

Tropical bat as mammalian model for skin carotenoid metabolism

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Animals cannot synthesize carotenoid pigments de novo, and must consume them in their diet. Most mammals, including humans, are indiscriminate accumulators of carotenoids but inefficiently distribute them to some tissues and organs, such as skin. This limits the potential capacity of these organisms to benefit from the antioxidant and immunostimulatory functions that carotenoids fulfill. Indeed, to date, no mammal has been known to have evolved physiological mechanisms to incorporate and deposit carotenoids in the skin or hair, and mammals have therefore been assumed to rely entirely on other pigments such as melanins to color their integument. Here we use high-performance liquid chromatography (HPLC) in combination with time-of-flight mass spectrometry (HPLC-TOF/MS) to show that the frugivorous Honduran white bat *Ectophylla alba* colors its skin bright yellow with the deposition of the xanthophyll lutein. The Honduran white bat is thus a mammalian model that may help developing strategies to improve the assimilation of lutein in humans to avoid macular degeneration. This represents a change of paradigm in animal physiology showing that some mammals actually have the capacity to accumulate dietary carotenoids in the integument. In addition, we have also discovered that the majority of the lutein in the skin of Honduran white bats is present in esterified form with fatty acids, thereby permitting longer-lasting coloration and suggesting bright color traits may have an overlooked role in the visual communication of bats.

bats | carotenoids | macular degeneration | skin coloration

Carotenoids are photosynthetic pigments animals use to avoid cellular oxidative damage and to color external structures (1–3). The inability of animals to synthesize de novo carotenoids means these pigments must be obtained only from dietary sources (4). Among vertebrates, the limiting nature of carotenoids is particularly marked in mammalian species, which exhibit a variable, but generally low, efficiency for carotenoid assimilation (5, 6). Here we show a mammal, the frugivorous Honduran white bat *Ectophylla alba*, that deposits amounts of carotenoids in the skin that are high enough to generate a conspicuous coloration. Indeed, up to this time, no mammal has been known to incorporate significant visible amounts of carotenoids in the integument (7, 8). This has prevented adequate animal models being found for carotenoid assimilation in the skin that would enable improvement of the bioavailability of carotenoids in the diet of humans (9), where these pigments are of utmost importance for health by having provitamin A value and fulfilling antioxidant and immunostimulatory functions (10, 11). Using high-performance liquid chromatography (HPLC) in combination with time-of-flight mass spectrometry (HPLC-TOF/MS), we found that the characteristic bright yellow coloration of the bare skin of the nose-leaf and ears in these bats is generated by the xanthophyll lutein in both free and esterified forms. This indicates that the Honduran white bat represents a change of paradigm in animal physiology, showing that some mammals actually have the capacity to accumulate dietary carotenoids in the integument. It is thus a mammalian model that may help in developing strategies to improve the assimilation

of lutein in humans (12), who depend on dietary contents to avoid macular degeneration (13) and skin oxidative damage (14).

The nocturnal Honduran white bat is a small phyllostomid distributed exclusively in lowland rainforests of Mesoamerica that exhibits bright yellow coloration in the bare skin of the nose-leaf, ears, and wings (Fig. 1 *A* and *B*). This distinctive coloration resembles the one generated by some carotenoids, mainly lutein and zeaxanthin, in the integument of other vertebrates, such as the bare integument and feathers of birds (4, 15). To the best of our knowledge, such yellow hues are not found in other mammals, which mostly rely on darker endogenous melanins to color their pelage and skin (16), with the exception of other tropical bats (see *Evolutionary Implications*). To determine the origin of the yellow color, we conducted in-depth chemical analyses of the bare skin of the ears and nose-leaf and of the liver of two male Honduran white bat specimens from Costa Rica.

