A Batesian mimic and its model share color production mechanisms

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Abstract  Batesian mimics are harmless prey species that resemble dangerous ones (models), and thus receive protection from predators. How such adaptive resemblances evolve is a classical problem in evolutionary biology. Mimicry is typically thought to be difficult to evolve, especially if the model and mimic produce the convergent phenotype through different proximate mechanisms. However, mimicry may evolve more readily if mimic and model share similar pathways for producing the convergent phenotype. In such cases, these pathways can be co-opted in ancestral mimic populations to produce high-fidelity mimicry without the need for major evolutionary innovations. Here, we show that a Batesian mimic, the scarlet kingsnake Lampropeltis elapsoides, produces its coloration using the same physiological mechanisms as does its model, the eastern coral snake Micrurus fulvius. Therefore, precise color mimicry may have been able to evolve easily in this system. Generally, we know relatively little about the proximate mechanisms underlying mimicry [Current Zoology 58 (4): 658–667, 2012].

Keywords  Adaptation, Coloration, Convergent evolution, Pteridine, Pigment

Batesian mimicry, where a harmless organism resembles a dangerous one and thus receives protection from predators, has long served as an exemplar of how natural selection can create complex adaptations (Bates, 1862; reviewed in Ruxton et al., 2004; Forbes, 2009). Mimicry also illustrates how two different taxa can converge on the same phenotype. This process can entail many difficulties, especially if mimics arise from cryptic species and must cross a selective valley between crypsis and mimicry, during which period they receive neither the benefits of crypsis nor mimicry (Leimar et al., in press). Even once mimicry evolves, it may retain imperfections (Sherratt, 2002; Ruxton et al., 2004). Both the difficulty of initially evolving mimicry, as well as its degree of fidelity to its model, depend ultimately on the underlying similarity between the proximate mechanisms that models and mimics use to produce their phenotypes. By this, we mean the underlying genetic architecture, developmental processes, and metabolic pathways used to produce the convergent phenotypes of models and mimics (Arendt and Reznick, 2007; Manseau et al., 2010).

When models and mimics share mechanisms of phenotype production, new mimics may evolve easily. In such cases, just a few mutations may be needed for the mimic to match the phenotype of its model, and low-fitness intermediate phenotypes may be bypassed (Leimar et al., in press). Moreover, in such a situation, high fidelity mimicry becomes more likely. By contrast, when models and mimics do not share mechanisms of phenotype production, high fidelity mimicry may be more difficult to evolve. The use of different proximate mechanisms by mimic and model may be especially likely to occur in populations in which intermediate phenotypes are not selected against (e.g. Kikuchi and Pfennig, 2010a) or in which perfect mimicry is not favored (e.g. Dittrich et al., 1992; Sherratt, 2002).

Little is known about how models and mimics produce their phenotypes. Most of what we do know comes from work on a Müllerian mimicry complex involving butterflies in the genus Heliconius. Recently, cis-regulatory elements in a single gene were shown to control covariation between the red elements of patterns in H. erato and H. melpomene, whose variously colored subspecies mimic each other throughout Latin America (Reed et al., 2011). However, these species are very closely related, and it is thus not necessarily surprising that co-mimic pairs use essentially the same mechanisms to produce their patterns.

The coevolution between Batesian mimics and their models may be much more fruitful for understanding adaptation in general, because in Batesian mimicry complexes, models and mimics tend to be more distantly related. Batesian models and mimics also have
intrinsic differences defined by their roles as defended
models and undefended mimics, so any mechanism
common to the production of secondary defenses and
warning signals may further decrease the likelihood of
closely shared proximate means of signal production.
For example, if the larvae of a model butterfly species
feeds on a plant that provides it with both a toxin and a
pigment, while its mimic feeds on a plant that provides
neither, the mimic might have to obtain an alternative
dietary pigment or manufacture its own in order to de-
ceive potential predators. The possibility that the envi-
ronment may play a role in generating the mimetic
phenotype further highlights the critical importance of
understanding how models and mimics produce their
phenotypes. Some Batesian mimics of the butterfly
clades Papilio and Dismorphiinae use different pig-
ments to produce their red coloration than do their mod-
els (Ford, 1953), so they use different mechanisms at
least for this aspect of phenotype. Moreover, some of
those pigments are environmentally derived.
Here, we report an analysis of the pigments used to
produce warning signals in a Batesian mimicry complex.
The defended model is the venomous eastern coral
snake, Micrurus fulvius, which is mimicked by the
harmless scarlet kingsnake, Lampropeltis elapsoides.
Both species are characterized by having bright red,
yellow, and black rings encircling their body (Fig. 1).
We therefore sought to determine whether or not the
same pigments were used in each of these color ele-
ments. Additionally, we explored the cellular structures
associated with coloration in the skin of models and
mimics. Much future work remains to be done to eluci-
date genetic and developmental aspects of color produc-
tion in this system, but this study represents a first step
in understanding the mechanistic basis of mimicry in a
well-studied Batesian mimicry complex, and it is also
the first detailed study of the mechanisms underlying
coloration in snakes.

