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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 ( ): –, 2012].

Key words 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; Manceau 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

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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 of producing 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 deceive potential predators. The possibility that the environment 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 pigments to produce their red coloration than do their models (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 elements. Additionally, we explored the cellular structures associated with coloration in the skin of models and mimics. Much future work remains to be done to elucidate genetic and developmental aspects of color production 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.

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 better 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, iridophores, and melanophores, collectively known as chromatophores (reviewed in Cooper and Greenberg, 1992). Iridophores contain guanine crystals that can cause both 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 erythrophores 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 pheomelanins, 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 pheomelanins 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 pheomelanins 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 phenomelanin. 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 pterdine 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 NH_4OH 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 using 1 N NH_4OH and 1:1 hexanes:TBME as blanks, respectively, on a Benkmann-Colture spectrophotometer. Most carotenoids have characteristic triplets of absorbance peaks between 400–500 nm (Britton, 1985; Macedonia et al, 2000).

1.4 Thin-layer chromatography of skin extracts

We next sought to identify the different 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 R_f 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% NH_4Cl (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 R_f 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.

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 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 R_f 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 R_f 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 R_f 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 R_f values of the violet fluorescent spot was 0.4 in 1:1 isopropanol:2% ammonium acetate, and 0.34 in 3% NH_4Cl. For the blue spot, they were 0.35 and 0.55, respectively. For both spots, the R_f values in 1:1 isopropanol:2% ammonium acetate were more variable than in 3% NH_4Cl. The closest R_f 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.

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 iridophores, 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 *Pleistodon* 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 *Pleistodon*, 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 underscore 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 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 strong 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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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.

