Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part B 149 (2008) 370–377
CBP
www.elsevier.com/locate/cbpb

Carotenoids and throat pouch coloration in the great frigatebird (*Fregata minor*)

Frans A. Juola [a,*], Kevin McGraw [b], Donald C. Dearborn [c]

[a] *Department of Biology, University of Miami, Coral Gables, FL, USA*
[b] *School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA*
[c] *Department of Biology and Program in Animal Behavior, Bucknell University, Lewisburg, PA, USA*

Received 4 September 2007; received in revised form 24 October 2007; accepted 24 October 2007
Available online 30 October 2007

Abstract

Carotenoid pigments are a common source of red, orange, and yellow coloration in vertebrates. Animals cannot manufacture carotenoids and therefore must obtain them in their diet to produce carotenoid-based coloration. Male great frigatebirds (*Fregata minor*) display a bright red inflated gular pouch as part of their elaborate courtship display. The basis of this coloration until now has not been investigated. Using high-performance liquid chromatography (HPLC), we investigated the types and concentrations of carotenoids that great frigatebirds circulate in their plasma and whether male gular pouch coloration was carotenoid-based. Great frigatebird plasma collected during the breeding season contained three carotenoid pigments in dilute concentrations—tunaxanthin, zeaxanthin, and astaxanthin—with astaxanthin accounting for nearly 85% of the carotenoids present. Astaxanthin was the only carotenoid present in gular pouch tissue, but the concentration is the highest reported for any carotenoid-pigmented avian tissue. Throat pouch reflectance curves were measured with a UV-VIS spectrophotometer, revealing a complex pattern of one UV peak (approx. 360 nm), two absorption valleys (approx. 542 and 577 nm), followed by a plateau at approx 630 nm. The reflectance curve suggests a role for additional pigments, in particular hemoglobin, in the production of color in this ornament.

Keywords: Carotenoid pigments; Astaxanthin; Color signals; Sexual selection; HPLC

1. Introduction

Carotenoids are natural pigments that produce yellow, orange and red coloration in animals and are known to be an important source of ornamental coloration in fish, lizards, and birds (Endler, 1980; Kodric-Brown, 1989; Hill et al., 2002; McGraw et al., 2004). Animals cannot manufacture carotenoids, which therefore must be obtained through the diet (Fox, 1979). In birds, carotenoid-based colors are common in feathers and bare parts (McGraw, 2005), and in some species, it has been demonstrated that color intensity is a direct product of the overall concentration of carotenoid pigments present (Saks et al., 2003). Furthermore, experimental evidence indicates that consumption of carotenoids enhances coloration (Hill et al., 2002; Blount et al., 2003; McGraw and Ardia 2003; Alonso-Alvarez et al., 2004).

In addition to their role in color production, carotenoids serve a variety of important physiological functions including multiple stimulating effects on the immune system (Bendich, 1989; Olson and Owens, 1998; Hill, 1999a). Empirical evidence in birds has demonstrated an association between carotenoids and immune activity (McGraw and Ardia, 2003, 2005; Alonso-Alvarez et al., 2004). The dual functions of color production and immunostimulation suggest that carotenoid-based coloration could convey honest information about individual quality by way of a physiological tradeoff between expression of ornamental color and health status (Lozano, 1994). Hypotheses on the costs associated with carotenoid-based coloration, however, remain controversial because so little is known about their intake (Lozano, 1994; Grether et al., 1999; Hill, 1999a; Hill et al., 2002; Goden and McDonough,

* Corresponding author. Department of Biology, Cox Science Center, Room 73, University of Miami, Coral Gables, FL 33146, USA. Tel.: +1 305 284 2041; fax: +1 305 284 3039.
E-mail address: frans@bio.miami.edu (F.A. Juola).

1096-4959/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2007.10.010

2003). Recent advances in spectrophotometric technology allow for more objective and quantitative assessment of color while considering the range of avian visual perception (Andersson and Prager, 2006).

In sexually dichromatic bird species, in which males exhibit brighter, carotenoid-based colors, males typically circulate higher levels of carotenoids in their blood than do females (Hill, 1995; Bortolotti et al., 1996; Figuerola and Gutierrez, 1998; Negro et al., 1998; McGraw and Ardia, 2005). Seasonal patterns of carotenoid concentrations have been documented in both sexes, with carotenoid concentrations in blood typically being highest during the breeding season when the expression of colorful traits is most pronounced (Hill, 1995; Negro et al., 1998).