![Fig. 1](image)

**Fig. 1** (A) The venomous eastern coral snake, *Micrurus fulvius*, is mimicked by (B) the nonvenomous scarlet kingsnake *Lampropeltis elapsoides*. Both snakes have brightly colored rings of red, yellow, and black, which deter predators. Photos by W. Van Devender and D. Kikuchi.

## 1 Materials and Methods

### 1.1 Reptile coloration

Our study system is attractive for asking whether or
not coloration has evolved using the same mechanisms
in models and mimics for at least three reasons. First,
scarlet kingsnakes are attacked by a wide array of both
mammalian and avian predators (Pfennig et al., 2007;
Kikuchi and Pfennig, 2010a). Therefore, mimics should
be under selection to be good color mimics of their
models due to the wide variety of photoreceptors that
their guild of predators possess. Second, the scarlet
kingsnake and eastern coral snake are separated by a
greater genetic distance than the Müllerian mimics in
the butterfly genus *Heliconius*, whose coloration is bet-
ter studied. The Kimura two-parameter genetic distance
for cytochrome oxidase unit 1 between the eastern coral
snake and *Elaphe carinata* (a colubrine snake in the
same clade as the scarlet kingsnake) is 0.2404, whereas
this same comparison made between *H. pachinus* and *H.
hewitsoni* returns a genetic distance of only 0.1013
(GenBank accession numbers GU045453.1, JF700159.1,
AY748076.1, and GQ398195.1). A third advantage to
our system is that a variety of different pigments and
cellular elements have been found to color the skin of
lower vertebrates. It is this last point on which we focus
here.

In fish, amphibians, and reptiles, color patterns are
composed of dermal cells called xanthophores, irido-
phores, and melanophores, collectively known as chro-
iridescence and structural coloration in reptiles (Gosner, 1989; Morrison et al., 1995; Kuriyama et al., 2006). Melanophores contain the dark, endogenous tyrosine pigment melanin. Xanthophores (also called erythropores when they hold red pigments) can sequester both pteridine and carotenoid pigments (Macedonia et al., 2000; Steffen and McGraw, 2009).

Pteridine and carotenoid pigments represent two different possible mechanisms of color production. Pteridines are metabolically derived from guanine triphosphate (Kim et al., 2006; Kim et al., 2009) and are found in taxa as diverse as insects, fish, amphibians, and reptiles (Watt, 1967; Fukushima, 1970; Silva and Mensu, 1988; Pfleiderer, 1992). In contrast, carotenoid pigments are environmentally derived and their concentration can vary greatly from individual to individual depending on diet (Olson and Owens, 1998). Both pteridines and carotenoids may contribute to coloration in the same tissue type (Macedonia et al., 2000; Grether et al., 2001; Steffen and McGraw, 2009), and the color achieved sometimes depends on their relative concentrations (Grether et al., 2005). Alternatively, color can be produced by just one type of pigment in isolation (Macedonia et al., 2000). Many different pteridines can be produced in the pteridine biosynthetic pathway, and although not all function in pigmentation, those that do range from red to ultraviolet in their hues. The type of carotenoids present depends on diet, but they typically have a red to yellow color.

Iridophores, which are found in a layer of cells below the xanthophores, can also contribute to coloration. When iridophores contain guanine crystals in parallel layers of uniform thickness, they can give rise to coloration by reflecting only the wavelength of light that corresponds to the thickness of the layers, referred to as thin-layer interference (Morrison et al., 1995). When iridophores contain less organized groups of small crystals, they reflect short wavelength light more than long wavelength light through a process known as Tyndall scattering. Regardless of whether crystals are organized to cause coloration though Tyndall scattering or thin-layer interference, iridophores typically contribute to reflectance in the ultraviolet, blue, and green areas of the spectrum (Gosner, 1989; Morrison et al., 1995; Kuriyama et al., 2006). In combination with melanophores and xanthophores that absorb other wavelengths, iridophores can contribute to colors produced by the reflection of narrow bands of light, such as green (Nielson and Dyck, 1978; Gosner, 1989).