The integument of mammals represents a striking example of the difference in the bioavailability of carotenoids among animals. Carotenoids are widespread pigments in the integument of all vertebrates except mammals, as only small amounts of carotenoids are found in the skin of humans and other mammalian species (7, 8), and in contrast to the feathers and scales of reptiles and fish, there seems to be a complete lack of carotenoids in mammalian hair (15). In humans, and aside from pathological conditions in which carotenoids accumulate in the skin (i.e., carotenoderma), these pigments only occur in low amounts in the skin (~200 ng/g tissue) (7), where they interact with the color generated by keratin, melanins, and blood (17). Carotenoids are present at higher concentrations in internal tissues (human macula: 1 mM; serum: 0.1–1 μM) (18). Therefore, the contribution of dietary carotenoids to the perceived variation in human skin coloration is negligible. To date, the role of carotenoids as the primary pigments responsible

Significance

We have discovered that a mammalian species, a bat called the Honduran white bat *Ectophylla alba*, displays a yellow carotenoid pigment called lutein in its bare skin. Even though carotenoid-based coloration has been found in birds, fish, amphibians, and reptiles, there are no reports of any extant mammals showing these pigments in their skin or hair. The implications of this finding may be profound for human health, as carotenoids are essential micronutrients. Lutein in particular is involved in the preservation of the macula of the eye. The Honduran white bat, with its ability to assimilate and deposit lutein in its bare skin, may be the sought-after mammalian model needed for enhancing studies on carotenoid function and metabolism.

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Fig. 1. Honduran white bat images. (A and B) Details of the bright yellow coloration of the bare skin in the ears, nose-leaf, and wings of a Honduran white bat from Costa Rica. (C) A Honduran white bat feeding on the ripe fruit of a fig tree *F. colubrinae*. Photographs by Sharlene Santana (A and B) and Marco Tschapka (C).

for the generation of distinctive patches of bright color in the integument, as in other vertebrates (4, 15), has never been reported in mammals. Beyond increasing our understanding of the evolution of animal coloration and appearance, finding mammals capable of coloring their integument with carotenoids would open new opportunities to obtain mammalian models that help improve our bioavailability of dietary carotenoids for a better fulfillment of human physiological functions (19).

Mammals obtain carotenoids through eating green plant products. Once ingested, these pigments are released from the food matrix by the action of bile salts and pancreatic lipases, which form lipid micelles that incorporate carotenoids and facilitate their transport to the mucosal cells of the duodenum by passive diffusion or through facilitated transport via scavenger receptor class B, type 1 (20). The capacity to assemble micelles with incorporated carotenoids partly determines the efficiency of carotenoid absorption, which has led to the advent of functional food technologies searching for food matrices that optimize the absorption of supplemented carotenoids in farmed fish (5, 21) and human diets (22). Once absorbed, carotenoids are transported by lipoproteins through the bloodstream to the target tissues, a process in which the abundance and nature of lipoproteins limit the capacity to obtain carotenoids (15). Furthermore, some animals can metabolize carotenoids into other forms, and occasionally esterify them with long-chain fatty acids (20). The result is that several factors, such as amounts and species of carotenoids ingested, molecular linkage and food matrix composition, nutrient status of the host, and genetic components, influence the

bioavailability of carotenoids (23), creating large differences between species in their capacity to obtain these pigments from their diets and make physiological use of them (15).

Results and Discussion

Our chromatographic analyses, performed with extracts from the ears and nose-leaf of Honduran white bats, clearly showed that their bright yellow color is caused by the xanthophyll lutein, including *trans* and *cis* isomers, and its epoxy derivative, which exclusively forms the carotenoid profile in the extracts of the two specimens analyzed. Thus, both specimens contained the same qualitative carotenoid profile. The identification was based on the chromatographic features, UV-visible absorbance spectra, and MS characteristics, including MS and MS² spectral behavior, of the molecular ion, which is in line with the data available in the literature (24). Fig. 2 depicts the HPLC analyses of the extracts from liver (Fig. 2A), ears, and nose-leaf tissue (Fig. 2B) for both specimens, whereas Fig. 3 shows the UV-visible absorbance spectra of the main peaks detected in the analyzed tissues. We compared the experimental data with those obtained from the analysis of lutein standard isolated from a vegetable source, obtaining equivalent results (Figs. 2C and 3). Unexpectedly, most of the lutein content in the ears and nose-leaf is present as esterified forms with saturated and unsaturated fatty acids, whereas almost all lutein content in liver is present as free form (Table 1). The probable source of dietary lutein for Honduran white bats is the fig tree *Ficus colubrinae*, the ripe red fruit of