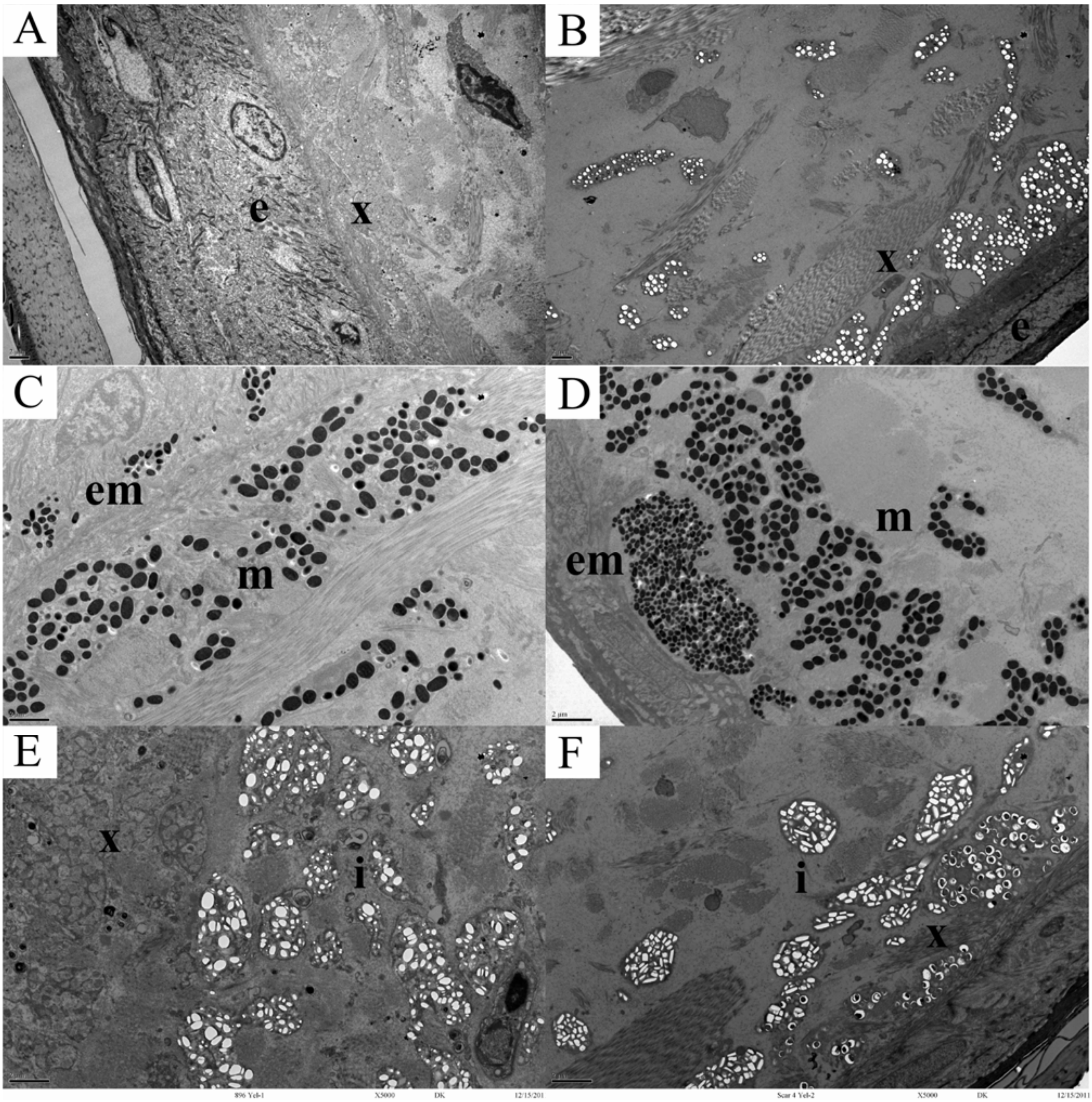

Fig. 2 These TEM micrographs of cross-sectioned snake skin show that in red skin of both (A) the eastern coral snake and (B) the scarlet kingsnake, xanthophores (marked with x) lie close to the epidermis (marked with e)

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.

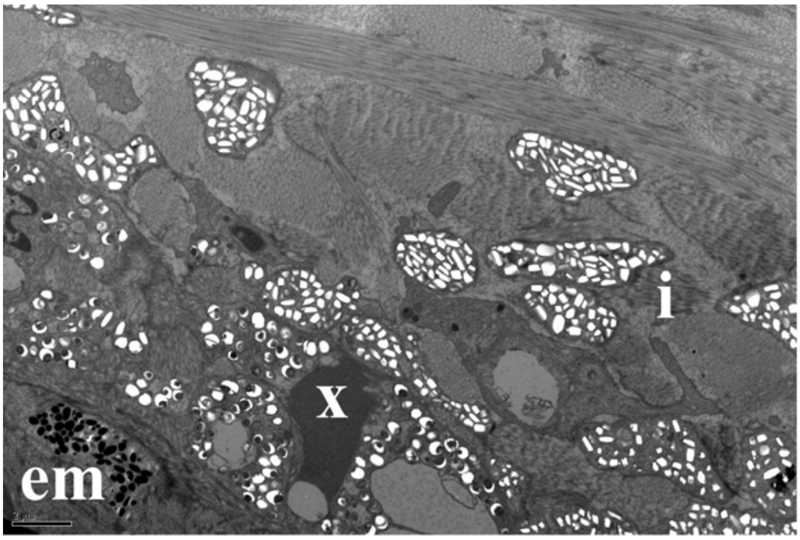

Fig. 3 This cross-section of yellow skin from the scarlet kingsnake reveals a epidermal melanophore (marked with em) that lies above the xanthophores (x) and iridophores (i), which allows it to absorb light of all wavelengths before it reaches the reflective iridophores

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.