Melanophores in lower vertebrates have to date only been shown to contain the blackish pigment eumelanin, which is found in animal tissues ranging from squid ink to mouse hair (Ito and Wakamatsu, 2003). However, melanin pigments also include the reddish-yellow pheomelansins, which are found in mammals and birds (Ito and Wakamatsu, 2003). In lower vertebrates, melanins have been poorly explored, and it is possible that the absence of pheomelansins from all taxa but birds and mammals is simply a product of incomplete sampling. Reptiles use eumelanin to darken skin because it absorbs relatively uniformly across the ultraviolet and visible spectrum (Shawkey et al., 2009), but the use of pheomelansins by some taxa to produce red or yellow hues remains an open possibility, and represents an alternative pathway by which taxa might converge in color production.

1.2 Histology of the eastern coral snake and scarlet kingsnake

To determine the nature and organization of chromatophores in each color of the model and its mimic, we used transmission electron microscopy (TEM). TEM can also be used to distinguish eumelanin from pheomelanin. Pheomelanin tends to be organized into more disorganized and diffuse granules than eumelanin (Brumbaugh, 1968). We obtained specimens of coral snakes from Florida that had been found recently after death (due to road kill) and immediately frozen. We also collected three scarlet kingsnakes (two from Florida and one from North Carolina) and sacrificed them by first anesthetizing them with chloroform and then severing the cervical vertebrae. Skin samples of each color were immediately collected from the sacrificed animals and fixed with 2.5% glutaradehyde. Samples were then sent to the University of North Carolina Microscopy Services Laboratory for further preparation.

We sampled color patches from one coral snake (the best-preserved specimen) and the three scarlet kingsnakes using TEM. In the coral snake, there are occasionally black speckles in the red rings. These black speckles were excluded from our samples of the red tissue. There was also some slight fringing of black and red on the scales of the yellow rings in some scarlet kingsnakes. When this occurred, it generally affected all the yellow scales on the snake and therefore we could not avoid including these color elements in our samples.

1.3 Absorbance spectra of skin extracts

To isolate pteridine and carotenoid pigments from skin samples of each color from each snake, we finely
diced them and then placed them in uniquely labeled microcentrifuge tubes. For each snake, we used approximately equal quantities of black and yellow skin because initial trials indicated that yellow skin contained unidentified pigments, and black skin served as a useful control for skin with the absence of soluble pigments. We added 1 mL 1 N NH₄OH to each tube and homogenized the tissue using a laboratory homogenizer (Steffens and McGraw, 2009). This extracted pteridine pigments, which are well extracted by basic aqueous solutions. We then added 0.5 mL 1:1 hexanes: tert-butyl-methyl ether (TBME) to each tube and vortexed for 30 seconds. This organic extraction was designed to remove any organic-soluble pigments such as carotenoids (McGraw et al., 2005). Samples were then centrifuged for 5 minutes at 8000 rpm. The organic and aqueous fractions were separated from each sample. Their absorbencies were measured from 200–800 nm (Steffens and McGraw, 2009). This extracted pteridine pigments that might be present in our aqueous extracts. Pteridines can be identified using thin-layer chromatography (TLC). In TLC, the components of a solution that has been absorbed onto a solid medium are separated into different spots on the medium when a solvent moves across it. Chromatography of pteridines is aided by ultraviolet light because it causes fluorescence in spots that would otherwise not have any optical activity. Spots can be identified by color and by their Rf values, which indicate how far they have travelled on the solid medium.

For each sample of skin, we spotted 10 µL of aqueous solution onto a corner of cellulose chromatography paper. We developed these chromatograms in two solvents running at right angles to each other. The first solvent was 1:1 propanol:2% ammonium acetate. After allowing each chromatogram to dry, we then developed them in 3% NH₄Cl (Wilson and Jacobson, 1977; Ferre et al., 1986). Once each chromatogram had been developed, we examined it under 365 nm light, which causes each type of pteridine to fluoresce a particular color. In this way, we were able to determine the locations of each pteridine spot, including those that typically have no optical activity. We compared the Rf values and fluorescent colors of the spots isolated in each sample to those of a standard extracted from Drosophila eyes (strain Oregon R-P2), whose pigment identities are known (Wilson and Jacobson, 1977; Ferre et al., 1986). We also ran conformational tests using standards of isoxanthopterin (isolated from the bodies of male Drosophila), sepiapterin (Sigma-Aldrich), xanthopterin, 7,8-dihydrobiopterin, and 2-amino-4-hydroxy-1H-pteridine (Fisher). In addition, when there was doubt about the identity of a spot, we repeated both the extractions and the chromatography using various solvents described in the literature (Ephrussi and Herold, 1944; Grether et al., 2001).