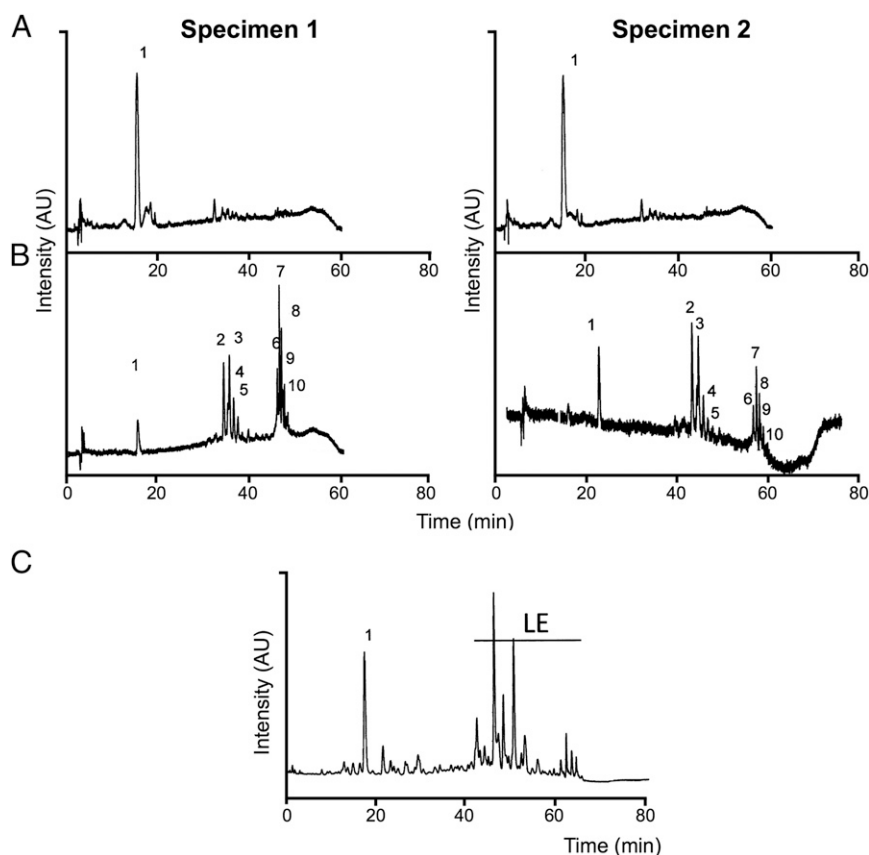


Fig. 2. Chromatogram traces. The graphs correspond to the analyses of liver (A) and ears+nose-leaf (B) extracts from two specimens of Honduran white bats, and to the analysis of marigold *Tagetes erecta* extract (C). Peak identification is the same as in Table 1. LE, lutein esters.

which is reported to be the bat's main food item (25) (Fig. 1C). The main carotenoids in fresh cultivated figs, from the congeneric species fig tree *Ficus carica*, are lutein followed by zeaxanthin, both of which are concentrated in the fruit peel (26).