A majority of studies investigating the significance of ornamental coloration in birds have focused on colorful species from the order Passeriformes (reviewed in Hill, 1999b, and Hill and McGraw, 2006). More recent studies are beginning to shed light on ornamental coloration in non-passerine birds (Negro et al., 2002; Massaro et al., 2003; Blas et al., 2006; Bortolotti et al., 2006; Velando et al., 2006; Mougeot et al., 2007). The great frigatebird (*Fregata minor*) is a large marine bird from the order Pelecaniformes that exhibits a high degree of sexual dimorphism. Males possess conspicuous sexual ornaments, including a bright red gular pouch that is inflated during elaborate courtship displays (Nelson, 1975). These courtship displays are conducted in dense aggregations where females can assess many potential mates at the same time (Nelson, 1975). Great frigatebird males exhibit dramatic seasonal variation in gular pouch appearance. At the onset of the breeding season, pouches become bright red and extend down greater than 5 cm from the throat (F. Juola pers. obs., Fig. 1B). Red coloration remains intense while males actively display over the course of weeks or months (Fig. 1A and B). Once a male secures a mate, the gular pouch retracts into the throat (Fig. 1C) and the color begins to fade rapidly from red to orange (Fig. 1D). This decline in ornamentation occurs over the course of several weeks (F. Juola pers. obs.). Throughout the non-breeding season, male gular pouches remain small and dull-orange in color. The rapid shift in coloration after mating suggests that development and/or maintenance of this bright red color is costly.

This striking ornament is assumed to be an example of sexual selection by way of female choice (Nelson, 1975; Dearborn and Ryan, 2002; Madsen et al., 2007). The goal of this study was to investigate the source of red coloration in male great frigatebird gular pouches in hopes of shedding light on the possible signaling functions of this color ornament. Specifically, we tested whether male gular pouch coloration was carotenoid-based through identification and quantification of what, if any, carotenoids were circulating in blood and deposited in gular pouch

Fig. 1. A. Upper left: Male great frigatebird (*F. minor*) displaying a bright red, inflated gular pouch on Tern Island, French Frigate Shoals. B. Upper right: Between bouts of displaying, gular pouch is extended from the throat and remains bright red. C. Lower left: After pair formation, the gular pouch is deflated and begins to retract into the throat. C. Lower left: Within days of pair formation, pouch coloration begins to fade from red to orange.

tissue. In addition, we tested whether differences exist in circulating carotenoid levels between males and females of three differing physiological states: courting, incubating, and non-breeding. Integumentary coloration is predicted by levels of carotenoids in plasma for at least six bird species (reviewed in McGraw, 2006). In dichromatic bird species that exhibit carotenoid-based coloration, colorful males commonly circulate higher levels of carotenoids than do females (Bortolotti et al., 1996; McGraw et al., 2003; McGraw and Ardia, 2005). In addition, females may circulate higher levels of carotenoids during the physiological state prior to egg-laying, in order to divert carotenoid pigments into egg yolk (Blount et al., 2000).

Finally, we describe the spectral curve of the male gular pouch and investigated whether any spectral properties of this curve might provide further insight into the basis of this colorful ornament. Pigments absorb light according to characteristic absorption functions, the patterns of which can provide insight into the types of pigments present in animal tissues (Andersson and Prager, 2006). In particular, carotenoids absorb light in the blue–green wavelengths (430–500 nm) and consequently produce distinct reflection peaks in the yellow, orange, and red wavelengths (McGraw et al., 2007).

2. Materials and methods

We collected blood and tissue samples for this study on Tern Island, French Frigate Shoals, in the northwestern Hawaiian Islands. There are roughly 2000 nesting attempts by great frigatebirds (*Fregata minor*) on this 14-ha island each year (Dearborn and Anders, 2006). One hundred and twelve adult great frigatebirds of known breeding status (61 displaying males, 10 incubating males, 11 non-breeding males, 10 searching females, 10 incubating females, 10 non-breeding females) were captured, either by hand or with a hand net, between 0900 and 1400 h from February–March 2006. Breeding status was determined by behavior and plumage. Displaying males exhibit bright red inflated gular pouches and freshly molted black plumage, while non-breeding males exhibit retracted, dull-orange pouches and an overall faded brownish-black plumage color. Females seeking mates fly low around the breeding colony and appear to be visually inspecting males with inflated throat pouches. These females also exhibit freshly molted black plumage; plumage of non-breeding females is also faded brownish-black in color. At the time of capture, approximately 1 mL of blood was collected from the brachial vein of each bird. Plasma was separated from blood by centrifugation of blood samples at 12,000 *g* for 5 min. Centrifugation was completed within 4 h of blood collection, and all plasma samples were then stored at −20 °C until carotenoid analysis approximately 4 months later. Eight gular pouch tissue samples were collected from adult male great frigatebirds found dead (within ~24 h) in the breeding colony from 1999–2006. Pouch samples were stored at −20 °C.