2 Results
2.1 Cross-sections of skin magnified under TEM
The images obtained by exploration of prepared TEM specimens indicated that all three types of chromatophores are present in the skin of both models and mimics (Fig. 2). In the scarlet kingsnake, red tissue contained xanthophores. Although most xanthophores were present near the epidermis, some specimens had xanthophores sparsely distributed deeper in the dermis. One specimen also had sporadic epidermal melanophores in the red tissue. Black tissue contained only melanophores, which were mostly large and found deep in the dermis, although some specimens exhibited some small epidermal melanophores. Yellow tissue consisted of an upper layer of sparsely distributed xanthophores.

We also observed a few epidermal melanophores close to the epidermis in the yellow tissue (Fig. 3). The scales that form yellow and red tissue can be tinged with black (especially, along their rear edges of these color rings); thus, the trace epidermal melanophores may relate to such spatial variability in scale coloration. Beneath the xanthophore layer in the yellow tissue, we found a much more extensive layer of disorganized guanine crystals in the iridophores. The crystals were of variable size and orientation. Notably, the yellow and red tissue did not appear to contain pheomelanin-bearing melanocytes.

We found that the xanthophores in the red tissue of the coral snake appeared to be located much deeper in the skin than in the red tissue of the scarlet kingsnake, due to the thicker epidermis of the coral snake (Fig. 2). The black skin of the coral snake contained both epidermal and dermal melanophores. The yellow coral snake skin contained iridophores deep in the dermis that appeared to be overlaid with xanthophores. Unfortunately, because our coral snake specimen was poorly preserved, its iridophore layer was a disorganized network of rounded holes, and its xanthophores did not contrast with surrounding tissue as well as they did in the scarlet kingsnake.
Fig. 2  These TEM micrographs of cross-sectioned snake skin show that in red skin of both (A) the eastern coral snake and (B) the scarlet kingsnake, xanthophores (marked with x) lie close to the epidermis (marked with e) Black skin of the (C) coral snake and (D) kingsnake contains a layer of large, dark melanophores (marked with m) that reside deeper in the dermis than the xanthophores found in red skin. It also contains smaller epidermal melanophores (marked with em). Yellow skin of the (E) coral snake and (F) kingsnake shows a layer of xanthophores above a layer of iridophores (marked with i), which contain guanine crystals of irregular size, shape, and orientation. Panels A and B are 2500x; Panels C, D, E, and F are 5000x.

2.2  Absorbance of organic and aqueous skin extracts

We used absorbance spectroscopy to determine the possible presence of carotenoids in the organic fractions of our skin extracts. None of the organic fractions that we examined from either the coral snake or the scarlet kingsnakes had such peaks. Therefore, we conclude that no appreciable quantity of carotenoids was present in the skin of either the model or the mimic.

The aqueous fractions of our extractions produced nearly identical results between the coral snake and kingsnake for red, black and yellow tissue (Fig. 4). Red tissue showed a strong, broad peak between 490–500 nm, which coincides with the absorbance maximum of drosopterin pigments. This is consistent with its red coloration. It also showed strong absorbance that increased into the ultraviolet, indicating the presence of other pigments, probably pteridines. Black tissue had nearly uniformly low absorbance across the visible spectrum that increased slightly towards the ultraviolet, which is consistent with most of its absorbance activity coming from melanin that would have been excluded from the aqueous extraction. Yellow tissue showed a minor shoulder beginning at 500 nm as its absorbance
This cross-section of yellow skin from the scarlet kingsnake reveals an epidermal melanophore (marked with em) that lies above the xan-thophores (x) and iridophores (i), which allows it to absorb light of all wavelengths before it reaches the reflective iridophores. It may represent some of the dark fringing visible on the edges of yellow scales. Its grains are far finer than those in dermal melanophores. Scale bar in upper left = 2 µm.

