless (with Jonathan Chang, personal communication). Red branches lead to aposematic species while blue lead to cryptic species. Nodes are labeled with Bayesian posterior probabilities.
Figure 8. Component loadings from principle component analysis of the spectral data of male and female *Godyris zygia* hindwing region. HWDF: Hindwing Discal Female; HWDM: Hindwing Discal Male; PC 1: brightness; PC2: Chroma; PC3: Hue.

Analyses of spectral data comparing males and females found significant differences (Fig 8). In spectrophotometric data, the first principal component corresponds to brightness or an overall measurement of light intensity, the second to chroma or the amount of grey or white light mixed in and the third principal component corresponds to hue or the shape of the spectrum (Grill and Rush 2000).

The forewing apical coloration was similar between males and females (Wilcoxon test, n=16, PC1: z=0.22, p=0.82; PC2: z=0.33, p=0.74; PC3: z=-1.62, p=0.1). The forewing discal coloration was also similar between males and females (Wilcoxon, n=16, PC1: z=0.87, p=0.39; PC2: z=0.87 p=0.39; PC3: z=-0.11, p=0.91). The hindwing apical coloration did not differ between males and females (Wilcoxon, n=16, PC1: z=1.74, p=0.08; PC2: z=-0.11, p=0.91; PC3: z=-0.22, p=0.83). The hindwing apical discal coloration differed across wavelengths between
males and females in all principal components (Fig 8, Wilcoxon, n=16, PC1: z=-3.25, \( p=0.001 \); PC2: z=3.25, \( p=0.001 \); PC3: z=2.5, \( p=0.012 \)).

Discussion

The previous work of Penney et al. (2012) and Wilson et al. (2013) found that both community diversity and body size were important factors driving mimetic fidelity, as did I in ithomiines. However, in contrast to the findings of Wilson et al. (2013), I did not find a relationship between the diversity of the color patterns found in the community and mimetic fidelity although I did find a relationship between increasing phylogenetic diversity within a community and increasing mimetic fidelity. Wilson et al. (2013) had predicted a decrease in mimetic fidelity in diverse communities because predators may generalize more when exposed to multiple mimicry groups. While perhaps it is true that predators generalize across mimicry patterns, higher phylogenetic diversity may result in fine scale differences within color patterns causing a less precise mental image of a mimicry pattern as opposed to a less specific image of a toxic butterfly. Alternatively, predators may be deterred by less precise mimicry in areas of high diversity, especially if there are many non-toxic Batesian mimics from other taxonomic groups found in conjunction with the Müllerian mimicry ring.

I found that body size correlated with mimetic fidelity; however, I found the opposite pattern to that found by Penney et al. (2012) and Wilson et al. (2013) with larger species found to be less mimetically faithful in comparison to smaller species. Large ithomiines, however, differ from smaller ithomiines in signaling strategy as well as body size and mimetic fidelity; large ithomiine species are aposematic while small ithomiine species are cryptic. Cryptic ithomiine species overall have higher mimetic fidelity than aposematic ithomiines. This same pattern of smaller
size and higher mimetic fidelity being associated with cryptic, as opposed to aposematic patterns, holds within a genus, indicating that changes in size are related to the color pattern shift or vice versa. Looking only at the genus *Ithomia*, where the phylogeny is well resolved, the same pattern holds true with reduced mimetic fidelity and larger size in aposematic species and higher levels of mimetic fidelity and crypsis in smaller species.

It is not clear why these traits of crypsis and mimetic fidelity have become correlated. The constraints on the clearwing pattern of transparency for low visibility on pattern variability may cause higher mimetic fidelity as a corollary of the ontogeny of the wing pattern. Alternatively, aposematic signaling per se may reduce selection pressure on aposematic species to be highly mimetically faithful or crypsis may increase selection pressure on clearwing species to be more mimetically faithful. Predators could require further experience or do not generalize as well about the toxicity of cryptic mimetic patterns in contrast to aposematic patterns, since aposematism may trigger more innate aversion than cryptic mimicry. Since clearwings are also unpalatable it would be interesting to better understand the learning process for predators on the different signaling strategies since naivety of predators has been shown to influence feeding preferences on Lepidoptera (Coppinger 1970). Potentially, different proportions of naïve versus experienced predators or different levels of prey scarcity could create different selective pressures for aposematism versus crypsis. Additionally, different ratios and perceptual abilities of predators, such as vertebrates versus invertebrates, could create conditions favoring transparency or aposematism since different predators rely less on visual cues than chemical cues and have variable memory. Lifespan and environmental factors also impact the usability of chemical versus visual signals by predators in prey assessment.
Signals used in communication may function in a compensatory or discrete capacity. Increasing mimetic fidelity to the mimicry group may enable increases in aposematic signal size due to protection of mimicry despite increasing conspicuousness. Conversely, although the larger bodied mimics are more detectable they are also presenting a larger warning signal and potentially a stronger signal in other modalities, such as chemical signaling of defense. Therefore, aposematic mimics, having other defenses, might be freed from the constraints of mimicry (Guilford and Stamp Dawkins 1991; Mappes and Alatalo 2005; Rowe and Halpin 2013). Perhaps for a predator to learn a cryptic mimetic pattern requires a higher level of mimetic fidelity between co-mimics than an aposematic mimetic pattern since predators may more easily learn to generalize within and across aposematic patterns.

