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Carotenoid Composition of Colorful Body Stripes and Patches in the Painted Turtle (*Chrysemys picta*) and Red-Eared Slider (*Trachemys scripta*)

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ABSTRACT. – **The objective quantification of integumentary color and its pigmentary basis have been relatively little studied in turtles. We used reflectance spectrometry to measure the color of the chin, neck, and leg stripes and spots of the painted turtle (*Chrysemys picta*) and the red-eared slider (Trachemys scripta), and we used absorbance spectrometry and high-performance liquid chromatography to identify pigments in these colored patches. Both species had stripes and patches that were predominantly yellow and orange, but the yellow stripes typically contained a small ultraviolet (UV) reflectance peak. Chin, neck, and leg stripes of the painted turtle and chin and neck stripes of the red-eared slider contained an unidentified apocarotenoid and a ketocarotenoid (astaxanthin), but the postorbital eyespot of the red-eared slider contained only astaxanthin. These findings add painted turtles and red-eared sliders to the growing list of animals that use carotenoids in part to generate red, orange, yellow, and UV colors. We discuss these findings in the context of dual pigment production, pigment metabolic production costs, immune system health, and visual signal use in emydid turtles and other vertebrates.**

KEY WORDS. – **Reptilia; Testudines: Cheloniidae; apocarotenoids; ketocarotenoids; UV-vis spectrometry**

Many species of emydid turtles, including common species such as the painted turtle (*Chrysemys picta*) and the red-eared slider (*Trachemys scripta*), have conspicuous red, orange, or yellow colors on their carapaces, throat patches, or stripes along the head, neck, and legs. In other animals, similar colors function as naturally or sexually selected visual signals (Andersson 1994; Searcy and Nowicki 2005) that are generated by many different pigments and structures (Bagnara and Obika 1965; Bagnara and Hadley 1973; McGraw 2006a, 2006b). Identifying the biochemical or molecular basis for naturally or sexually selected colors is key for understanding the different production or accumulation challenges associated with various mechanisms of color generation (McGraw 2002; McGraw et al. 2005a) and their metabolic costs and benefits (McGraw 2004).

The pale yellow and orange colors of turtles result from the presence of carotenoids and pterins in dermal xanthophores (Bagnara 1983; Bagnara and Matsumoto 2006). Carotenoids are lipid-soluble photosynthetic pigments in plants that animals must acquire from the diet (Goodwin 1984). In contrast, pterins are nitrogen-rich, ultraviolet- (UV) fluorescent compounds that animals synthesize from basic purine (e.g., guanine) precursors (McGraw 2006b). Carotenoids play diverse and important roles in photoprotection, free-radical scavenging, immunomodulation, visual tuning, and animal coloration (Vershinin 1999), and function as honesty-reinforcing mechanisms that underlie costly yet beneficial colorful ornamental traits (Lozano 1994; Hill 2002). Among vertebrates, carotenoids are commonly found to generate yellows, oranges, and reds in the integuments of birds, whereas pterins more typically generate red, orange, and yellow coloration in nonavian reptiles and amphibians. Some species exhibit colors that contain both carotenoids and pterins (e.g., anoles [*Anolis* spp.]; Steffen and McGraw 2007, 2009), and these 2 pigments are distinguished from each other based on their solubilities in polar and nonpolar solvents and by their chromatographic characteristics.