Increased towards the ultraviolet, which may reflect trace drosopterins from red fringing of the scales. As with the red tissue, yellow tissue absorbed very strongly at wavelengths < 400 nm. The two skin colors appear to share a pigment that has an absorbance peak around 340 nm in our strongly basic extraction, and which does not appear to be a pteridine produced in quantity by Drosophila (Fig. 5), although there is a slight increase in absorbance at that wavelength in the extraction of male Drosophila bodies, which contain mainly isoxanthopterin.

2.3 TLC chromatography

The spots on chromatograms of red, yellow, and black-colored tissue in coral snakes and kingsnakes showed an exact correspondence of color and Rf values. In red skin, four drosopterins (neodrosopterin, drosopterin, isodrosopterin, and aurodrosopterin) produced visible red, orange, and yellow fluorescent spots of great intensity. These pigments are responsible for the red coloration of Drosophila eyes, and the Rf values of spots from red skin extracts corresponded well to that of our Drosophila standard. Additionally, two broad spots—one violet and the other blue—were also present. These spots did not correspond well to those on our Drosophila standard in Rf values, nor did they appear to match our other pteridine standards. In the yellow tissue, only trace drosopterins were present, but two unidentified spots (also present in the red tissue) gave an intense fluorescence. The mean Rf values of the violet fluorescent spot was 0.4 in 1:1 isopropanol:2% ammonium acetate, and 0.34 in 3% NH₄Cl. For the blue spot, they were 0.35 and 0.55, respectively. For both spots, the Rf values in 1:1 isopropanol:2% ammonium acetate were more variable than in 3% NH₄Cl. The closest Rf values of a violet spot in Drosophila were for isoxanthopterin, which had values of 0.3 and 0.35 in the first and second phases, respectively.

Neither sepiapterin nor xanthopterin, which are both yellow in color, was present in any of the samples we examined. The unidentified pigments in the yellow tissue exhibited slight absorbance in the visible range (Fig. 5). The black tissue did not show significant amounts of pigmentation.
Fig. 5  Absorbance of yellow and red skin from the scarlet kingsnake in the ultraviolet, showing identical peaks near 340 nm, followed by very strong absorbance in the mid ultraviolet

Solid line = extract of male Drosophila bodies, which contain isoaxanthopterin; dashed line = red skin; dotted line = yellow skin; dotted-dashed line represents Drosophila head extract.

3 Discussion

We studied the physiological mechanisms by which a Batesian mimic and its model produced their distinctive phenotypes. We found that coloration in the eastern coral snake and its mimic, the scarlet kingsnake, is produced via the same pigments. Specifically, red skin is colored mainly by drosopterin pigments that are sequestered in xanthophores. Black coloration is produced by eumelanin, which is contained within melanosomes in the dermis and epidermis. Yellow coloration is the product of two unidentified pteridines in a layer of xanthophores, and also a disorganized assemblage of guanine crystals beneath the xanthophores. Moreover, on the basis of data obtained from TLC and spectrophotometry, we have established that in all color patches the scarlet kingsnake and coral snake employ the same pigments, even in the yellow tissue where we were unable to identify the specific pigments. We found no evidence of environmentally derived pigments in either snake. Although there are minor differences between the two species in ultrastructure, the scarlet kingsnake’s use of the same pigments for color production as its model may have facilitated the evolution of mimicry in this system. Such similarity in underlying mechanisms may also permit very precise color mimicry, which may be advantageous in this system, considering the diverse predator guild responsible for exerting selection on the mimic.

To understand the significance of such closely shared mechanisms of color production between a mimic and its model, we must place the mechanisms used in our system within the context of coloring mechanisms available to animals in general. There are a number of deeply conserved metabolic pathways associated with red and yellow coloration, including carotenoids, pteridines, and pheomelanins (McGraw et al., 2005). Within these biochemically complex metabolic pathways, a variety of pigments with different optical properties can be produced. Thus, even if the same metabolic pathway is involved in coloring two species, it does not necessarily mean that it will yield the same end products. Moreover, even if the same end products are produced, they may not be incorporated into tissues the same way. Very different colors can be conferred upon tissues when pigment deposition varies on the level of ultrastructure (Hoekstra, 2006; Shawkey et al., 2009). Therefore, even if two species manufacture the same pigments in the same pathways, we should not necessarily anticipate that histological examination will reveal much similarity in their tissues.