An interesting case under consideration, in terms of the multiple pressures at work on aposematism and crypsis and mimetic fidelity, is the case of sexual dimorphism. Although most ithomiines are not sexually dimorphic, several instances exist in the amber winged butterflies in which the male and the female are significantly different in coloration. One such instance is *Godyris zygia*, in which the males and females are equally good mimics of the amber winged pattern despite differing strikingly from one another in coloration (Figure 9). The male is more transparent and more yellow than orange and the female’s pattern is more opaque and orange with a contrast between the more yellow forewing and the orange and white hindwing. Other species showing a similar pattern are *G. zavaleta, Dirceenna dero, D. klugii, Pteronymia notilla*, and *Ithomia xenos*. In all cases, the female is more opaque and orange and less luminous than the male. Either natural selection or sexual selection may be the driving force in the evolution of sexually dimorphic aposematism in ithomiine butterflies. Under natural selection, females are advertising to predators in two channels: mimicry and aposematism. Sexual selection, however,
may drive a decrease in aposematism in males. In ithomiines, overall pyrrolizidine alkaloid content does not differ between sexes in these species, although male variance is higher (Trigo et al. 1996). Therefore, the sexes differ in aposematic signal despite, on average, not differing in toxicity. Since females have lower variance, and the lowest and highest levels of pyrrolizidine alkaloids were found in males, female aposematism is therefore a more honest and consistent signal. Since females are more conspicuous, aposematic signaling results in a relatively increased risk for faster moving males than females (Hatle and Faragher 1998; Hatle et al. 2002).

Figure 9. *Godyris zygia*. Female (left) and male (right)

In conclusion, I found additional evidence for a relationship between diversity and mimetic fidelity and for a relationship between body size and mimetic fidelity. Although the details and directions of the relationships found in ithomiines were not the same as those found in previously studied taxa, it seems to hold that prey desirability and diversity are important factors in driving mimetic fidelity. In addition, I found a difference in levels of aposematism between male and female *Godyris zygia*. It is still unclear what precisely has driven the sexual dimorphism in some species in this group, however. Perhaps it is similar to the processes still under debate which drive sex limited mimicry (Kikuchi and Pfennig 2013; Long et al. 2015).
Overall, while the origins of aposematic and non-aposematic mimicry in ithomiine butterflies remains mysterious, the system is a tantalizing example of the variety of selection pressures and natural history traits influencing the relative risk of predation within and between species. Future studies of predator learning and signal perception, as well as the evolution of the transparent pattern and the dimorphic patterns, would further elucidate the cost levels of aposematism in mimicry and the differences in cost and signaling capacity of these strategies. There is much variation in aposematic ithomiines in within-species levels of aposematism and size of signals and it would be and the function and consequences of levels of variation deserve further consideration.

References


alkaloids: different acquisition and use patterns in Apocynaceae and Solanaceae feeding ithomiine butterflies (Lepidoptera: Nymphalidae). Biological Journal of the Linnean Society 58:99–123.


Species Diversity Tables

Table S1 shows the species, abundances and relative frequencies of species I netted in surveys in San Vito in Southern Costa Rica. Table S2 shows a Shannon Diversity index for color patterns, where each color pattern is treated as a unit in which each color pattern contains all individuals from all species within a given mimicry group, from species from San Vito in the Southern Costa Rica. Table S3 shows the species, abundances and relative frequencies of species I netted in surveys in the Central Valley of Costa Rica. Table S4 shows a Shannon Diversity index for color patterns, where each color pattern is treated as a unit from species in the Central Valley of Costa Rica. The diversity of species in San Vito (Table S1) is slightly higher than in the Central Valley (Table S2) as is the diversity of color patterns (Table S3 and S4).