The function of these colors has just begun to be investigated in testudinid (Galeotti et al. 2011), geoemydid (Ibañez et al. 2013a, 2013b, 2014), and emydid turtles (Bulté et al. 2013; Polo-Cavia et al. 2013; Ibañez et al. 2014). Spectrometric studies have shown that in *T. scripta,* yellow stripes have a small UV peak (Polo-Cavia et al. 2013), sexes differ in UV reflectance (Wang et al. 2013), postorbital eye spot redness and chin stripe yellowness positively correlate with immunocompetence (Polo-Cavia et al. 2013), and yellow-red color correlates with body condition (Wang et al. 2013). Surprisingly, the pigmentary basis to these colors has not been described in detail in *T. scripta*. Furthermore, no similar color studies have been performed on *C. picta*. The aims of this article are to 1) use spectrometry to spectrally describe the colorful chin, neck, and leg stripes of *C. picta* and the chin, neck and eye spots in *T. scripta;* and 2) use analytical chemistry to determine a more precise identity

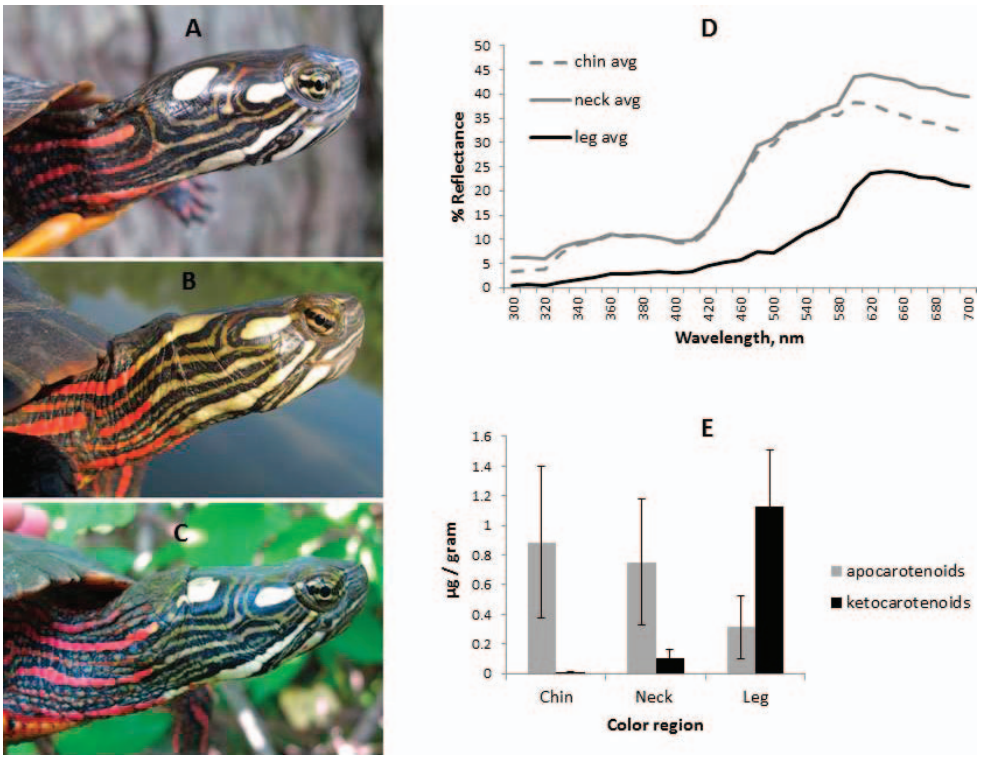

Figure 1. (A–C) Representative variation in extent of yellow chin and orange neck stripes of 3 painted turtles (*Chrysemys picta*). (D) Average smoothed reflectance spectra of the chin, neck, and leg stripes of *C. picta*. (E) Relative apocarotenoid and ketocarotenoid (astaxanthin) concentrations (expressed as µg pigment/g of tissue) in chin, neck, and leg of *C. picta*. Bars represent SE of the mean. Photo copyright 2013 by Scott Boback.

of the carotenoids contributing to the generation of colors in the dermal integument of turtles.