The eastern coral snake and scarlet kingsnake not only both use pteridines to color their tissues, they use the same ones among many such pigments that can be synthesized. At least two red pigments (erythropterin and drosopterins) and two yellow pigments (xanthopterin and sepiapterin) can be produced. The model and mimic both use drosopterins in red skin and the same two unidentified pteridines in yellow skin. We suggest that the unidentified violet spot revealed by TCL of red and yellow skin extracts may be isoxanthopterin, due to its UV absorbance peak at 340 nm (Albert, 1953). To produce yellow coloration, the absorbance of isoxanthopterin may be shifted towards the visible range under physiological conditions, such as lower pH or the binding of the pigment to other elements (Wijnen et al., 2007). Finally, the model and mimic also show similarity in the histology of their chromatophores. Thus, there is similarity on multiple organizational levels.

It is also helpful to understand color production in a comparative context. Because the present study was the first to identify pigments in the skin of snakes and associate them with chromatophore structure, fine-scale inference is limited. The only other study to explore the histology of snake skin was that of Gosner (1989), who used samples of Bothrops vipers from museum specimens which had long lost their colors. Still, his microscopy revealed that specimens that had been green in life had a layer of xanthophores near the surface, followed by a layer of disorganized guanine crystals in the irido-
phores, and underneath both of those a layer of melanophores. The arrangement of chromatophores found in the green Bothrops by Gosner (1989) is described by a model for color production proposed by Nielsen and Dyck (1978): xanthophores remove violet and blue light from the spectrum, iridophores reflect green light through Tyndall scattering, and melanophores remove any red and yellow light that would otherwise be reflected by the white collagen lying beneath them. As a result, only green is reflected. Yellow can be produced by removing the melanophores, allowing the red and yellow light to be reflected with the green light (Nielsen and Dyck, 1978). This model for the production of yellow coloration corresponds to our histological analysis of the coral snake and scarlet kingsnake as well as to a yellow Bothrops examined by Gosner (1989).

Given the similarity between histology of the snakes studied here and the only other snake studied to date, it may not seem surprising that the coral snake and its mimic share such a close resemblance. However, the arrangement of chromatophores in snakes and lizards can be quite diverse. In terms of histology, lizards have more organized iridophores arranged into discrete layers of guanine crystals (Taylor and Hadley, 1970; Kuriyama et al., 2006). This organization of iridophores may reflect light using thin-layer interference rather than Tyndall scattering, providing a tighter band of reflectance (Morrison, 1995). Additionally, in yellow or white skin, both Sceloporus and Plestiodon lizards have melanophores present under the iridophore layer, albeit fewer than in brown skin (Morrison et al., 1995; Kuriyama et al., 2006). Green skin in Plestiodon contains a mixed upper layer of iridophores and xanthophores, either of which may be closest to the epidermis (Kuriyama et al., 2006), which contrasts with the mechanism of production of green in Bothrops and Anolis (Gosner, 1989; Taylor and Hadley, 1970). As reported by Kuriyama et al. (2006) in Plestiodon, we found some epidermal melanophores in yellow and black skin, but there are striking differences between the histology of snake skin observed here and those of other snakes and lizards thus far studied.

The pteridine and carotenoid pigments deposited in xanthophores can also vary widely among taxa. Within Anolis, those from Jamaica lack xanthopterin entirely (Macedonia et al., 2000), whereas about two-thirds of species from Puerto Rico possess xanthopterin (Ortiz and Maldonado, 1966). All possess isoxanthopterin (Macedonia et al., 2000). There is also widespread interspecific variation in the production of optically active pteridines and in the sequestration of carotenoid pigments (Ortiz et al., 1963; Ortiz and Maldonado, 1966; Macedonia et al., 2000). In Sceloporus undulatus, skin on yellow chins contains xanthopterin and the yellow vitamin riboflavin, orange chins contain drosopterins, and both types of skin contain isoxanthopterin (Morrison et al., 1995). Taken as a whole, the panoply of pigments and variety of chromatophore arrangements found throughout the reptiles underscores the close concordance between the color production mechanisms of the eastern coral snake and the scarlet kingsnake. However, the production of yellow coloration in the distantly related related viper Bothrops is also very similar (Gosner, 1989), so our results may speak more to a conserved system of coloring mechanisms used by snakes in general rather than a particularly tight match between model and mimic.

In other words, it is unclear if the similarity between the mimic and its model in mechanisms of color production reflects convergence (by the mimic on the model), or if it reflects homology (e.g., all snakes may share the same mechanisms of color production). Future studies will be needed to clarify this matter. Specifically, it remains to be seen if a wider taxonomic sampling of color production in mechanisms in snakes reveals as much diversity as has been found in lizards.