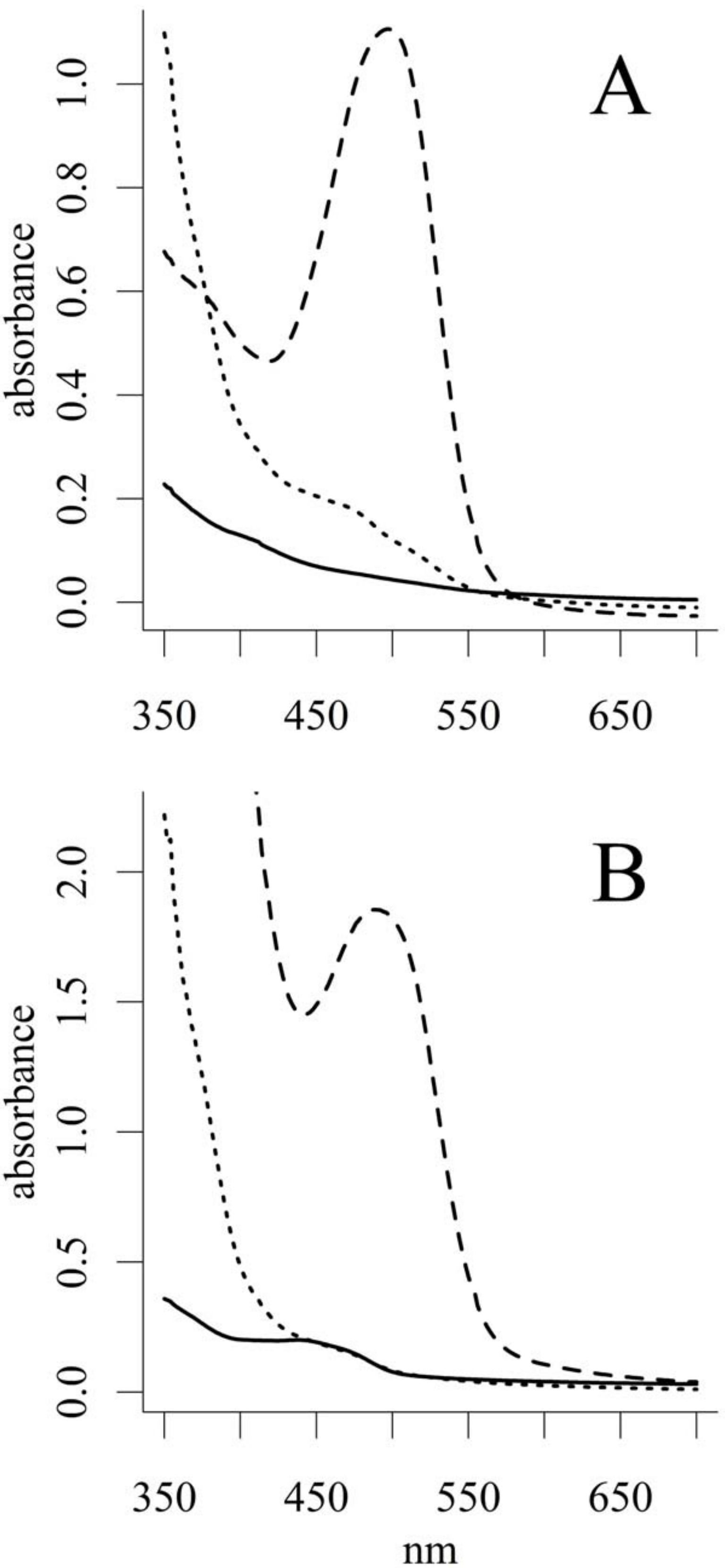

Fig. 4 Absorbance of water-soluble pigments in skin samples of the (A) eastern coral snake and (B) scarlet kingsnake

Absorbance of red, yellow, and black skin samples is plotted in the corresponding color. The strong peaks of absorbance between 490–500 nm are indicative of drosopterin pigments in the red skin, which is absent in other colors of skin. The yellow and red skin contains unidentified pigments (probably pteridines) that absorb light < 400 nm. Solid line = black skin, dashed line = red skin, dotted line = yellow skin.