METHODS

Study Organisms. — Painted turtles (*C. picta*) are common turtles inhabiting a variety of shallow-water habitats across much of the United States and southern Canada (Conant and Collins 1998). Their chins and necks are striped with yellow-orange, whereas their fore- and hind-legs have red stripes against black integumentary backgrounds (Fig. 1). Red-eared sliders (*T. scripta*) are native to ponds and lakes throughout the Mississippi Basin (Ernst 1990; Iverson 1992), but have been introduced to freshwater bodies worldwide (Polo-Cavia et al. 2013). Their legs, chins, and necks are yellow or orange against gray to black integumentary backgrounds and their postorbital eye patches can be orange or red (Fig. 2A, B).

Specimen Collection. — Adult animals were collected from 3 Pennsylvania counties: 2 *T. scripta* (1 female, 1 male) were collected from ponds in Cumberland and Dauphin counties and 4 *C. picta* (2 females and 2 males) were collected as 1-d-old roadkill in Cumberland and Erie counties. Both *T. scripta* were euthanized with an overdose of sodium pentobarbitol solution (390 mg/ml) injected intracoelomically at 100 mg/kg. Specimens were immediately transported in an ice-filled cooler for no more than 1 hr to −20°C freezers at the Tom Ridge Environmental Center, in Erie, Pennsylvania, and Dickinson College in Cumberland County, Carlisle, Pennsylvania. Animals were kept in −20°C freezers for no longer than 3 mo, at which point we thawed them out and rubbed water on the integument in preparation for spectrophotometric measurement. The skin was intact, showed no wear or fading, and looked similar to tissue observed on live animals (J.E. Steffen, *pers. obs.*).

Reflectance Spectrometry. — We measured ultraviolet-visible (UV-Vis) coloration of the chin, neck, and leg stripes and the postorbital eye spot of deceased turtles using an Ocean Optics S2000 spectrometer (Dunedin, FL) coupled to a tungsten–deuterium light source. We used a bifurcated fiber-optic cable (400 µm inner diameter) mounted in a black plastic sleeve that was fastened to the probe tip and that was placed at an angle of 90° to, and 2 mm away from, the measured skin surface. Prior to the reflectance measurement, we dried the turtles' skin surfaces, and in each body region, we took 3 unique, adjacent, and nonoverlapping measurements. Color data were gathered as percent reflectance at 1-nm wavelength increments from 300 to 700 nm (integration time = 2 msec, scans to average = 22) and this output was smoothed and processed using CLR software v 1.0 (Montgomerie 2006). From the smoothed spectral reflectance data, we constructed spectral reflectance curves for chin, neck, leg, and postorbital color patches. We describe the 'color' of turtle integument using Hue (H) where