Potential Applications for Human Health. The presence of esterified lutein and epoxy-lutein in the yellow skin of Honduran white bats, but not in the liver, suggests bats possess a physiological mechanism that allows them to esterify those xanthophylls that accumulate in the integument. The bioavailability of xanthophylls to humans partly depends on the efficiency with which the esterified forms of these carotenoids are hydrolyzed by enzymes during digestion, and in fact only free xanthophylls are normally found in human serum and peripheral tissues (27). Although the nutritional use of xanthophylls may require esters to be metabolized (27), the fact that bats are able to esterify them into their integument opens up interesting possibilities for improving the benefits of xanthophylls for human health. This is because, in plants, the esterification of xanthophylls enhances their stability (27). Together with zeaxanthin, lutein forms the macular pigment and plays a prominent role in human vision by acting as a filter of pro-oxidant short-wavelength light and as an antioxidant to prevent damage of free radicals in the retina. Lutein in the human macula is not esterified (28), but if humans had the mechanism to esterify this carotenoid as bats have, it could be speculated that the stability of macular lutein, and therefore its capacity to avoid eye damage, may be improved. A recent study in birds shows that the genes involved in the conversion of dietary yellow carotenoids to red ketocarotenoids are expressed in both integumentary tissues that are red colored by these pigments and in the retina, where they fulfill a role in color vision by

forming red oil droplets (29). Similarly, if esterified lutein also had a visual function in Honduran white bats, it would be expected that the genes regulating the metabolic transformation of lutein, and particularly its esterification, were expressed in both the integument and the eye. Almost nothing is known about the physiological mechanisms by which some animals esterify carotenoids (30), but proving that genes involved in esterification are expressed in the macula of Honduran white bats would represent a body of evidence of the physiological value of this metabolic transformation. This would be the precursor of future treatments in humans that may consider modifying the levels of the proteins that are involved.

The Honduran white bat therefore represents a potential mammalian model that could be used to understand these mechanisms, which may help improve the stability and bioavailability of carotenoids, and thus their benefits for human health. Although Honduran white bats should not be expected to become laboratory animals, they can be maintained in captivity if provided with adequate specific conditions (31). Furthermore, this species is considered as near threatened (i.e., not threatened with extinction) by the International Union for Conservation of Nature and is locally abundant, despite its restricted distribution (32), meaning it should be a valid model species for at least pioneering studies on the genetic basis of carotenoid esterification.

Evolutionary Implications. The esterification of lutein that likely occurs directly in the skin in Honduran white bats suggests this process has evolved because it provides some adaptive value. Given the stability that esterified xanthophylls are known to confer to plants, Honduran white bats may benefit from achieving a longer-lasting skin coloration, which may be related to a role in

Table 1. Chromatographic, UV-visible, and MS characteristics of main carotenoids from tissues of Honduran white bats *E. alba*

Peak	Carotenoid	t _R (min)	λ _{max} (nm)	[M+H] ⁺ *	Main product ion†
1 (L, E+n-l)‡	Lutein	15.7	420, 444, 472	569.4335 (−3.1/28.8)	551.4281 [M+H-18] ⁺ ; 495.3642
2 (E+n-l)	Dilauroyl-5,6-epoxy-lutein	33.8	418, 443, 469	949.7668 (2.6/42.3)	931.7601 [M+H-H ₂ O] ⁺ ; 749.5791 [M+H-FA] ⁺ 549.4184 [M+H-2FA] ⁺
3 (E+n-l)	Unknown‡	35.3	420, 444, 472	—	—
4 (E+n-l)	Lauroyl-myristoyl-5,6-epoxy-lutein	36.2	418, 443, 469	977.7970 (−1.4/39.2)	959.7912 [M+H-H ₂ O] ⁺ ; 777.6150 [M+H-FA] ⁺ 749.5890 [M+H-FA] ⁺ ; 549.4179 [M+H-2FA] ⁺
5 (E+n-l)	Myristoyl-oleyl-5,6-epoxy-lutein	37.1	418, 443, 469	1,059.8695 (−2.9/45.6)	1,041.8592 [M+H-H ₂ O] ⁺ ; 831.6697 [M+H-FA] ⁺ 777.6196 [M+H-FA] ⁺ ; 549.4162 [M+H-2FA] ⁺
6 (E+n-l)	Lauroyl-linoleoyl-lutein	45.5	420, 444, 472	1,013.8339 (−1.96/29.3)	995.8237 [M+H-H ₂ O] ⁺ ; 813.6589 [M+H-FA] ⁺ 733.5929 [M+H-FA] ⁺ ; 533.4187 [M+H-2FA] ⁺
7 (E+n-l)	Palmitoyl-linoleoyl-lutein [§]	46.3	420, 444, 472	1,067.8813 (1.74/30.2)	1,049.9804 [M+H-H ₂ O] ⁺ ; 811.6524 [M+H-FA] ⁺ 789.6613 [M+H-FA] ⁺ ; 533.4249 [M+H-2FA] ⁺
8 (E+n-l)	Palmitoyl-linoleoyl-lutein [§]	47.0	420, 444, 472	1,067.8859 (1.84/29.5)	1,049.9797 [M+H-H ₂ O] ⁺ ; 811.6537 [M+H-FA] ⁺ 789.6595 [M+H-FA] ⁺ ; 533.4262 [M+H-2FA] ⁺
9 (E+n-l)	Palmitoyl-linoleoyl-lutein [§]	47.5	420, 444, 472	1,067.8806 (1.06/32.8)	1,049.9992 [M+H-H ₂ O] ⁺ ; 811.6529 [M+H-FA] ⁺ 789.6604 [M+H-FA] ⁺ ; 533.4236 [M+H-2FA] ⁺
10 (E+n-l)	Oleoyl-linoleoyl-lutein	48.4	420, 444, 472	1,095.9183 (1.35/19.8)	1,077.9015 [M+H-H ₂ O] ⁺ ; 813.6593 [M+H-FA] ⁺ 815.6758 [M+H-FA] ⁺ ; 533.4278 [M+H-2FA] ⁺