The lack of environmentally derived pigments (e.g., carotenoids) in the skin of either the mimic or its model suggests that diet-mediated phenotypic plasticity may not have played a direct role in the evolution of mimetic coloration. This finding was somewhat surprising, because not only has the scarlet kingsnake evolved mimicry, it has also converged on its model in diet. In particular, both the scarlet kingsnake and the eastern coral snake eat primarily ectothermic prey such as Plestiodon skinks and other small snakes (Bartlett and Bartlett, 2003). An ancestral character state reconstruction of the snake clade Lampropeltini, in which the scarlet kingsnake is found, indicated that it probably arose from a larger snake that consumed a diet richer in endothermic (e.g., mammalian) prey (Pyron and Burbrink, 2009). Despite this dietary convergence, the absence of environmentally derived pigments reduces the likelihood that mimicry arose as a plastic response to diet. However, it remains possible that an environmental cue, perhaps one derived from a snake’s diet, could nonetheless play a role in the induction of the mimetic phenotype.

The use of exactly the same endogenous pigments in producing coloration could suggest that mimics have responded to selection for precisely matching their
models in that aspect of phenotype. However, the actual colors of these two species can vary, owing to different concentrations of pigments and spatial irregularities in the distribution of color. For example, larger scarlet kingsnakes tend to have deeper yellow coloration than smaller ones, and the red rings of coral snakes are often speckled with black (Bartlett and Bartlett, 2003). Ideally, one should quantify the reflectance spectra of skin samples from live snakes to compare the coloration of models and mimics objectively. It remains an open question as to how precisely the reflectance spectra of coral snakes and scarlet kingsnakes match and how strongly selection favors such a resemblance.

Much is known about how natural selection acts on color pattern in this system. Specifically, the relative width of black and red rings on the bodies of the snakes is a target of selection, and this finding is emphasized by stronger selection where coral snakes are rare relative to where they are common (Harper and Pfennig, 2007). We would expect to find a similar trend in coloration Nevertheless, the order in which rings are arranged (eastern coral snakes and scarlet kingsnakes always differ in the order of their rings) is not under selection by predators (Kikuchi and Pfennig, 2010b). We might therefore anticipate that genes controlling the arrangement of colored rings will differ between the model and the mimic. Although there should be strong selection on genes controlling the width of the rings, these genes may not necessarily be the same in the two snakes. At present, genes controlling patterns involving pteridine pigments are poorly known (Hubbard et al., 2010).

In sum, our results reveal that a Batesian mimic, the scarlet kingsnake Lampropeltis elapsoides, produces its distinctive coloration using the same physiological mechanisms as its model, the eastern coral snake Micrurus fulvius. Precise color mimicry may therefore have evolved relatively easily in this mimicry complex. However, we know relatively little about the genetic mechanisms underlying mimicry in this system. Future studies are needed to resolve whether the same genes regulate color production in the mimic as in the model, whether these genes involve substitutions in cis-regulatory regions or in coding sequence, and (perhaps most importantly) whether the observed similarity in color production mechanisms reflects homology or convergence (see above). These are some of the issues that pigment research can address (Protas and Patel, 2008), and doing so in a Batesian mimicry complex may be particularly informative.