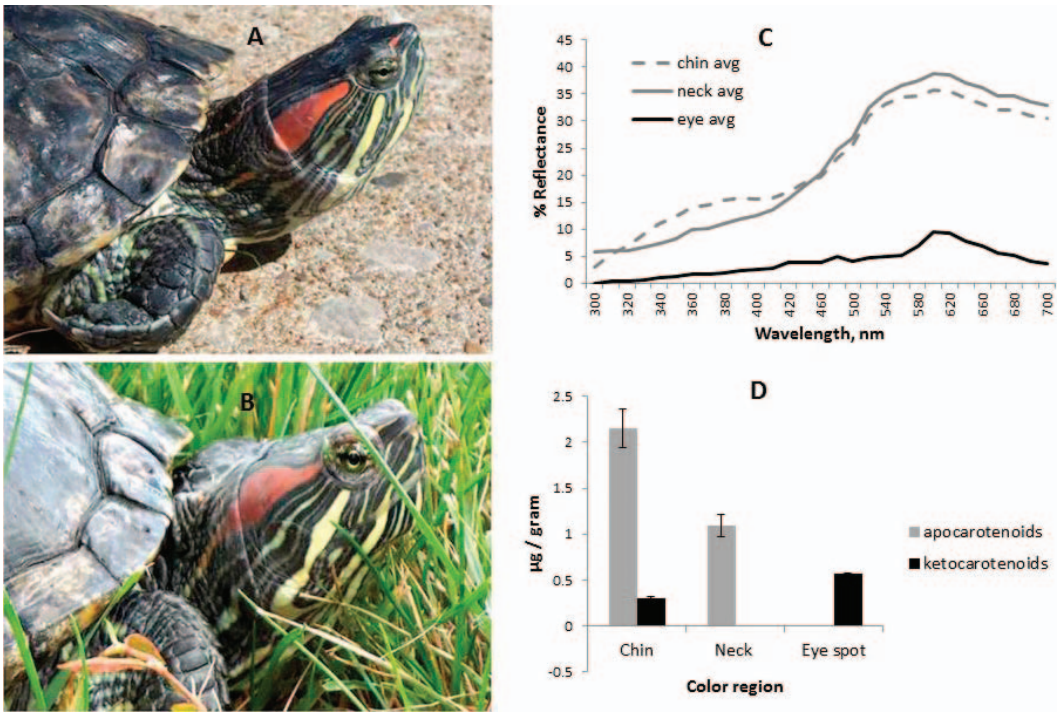

Figure 2. (A, B) Representative variation in extent of yellow chin and neck stripes and red postorbital spots of 2 red-eared sliders (*Trachemys scripta*). (C) Average smoothed reflectance spectra of the chin and neck stripes and postorbital eye spot of *T. scripta*. (D) Relative apocarotenoid and ketocarotenoid (astaxanthin) concentrations (expressed as µg pigment/g of tissue) in chin, neck, and postorbital eye spot of *T. scripta*. Bars represent SE of the mean. Photo copyright 2013 by Mary Ann Brown.

H = λRmax (Montgomerie 2006) and is the wavelength of maximum reflectance. We use the following wavelength ranges to delimit hue for 4 wavebands: 300–399 nm = UV, 400–474 nm = blue, 475–549 nm = green, 550–599 nm = yellow, 600–649 nm = orange, and 650–700 nm = red. We use UV chroma to describe spectral purity in the UV waveband (300–399 nm) and carotenoid chroma (S9 = (λ450 nm − λ700 nm)/λ700 nm; Montgomerie 2006) to describe spectral purity in the portions of the curve that capture light absorbance by carotenoids (i.e., 350–500 nm; Montgomerie 2006). Values for carotenoid chroma are negative if reflectance at λ700 nm is greater than reflectance at 450 nm and become more negative as amount of reflectance at λ700 nm increases relative to the proportion of reflectance at λ450 nm.

Immediately after taking spectral measurements from each color patch, approximately 1 cm^2 of the measured skin area was removed, washed with deionized water, and stored in a 70% ethyl-alcohol–filled 1.7-ml microfuge tube. Tissue was frozen in a −80°C freezer and shipped on dry ice to KJM for pigment analysis (see below). The tissues remained on dry ice for approximately 36 hrs and were held in a −80°C freezer for 1 mo prior to analysis.

Chemical Extraction and Identification of Skin Pigments. — Colorful (yellow, orange, or red) portions of the tissue were separated from dark regions, weighed to the nearest 0.00001 g with a digital balance, and ground in the presence of 1 ml hexane:methyl tert butyl ether (MTBE) and stainless-steel grinding balls using a mixer mill (McGraw et al. 2005a, 2005b). The ground material and solvent were transferred to a fresh tube, at which point 1 ml of 1% NH_4OH in water was added to partition pterins from carotenoids. The solution was then shaken for 1 min, centrifuged, and the 2 solvents separated for pterin and carotenoid quantification (see below). Carotenoids separated into the top layer and pterins, if present, were separated into the bottom layer (McGraw et al. 2005b).

We analyzed aqueous layers for pterins by transferring the aqueous NH_4OH into a quartz cuvette and placing it into an absorbance spectrometer (Cary 300 Bio Spectrophotometer). Spectra were analyzed from 300 to 700 nm for the presence of distinct absorbance peaks (i.e., comparable to those of pterins in animals, such as xanthopterin and erythropterin; Pfleiderer 1992). Unfortunately, we could not unequivocally confirm pterins in the bottom (i.e., ammonium-hydroxide) layer, because of low concentrations and failure to detect any peaks using absorbance spectrophotometry (see more below).