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callithomia hezia</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Callithomia hydra</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Ceratinia tutia</td>
<td>9</td>
<td>0.03</td>
</tr>
<tr>
<td>Dirccenna relata</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Godyris zygia</td>
<td>73</td>
<td>0.26</td>
</tr>
<tr>
<td>Greta andromica</td>
<td>11</td>
<td>0.04</td>
</tr>
<tr>
<td>Greta anette</td>
<td>6</td>
<td>0.02</td>
</tr>
<tr>
<td>Greta morgane</td>
<td>8</td>
<td>0.03</td>
</tr>
<tr>
<td>Greta nero</td>
<td>12</td>
<td>0.04</td>
</tr>
<tr>
<td>Greta polisenna</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Species</td>
<td>Count</td>
<td>Diversity</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>Heterosais edessa</td>
<td>3</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypoleria cassotis</td>
<td>5</td>
<td>0.02</td>
</tr>
<tr>
<td>Hyposcada virginiana</td>
<td>36</td>
<td>0.13</td>
</tr>
<tr>
<td>Hypothyris lycaste</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Ithomia heraldica</td>
<td>26</td>
<td>0.09</td>
</tr>
<tr>
<td>Ithomia patilla</td>
<td>15</td>
<td>0.05</td>
</tr>
<tr>
<td>Mechanitis lysimnia</td>
<td>7</td>
<td>0.03</td>
</tr>
<tr>
<td>Mechanitis menapis</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Napeogenes tolosa</td>
<td>3</td>
<td>0.01</td>
</tr>
<tr>
<td>Oleria paula</td>
<td>3</td>
<td>0.01</td>
</tr>
<tr>
<td>Oleria rubescens</td>
<td>14</td>
<td>0.05</td>
</tr>
<tr>
<td>Oleria vicina</td>
<td>17</td>
<td>0.06</td>
</tr>
<tr>
<td>Pseudoscada utillia</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>Pteronymia agalla</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Pteronymia artena</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Pteronymia fulvescens</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Pteronymia notilla</td>
<td>12</td>
<td>0.04</td>
</tr>
<tr>
<td>Tithorea tarricina</td>
<td>3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table S1. Species diversity in the Central Valley, Costa Rica (Shannon diversity index=2.61).
<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callithomia hezia</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Dircenna chiriquensis</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>Dircenna dero</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Dircenna klugii</td>
<td>17</td>
<td>0.03</td>
</tr>
<tr>
<td>Dircenna relata</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Episcada salvinia</td>
<td>40</td>
<td>0.06</td>
</tr>
<tr>
<td>Godyris zygia</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Greta andromica</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Greta anette</td>
<td>21</td>
<td>0.03</td>
</tr>
<tr>
<td>Greta morgane</td>
<td>122</td>
<td>0.18</td>
</tr>
<tr>
<td>Greta nero</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Hyposcada virginiana</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Hypothyris lycaste</td>
<td>9</td>
<td>0.01</td>
</tr>
<tr>
<td>Ithomia heraldica</td>
<td>94</td>
<td>0.14</td>
</tr>
<tr>
<td>Ithomia patilla</td>
<td>122</td>
<td>0.18</td>
</tr>
<tr>
<td>Ithomia xenos</td>
<td>26</td>
<td>0.04</td>
</tr>
<tr>
<td>Mechanitis lysimnia</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td>Mechanitis menapis</td>
<td>3</td>
<td>0.00</td>
</tr>
<tr>
<td>Mechanitis polymnia</td>
<td>13</td>
<td>0.02</td>
</tr>
<tr>
<td>Napecogens cranto</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>Napecogens tolosa</td>
<td>10</td>
<td>0.02</td>
</tr>
<tr>
<td>Oleria paula</td>
<td>4</td>
<td>0.01</td>
</tr>
<tr>
<td>Oleria rubescens</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>Oleria vicina</td>
<td>42</td>
<td>0.06</td>
</tr>
<tr>
<td>Pteronymia agalla</td>
<td>37</td>
<td>0.06</td>
</tr>
<tr>
<td>Pteronymia donata</td>
<td>12</td>
<td>0.02</td>
</tr>
<tr>
<td>Pteronymia notilla</td>
<td>14</td>
<td>0.02</td>
</tr>
<tr>
<td>Pteronymia simplex</td>
<td>41</td>
<td>0.06</td>
</tr>
<tr>
<td>Pteronymia lonera</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Thyridia psidii</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>Tithorea harmonia</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>Tithorea tarricina</td>
<td>2</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table S2. Species diversity in the Central Valley, Costa Rica (Shannon diversity index=2.61).

<table>
<thead>
<tr>
<th>Color pattern</th>
<th>Number</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color pattern</td>
<td>Number</td>
<td>Relative Abundance</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Amber</td>
<td>122</td>
<td>0.44</td>
</tr>
<tr>
<td>Clearwing</td>
<td>39</td>
<td>0.14</td>
</tr>
<tr>
<td>Orange and Black</td>
<td>41</td>
<td>0.15</td>
</tr>
<tr>
<td>Small Dark</td>
<td>61</td>
<td>0.22</td>
</tr>
<tr>
<td>Tiger</td>
<td>15</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table S3. Color pattern diversity, San Vito, Costa Rica (Shannon diversity index=1.41)

<table>
<thead>
<tr>
<th>Color pattern</th>
<th>Number</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amber</td>
<td>76</td>
<td>0.27</td>
</tr>
<tr>
<td>Clearwing</td>
<td>40</td>
<td>0.14</td>
</tr>
<tr>
<td>Orange and Black</td>
<td>9</td>
<td>0.03</td>
</tr>
<tr>
<td>Small Dark</td>
<td>145</td>
<td>0.52</td>
</tr>
<tr>
<td>Tiger</td>
<td>7</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table S4. Color pattern diversity in the Central Valley, Costa Rica (Shannon diversity index=1.18).