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References
Albert A, Wood HCS, 1953. Pteridine synthesis. II. Isoxanthopterin. J. Appl. Chem. 3: 521–523.
Arendt J, Reznick D, 2008. Convergence and parallelism reconsidered: What have we learned about the genetics of adaptation? Trends Ecol. Evol. 23: 26–32.
Bartlett RD, Bartlett PP, 2003. Florida's Snakes: A Guide to Their Identification and Habits. Gainesville: University Press of Florida.
Bates HW, 1862. Contributions to an insect fauna of the Amazon valley (Lepidoptera: Heliconiidae). Trans. Linn. Soc. Lond. 23:495–556.
Britton G, 1985. General carotenoid methods. Methods Enzymol. 111: 113–149.
Brumbaugh JA, 1968. Ultrastructural differences between forming eumelanin and pheomelanin as revealed by pink-eye mutation in fowl. Dev. Biol. 18: 375–390.
Cooper WE, Greenberg N, 1992. Reptilian coloration and behavior. In: Gans C, Crews D ed. Physiology, Hormones, Brain and Behavior. Chicago: University of Chicago Press, 298–422.
Dittrich W, Gilbert F, Green P, McGregor P, Grenczock D, 1993. Imperfect mimicry: A pigeon's perspective. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 251: 195–200.
Ephrussi B, Herold JL, 1944. Studies of eye pigments of Drosophila. I. Method of extraction and quantitative estimation of the pigment components. Genetics 29: 148–175.
Ferre J, Silva FJ, Real MD, Mensua JL, 1986. Pigment patterns in mutants affecting the biosynthesis of pteridines and xanthomatin in Drosophila melanogaster. Biochem. Genet. 24: 545–569.
Forbes P, 2009. Dazzled and Deceived: Mimicry and Camouflage. New Haven: Yale University Press.
Ford EB, 1953. The genetics of polymorphism in the Lepidoptera. Adv. Genet. 5: 43–87.
Fukushima T, 1970. Biosynthesis of pteridines in tadpoles of the bullfrog Rana catesbeiana Arch. Biochem. Biophys. 139: 361–369.
Gosner KL, 1989. Histological notes on the green coloration of arboreal pit vipers: Genus Bothrops. J. Herpetol. 23: 318–320.
Grether GF, 2005. Environmental change, phenotypic plasticity, and genetic compensation. Am. Nat. 166: E115–E123.
Grether GF, Hudon J, Endler JA, 2001. Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies Poecilia reticulata. Proc. R. Soc. B 268: 1245–1253.
Harper GR, Pfennig DW, 2007. Mimicry on the edge: Why do mimics vary in resemblance to their model in different parts of their geographical range? Proc. R. Soc. B 274: 1955–1961.
KIKUCHI DW, PFENNIG DW: Color production in mimicry 667

Olson VA, Owens IPF, 1998. Costly sexual signals: Are carotenoids rare, risky or required? Trends Ecol. Evol. 13: 510–514.

Ortiz E, Bächli E, Price D, Williams-Asman HG, 1963. Red pteridine pigments in the dewlaps of some anoles. Physiol. Zool. 36: 97–103.

Ortiz E, Maldonado AA, 1966. Pteridine accumulation in lizards of the genus Anolis. Caribb. J. Sci. 6: 9–13.

Pfennig DW, Harper GR, Bruno AF, Harcombe WR, Pfennig KS, 2007. Population differences in predation on Batesian mimics in allopatry with their model: Selection against mimics is strongest when they are common. Behav. Ecol. Sociobiol. 61: 505–511.

Pfleiderer W, 1992. Pteridines: Properties, reactivities and biological significance. J. Heterocycl. Chem. 29: 583–605.

Protas ME, Patel NH, 2008. Evolution of coloration patterns. Annu. Rev. Cell. Dev. Biol. 24: 425–446.

Pyron RA, Burbrink FT, 2009. Body size as a primary determinant of ecomorphological diversification and the evolution of mimicry in the lampropeltidine snakes (Serpentes: Colubridae). J. Evol. Biol. 22: 2057–2067.

Reed RD, Papa R, Martin A, Hines HM, Counterman BA et al., 2011. Optix drives the repeated convergent evolution of butterfly wing pattern mimicry. Science 333: 1137–1141.

Ruxton GD, Sherratt TN, Speed MP, 2004. Avoiding Attack. New York: Oxford University Press.

Shawkey MD, Morehouse NJ, Vukusic P, 2009. A protein palette: Colour materials and mixing in birds and butterflies. J. R. Soc. Interface 6: S221–S231.

Sherratt TN, 2002. The evolution of imperfect mimicry. Behav. Ecol. 13: 821–826.

Silva FJ, Mensua JL, 1988. Effect of some tryptophan metabolites on the biosynthesis of pteridines in the mutant red malphigian tubules of Drosophila melanogaster. Insect Biochem 18: 675–679.

Steffen JE, McGraw KJ, 2009. How dewlap color reflects its carotenoid and pterin content in male and female brown anoles Norops sagrei. Comp. Biochem. Physiol. B: Biochem. & Mol. Biol. 154: 334–340.

Taylor JD, Hadley ME, 1970. Chromatophores and color change in the lizard Anolis carolinensis. Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie 104: 282–294.

Watt WB, 1967. Pteridine biosynthesis in the butterfly Colias eurytheme. J. Biol. Chem. 242: 565–572.

Wijnen B, Leertouwer HL, Stavenga DG, 2007. Colors and pterin pigmentation of pierid butterfly wings. J. Insect Physiol. 53: 1206–1217.

Wilson TG, Jacobson KB, 1977. Isolation and characterization of pteridines from heads of Drosophila melanogaster by a modified thin-layer chromatography procedure. Biochem. Genet. 15: 307–319.