Hexane:MTBE fractions were transferred to a fresh tube and evaporated to dryness under a stream of nitrogen. We resuspended the pigment residue in 200 µl HPLC mobile phase (42:42:16, methanol:acetonitrile:dichloromethane, v:v:v) and injected 50 µl into a Waters Alliance autosampler HPLC fitted with a YMC-30 carotenoid column (4.6 × 250 mm) and a column heater set to 30°C. Flow rate was always held at 1.2 ml/min and runs consisted of a isocratic phase using the aforementioned

Table 1. Carotenoid concentrations, colorimetrics, and presence of small quantities of pterins in tissue from 3 colored regions of painted turtle and red-eared slider skin. [Apo] refers to apocarotenoid concentration, [Keto] refers to ketocarotenoid concentrations. Hue (H) is the wavelength of maximum reflectance. Color refers to the qualitative description of the tissue's major hue. Carot C refers to carotenoid chroma, which is the spectral purity under the spectral curve between 450 and 700 nm. It is negative when the amount of reflectance at λ700 nm is greater than reflectance at λ450 nm. The magnitude of the negative value increases as the proportion of reflectance at λ700 nm increases relative to proportion of reflectance at λ450 nm. UV chroma is the spectral purity under the spectral curve between 300 and 399 nm. Pterins refers to the presence of very small concentrations of unidentifiable pterins; Y = yes, N = no. See text for details.

Species	Region	*N*	[Apo] ± SE	[Keto] ± SE	Hue (λRmax)	Color	Carot C	UV C	Pterins?
Painted turtle	Chin	4	0.884 ± 0.510	0.007 ± 0.007	579 nm	yellow	−0.477	0.081	Y
	Neck	4	0.750 ± 0.424	0.101 ± 0.061	608 nm	orange	−0.543	0.083	Y
	Leg	4	0.313 ± 0.215	1.126 ± 0.381	642 nm	orange	−0.776	0.044	Y
Red-eared slider	Chin	2	2.153 ± 0.215	0.296 ± 030	569 nm	yellow	−0.224	0.111	Y
	Neck	2	1.096 ± 0.118	0.0001 ± 0.001	576 nm	yellow	−0.669	0.084	Y
	Eye spot	1	0.000 ± 0.000	0.570	608 nm	orange	−0.330	0.064	N

mobile phase through minute 11, following by a linear gradient reaching 42:23:35 methanol:acetonitrile:dichloromethane (v:v:v) at minute 21, at which point these conditions were held through minute 25 and then returned to initial conditions until minute 29.5. Carotenoids were compared with authentic reference carotenoids (obtained from CaroteNature GmbH, Ostermundigen, Switzerland) for identification and to external curves of these for quantification using Empower software.

RESULTS

Reflectance Spectrometry. — Chin stripes of *C. picta* reflected maximally (approx. 40% reflectance) in the yellow waveband and had a yellow hue, whereas neck stripes reflected maximally (approx. 45% reflectance) in the orange waveband and had an orange hue. Both spectral reflectance curves showed low levels of UV chroma (Table 1; Fig. 1D). Leg stripes showed a comparatively lower maximal reflectance (approx. 25% reflectance) in the orange (nearly red) waveband, had an orange-red hue (Table 1; Fig. 1D), and contained little UV reflectance or chroma (Table 1; Fig. 1D). Carotenoid chroma was smallest in the chin stripes, intermediate in the neck stripes, and highest in the leg stripes (Table 1; Fig. 1D).