**m/z* experimental value of the molecular ion. Numbers in parenthesis are mass error in ppm and SigmaFit value.

†Main products ion arising from MS²-based reactions.

‡Peak present in liver extract (L) and/or in ears+nose-leaf (E+n-l) extract.

§Peak not identified.

§*Trans* and *cis* isomers.

separation. The mobile phases were mixtures of methanol:tert-butyl methyl ether:water (A, 81:15:4 and B, 7:90:3), starting with 10 min isocratic A (100%), then a gradient to B (50%) at 40 min, B (100%) at 50 min, A (100%) at 55 min, and isocratic A (100%) from 55 to 60 min. The flow rate was 1 mL/min, and the injection volume was 30 μL. UV-visible spectra were recorded from 350 to 600 nm with a photodiode array spectrometer. A split postcolumn of 0.4 mL/min was introduced directly onto the mass spectrometer ion source. MS was performed in a micrOTOF-QII high-resolution TOF mass spectrometer (UHR-TOF) with quadrupole (qQ)-TOF geometry (Bruker Daltonics) equipped with atmospheric pressure chemical ionization (APCI) source. The instrument was operated in positive ion mode, using a scan range of *m/z* 50–1,200. Mass spectra were acquired through the broadband collision-induced dissociation mode, providing MS and MS/MS spectra simultaneously. The instrument control was performed using Bruker Daltonics Hystar 3.2.

The strategy for the identification of pigment catabolites in plant tissues was adapted to the identification of carotenoids in animal tissues (38). The in-house mass database created *ex professo* comprises monoisotopic masses, elemental composition, and optionally, retention time and characteristic product ions for 360 carotenes, xanthophylls, and xanthophyll esters. Data evaluation was performed with Bruker Daltonics DataAnalysis 4.0. From the

HPLC/TOF-MS data, an automated peak detection on the extracted ion chromatograms expected for the [M+H]⁺ ion of each compound in the database was performed with a script command editor. The identification of carotenoids was performed on the basis of their chromatographic behavior, UV-visible data, and high-resolution MS data. Thus, positive identification rely on mass accuracy of the protonated molecule, mass error in comparison with the calculated mass for the target compound, and comparison of the theoretical and experimental isotope patterns for the assumed protonated molecule. The latter was automatically calculated by the software module SigmaFit, included in the data evaluation software. The SigmaFit value is a measure of the goodness of fit between theoretical and experimental isotopic patterns, with smaller values indicating a better isotopic matching.

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