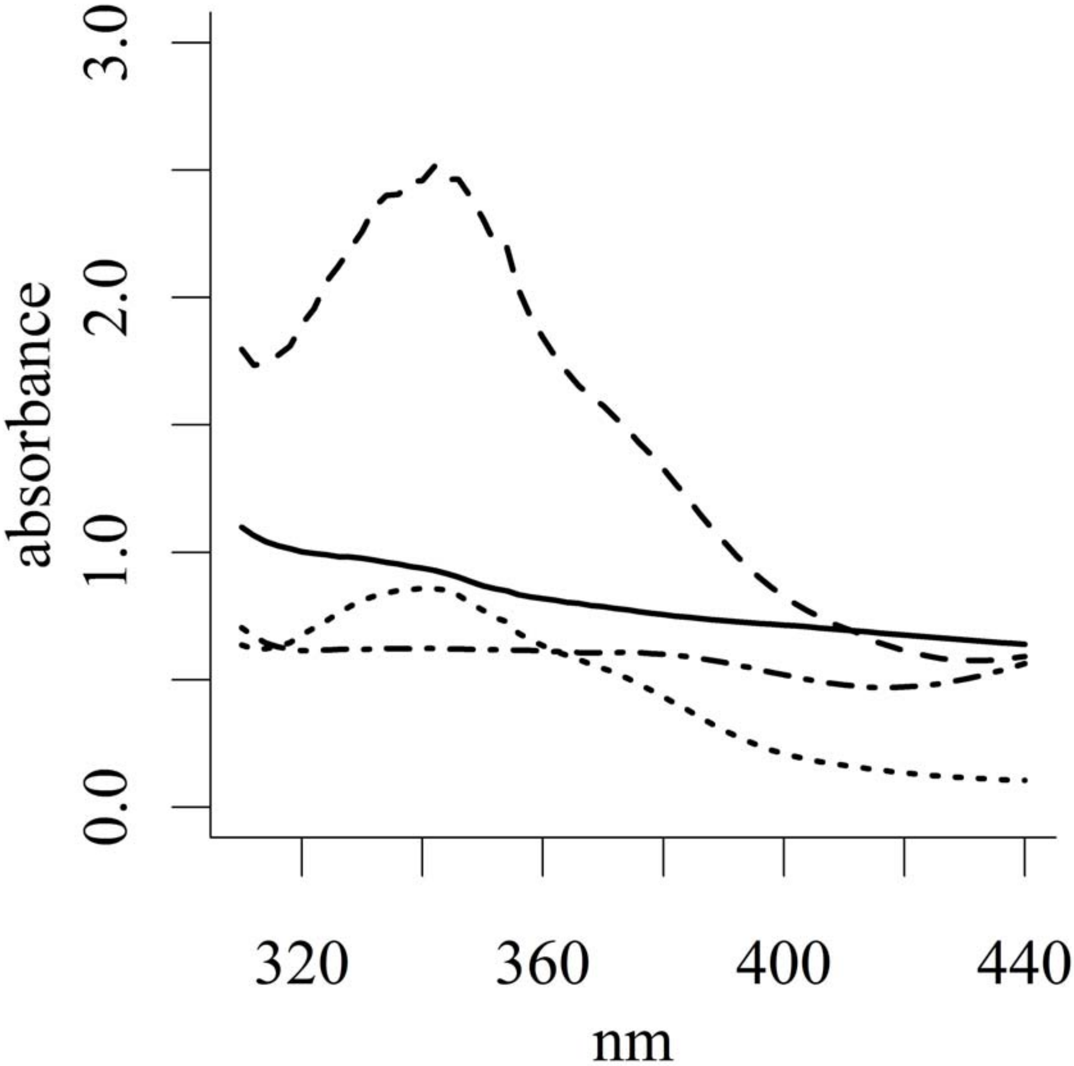

Fig. 5 Absorbance of yellow and red skin from the scarlet kingsnake in the ultraviolet, showing identical peaks near 340 nm, followed by very strong absorbance in the mid ultraviolet

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