Chin and neck stripes in *T. scripta* reflected maximally (approx. 40%) in the yellow waveband and had a yellow hue (Table 1; Fig. 2C). The *T. scripta* chin stripes had moderate levels of UV chroma (340–430 nm) and the neck stripes had low levels of UV chroma (Table 1; Fig. 2C). The postorbital eye patch reflected maximally within the orange waveband and had an orange hue but showed a lower maximal reflectance (approx. 5%) than the chin or neck stripes and had low UV reflectance and chroma (Table 1; Fig. 2C). Carotenoid chroma was lowest in the chin stripes, intermediate in the eye spot, and highest in the neck stripes (Table 1; Fig. 2C).

Biochemical Identity of Pigments. — High-performance liquid chromatography analyses of the carotenoid fractions revealed that tissue from the yellow *C. picta* chin and the orange neck stripes contained 2 pigments: the first absorbing light maximally at 436 nm (t_R = 3.5 min) and the second at 477 nm (t_R = 6.3 min). By comparison to known standards, we confirmed that the second peak is the ketocarotenoid astaxanthin. Based on comparison with published lambda-max and retention-time values for other types of carotenoids (Britton 1985), we hypothesize that the first pigment is an apocarotenoid that could not be specifically identified. The apocarotenoid was more highly concentrated than astaxanthin in *C. picta* chin and neck tissues (Table 1; Fig. 1E). Tissue from the orange (nearly red) *C. picta* leg stripes also contained both the apocarotenoid and astaxanthin, but astaxanthin was more highly concentrated than the apocarotenoid (Table 1; Fig. 1E).

Tissue from yellow chin and neck stripes of *T. scripta* also contained the apocarotenoid and astaxanthin, with the apocarotenoid being more concentrated than astaxanthin (Table 1; Fig. 2D). However, tissue from the orange postorbital patch of *T. scripta* contained astaxanthin, in comparatively moderate concentration (Table 1; Fig. 2D).

DISCUSSION

Colored skin patches of painted turtles and red-eared sliders showed spectral curves with high percent reflectance in the yellow and orange hues. Freshwater lakes and ponds that these 2 species inhabit probably transmit light maximally at longer wavelengths (Lythgoe 1984). Thus these yellow and orange stripes and patches are likely to be highly visible to conspecific individuals in the aquatic environment of both species. In addition, yellow chin and neck stripes of *C. picta* and the yellow chin stripes of *T. scripta* reflect UV light, albeit in relatively small amounts. *Trachemys scripta* possesses UV-sensitive photoreceptors (Loew and Govardovskii 2001), and presumably other emydid turtles (including *C. picta*) possess similar photoreceptors. Light conditions at the surface of lake and pond waters these turtles inhabit render these colors visible to conspecific turtles and predators; the surface of the water is relatively rich in UV wavelengths (Leech and Johnsen 2009) and the UV

reflectance of these stripes and patches is visible if they are viewed horizontally. The UV signals might also be visible to conspecifics when viewed from above, because absorption, scatter, and attenuation of UV wavelengths increase with increasing depth, such that the background has a dearth of UV compared with the UV reflecting stripe or patch. This has been shown to create UV contrast in UV patches of fish when viewed against the bottom (Lythgoe 1968; Leech and Johnsen 2009), and this same phenomenon should occur for turtles.

Courtship behavior of *C. picta* and *T. scripta* has been described (Ernst 1971; Jackson and Davis 1972; Thomas 2002; Thomas and Altig 2006) and includes a female facing a male's colorful head under the surface of the water while the male vigorously strokes the female's head with his elongated foreclaws (titillation). After repeating this head-on foreclaw display multiple times, the female sinks to the bottom (Ernst and Lovich 2009) in *C. picta,* and the male swims behind and mounts her, and copulation begins (Ernst and Lovich 2009). *Trachemys scripta* has similar behaviors, but the male and female slowly sink to the bottom together as they are looking at each other during titillation. Copulation occurs at the bottom for *T. scripta* as well (Ernst and Lovich 2009). Thus, these colorful stripes and patches should be visible to conspecifics during courtship. Although the functional role of these yellow, orange, and UV colors in social interactions has not been explored, the ability of turtles to see UV-reflecting patches (Loew and Govardovskii 2001) may aid in color discrimination and contrast enhancement against background illumination (Leech and Johnsen 2009).

We found that the yellow and orange hues present in the color patches of these turtles are generated, at least in part, by carotenoids. Carotenoids are presumably easy to obtain for *C. picta* and *T. scripta* because many of the plants and animals that are commonly found in aquatic habitats contain abundant carotenoids (Goodwin 1980, 1984). Moreover, *C. picta* and *T. scripta* are omnivorous generalists and consume a wide variety of carotenoids by eating different species of plants and animals found in their aquatic habitat (Rowe and Parsons 2000; Rowe and Bowen 2005; Ernst and Lovich 2009) in relative amounts that are proportional to local dietary plant and animal abundance (Hart 1983; Pearson et al. 2013).

Two types of carotenoids, a short-wave–absorbing apocarotenoid and a longer-wave–absorbing ketocarotenoid (astaxanthin), generate color in the integumentary patches of *C. picta* and *T. scripta*. The yellow and orange hues present in *C. picta* and the yellow chin and neck colors of *T. scripta* were probably due to combined effects and varying amounts of the apocarotenoid (likely conferring a yellow-green color) and astaxanthin (a red pigment), but the orange hue of the postorbital eye (*T. scripta*) spot reflects astaxanthin alone. The carotenoids responsible for generating pale yellow and orange colors have been found to reside in xanthophores and lipophores that are located in the loose dermis of chelonian skin (Alibardi 2013), but the types of carotenoids found in the turtle dermis had not previously been described.

Pigments are produced via various metabolic pathways and may differ in their metabolic transformation cost (Hill 2002); thus, it is important to know the dietary versus metabolic identity of integumentary carotenoids. For example, xanthophyll-based yellow color patches are generated by dietary intake of lutein and zeaxanthin in many animals (Hill 2002; McGraw 2006a). Presumably there is very little transformation cost associated with the dehydrogenative conversion of lutein and zeaxanthin to xanthophylls, including apocarotenoids, because lutein and zeaxanthin's end-ring hydroxyl groups are converted to a carbonyl group (Hill 2002; McGraw 2006a). Ketocarotenoids, on the other hand, are probably not ingested by most animals, except in crustacean-consuming aquatic and marine organisms. These pigments are likely to incur metabolic conversion costs because the end-ring functional groups are transformed from a hydroxyl group (conferring yellow color) to a red carotenoid through oxidization of the end-rings by introduction of a keto-group in the 4 position on the end rings (Inouye 1999; McGraw et al. 2001; McGraw 2006a). Because we did not study turtle diets, we cannot rule out ingestion of either pigment type, but future research should investigate the endogenous versus exogenous origin to these carotenoids and determine whether one or both carotenoid types we detected here are costly to acquire or manufacture (Hill 1996). Interestingly, these 2 types of carotenoids have previously been reported together only in the eye tissues of wild birds (e.g., avian oil droplets; Wald 1948; Toomey and McGraw 2007).

In addition to carotenoids, we suspect that pterins are also present in very small quantities in the turtle integuments studied. Pterins and carotenoids have previously been found in the colorful tissues of various amphibians and reptiles (Bagnara and Hadley 1973), including turtles (Bagnara 1983; Bagnara and Matsumoto 2006). The apparently low concentrations of pterins detected here suggest that they play a minor role in color generation for both turtle species, but we cannot discount the possibility that the low concentrations of pterins found here are due to the low sample size of turtles used for this study. Still, their presence in even small quantities leads one to ask the question: why have pterin pigments at all? Using 2 different pigment types might allow for a wider range of colors to be produced in the integument. Yellow pterins, for example, absorb light at shorter wavelengths than the carotenoids detected here (McGraw 2006b), and if turtle integument contains both pterins and carotenoids, then the skin can absorb maximally across multiple visible wavebands. Using different pigments might also be a way of broadcasting different types of physiological information. For example, Grether et al. (2005) considered carotenoid and pterin color production in the sexually selected orange spots of Trinidadian guppies (*Poecilia reticulata;* Houde 1987; Kodric-Brown 1989,

1993). Carotenoid expression was sensitive to variation in the diet but pterin production was not, being instead under strong genetic control (Grether et al. 2005). Neither the relationships between pterins and carotenoids nor the evolutionary significance of dual pigment usage have been studied in emydid turtles, but if carotenoid expression in the integument of these turtle species varies according to dietary access to carotenoids, while pterin expression varies according to another fitness-related mechanism (e.g., sex and age, as is the case in the irises of some birds; McGraw 2006b), then integumentary color might jointly communicate information about dietary access to carotenoids and some other factor.

Postorbital eye-spot redness and chin stripe yellowness have been shown to positively correlate with immunocompetence in *T. scripta,* even though the pigmentary basis to color was not described (Polo-Cavia et al. 2013). Postorbital eye spots that had higher values of long-wavelength (i.e., red) reflectance and chin stripes that had higher values of medium-wavelength (i.e., yellow) reflectance correlated with higher individual immune responses (e.g., blood hematocyte/leukocyte ratios and cutaneous immune activity). Interestingly, sexes did not differ in color of the patches and stripes, but Polo-Cavia et al. (2013) suggest that information about immune health communicated in this visual signal might be used by both sexes in intrasexual and intersexual communication. Wang et al. (2013) found UV sexual dichromatism in *T. scripta,* as well as yellow-red colors correlating with body condition. The authors suggest that females might recognize sex based on sexual differences in UV reflectance and evaluate male body condition by the yellow and red colors of the forelimbs. Finally, Ibañez et al. (2014) found that an immune challenge—an injection of a bacterial antigen—reduced overall brightness, long-wavelength reflectance, and chroma in *T. scripta elegans;* they speculated that a trade-off between immune system function and visual ornament expression may allow turtles to honestly signal individual quality in intersexual selection processes.

If male and female integumentary color expression varies according to some physiological or genetic mechanism and coloration is important to male and female courtship preferences, then perhaps sex-specific colors (anterior chin–head–neck stripes, postorbital eye patches, and posterior leg stripes) are used to communicate fitness-related information during courtship interactions. This possibility has also been suggested by Wang et al. (2013) and Ibañez et al. (2014).

Other types of pigment (e.g., melanin) might be important to color generation in turtles (Alibardi 2013). Melanin-based brown, gray, and black colors are very common in turtles and provide crypsis through substrate–color convergence (Endler 1990) in *C. picta* (Rowe et al. 2006, 2009, 2013) and *T. scripta* (Rowe et al. 2009, 2013). In addition, the brown, gray, and black colors vary among populations that live in environments with different substrate colors and can vary over time when exposed to different substrate colors in the lab (Rowe et al. 2009). The adjacent placement of the yellow, orange, and UV colors against these melanin-based brown, gray, and black colors may serve to maximize signal conspicuousness (Endler 1992). Therefore variation in these dark background colors among populations could alter the conspicuousness of these stripes.

In conclusion, the results presented here show small UV peaks associated with the yellow stripes of *C. picta* and *T. scripta,* and a richer range of yellows and oranges than previously described (e.g., Polo-Cavia et al. 2013; Wang et al. 2013; Ibañez et al. 2014). Moreover, the generation of yellows and oranges in these 2 species is at least in part due to the combined effects of apo- and ketocarotenoids. Future research in chelonian coloration is rich with possibilities. Our results point to particularly interesting avenues, including correlations among integumentary color and pigment concentrations, pigmentary transformation costs, and the use of integumentary color as visual signals in intra- and intersexual interactions in both turtle species (e.g., Ibañez et al. 2014).

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