The types and amounts of carotenoids in plasma and gular pouch tissue were analyzed using high-performance liquid chromatography (HPLC). Plasma analysis followed previously published methods (McGraw et al., 2002, 2006), with the exception of using 50 μL of plasma from each sample for carotenoid extractions (because pigment concentrations were so low). Briefly, reverse-phase chromatography was run with a Waters Alliance 2695 autosampler instrument (Waters Corporation, Milford, MA, USA) equipped with a Waters YMC Carotenoid C-30 5 um column (4.6×250 mm) that was heated to 30 °C with a built-in column heater. We used an isocratic solvent system (42:42:16, methanol:acetonitrile:dichloromethane, v/v/v), at a flow rate of 1.2 mL/min for 12 min, to analyze polar carotenoids after initial tests revealed that no non-polar carotenoids (i.e. cryptoxanthins or carotenes) were present. The detection limits of our system were 0.01 μg/mL for plasma analyses and 0.01 μg/g for pouch analyses.

For each gular pouch, a 0.5 g cube of flesh was sectioned and processed as follows: The gular pouch tissue was ground in presence of 2 mL hexane:TBME (1:1) for 5 min. The solvent was transferred to a new tube, while the grinding jar was rinsed with 1 mL hexane: MTBE to recover any residual pigment. The solvent was then centrifuged at ca. 500 *g* for 5 min. and transferred to a new tube where it was evaporated to dryness under nitrogen, then resuspended in 1 mL 0.02 M KOH in order to saponify (attempts to analyze carotenoids from unsaponified material were unsuccessful). It was then placed in darkness at room temperature for 4 h, after which 1 mL saturated NaCl was added. The solution was vortexed, 2 mL ddH2O was added, and the solution was again vortexed. Next, 2 mL MTBE was added and the solution was shaken vigorously for 1 min. This solution was then centrifuged at ca. 500 g for 5 min, and finally evaporated to dryness. Standard HPLC methods (McGraw et al., 2006) were then followed exactly as for the plasma samples. Reported carotenoid concentrations in pouch tissues refer to dry weight of the starting material.

During the mating season of 2007, reflectance curves were obtained from 10 displaying male and 10 incubating male great frigatebirds using a UV-Vis spectrophotometer (USB4000, configured range 250–850 nm, Ocean Optics Inc.) with a pulsed xenon lamp (PX-2) as a light source. Using a black rubber sheath that excluded ambient light, we held a bifurcated micron fiber-optic probe 3 mm from and perpendicular to the pouch tissue. All measurements were conducted indoors, in a dark

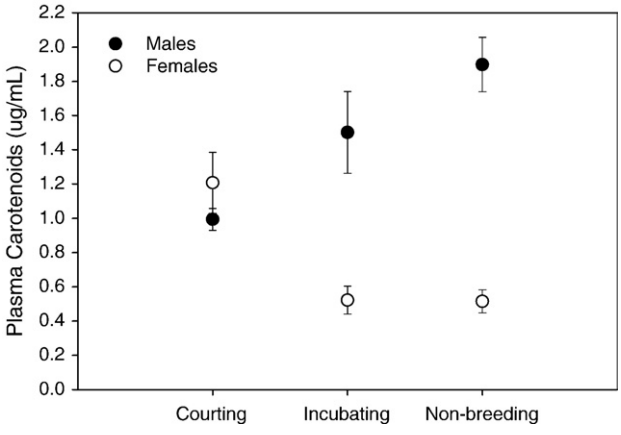

Fig. 2. Representative two-dimensional HPLC chromatogram depicting carotenoids detected in the plasma of frigatebirds.

Table 1
Comparison of plasma carotenoid concentrations and percent composition of each carotenoid present in male and female great frigatebirds

	Males (n=82)	Females (n=30)	Z	P
Concentration (μg/mL)				
Astaxanthin	0.97±0.05	0.59±0.07	−4.69	<0.001
Tunaxanthin	0.18±0.03	0.13±0.02	−0.83	0.41
Zeaxanthin	0.04±0.004	0.03±0.01	−0.53	0.60
Total carotenoids	1.18±0.07	0.75±0.49	−3.86	<0.001
Percent composition (of total plasma carotenoids)				
Astaxanthin	82.00±0.01	78.88±0.02	−2.10	0.04
Tunaxanthin	14.94±0.01	16.71±0.02	−1.70	0.90
Zeaxanthin	3.06±0.003	4.41±0.01	−0.86	0.39

Mann–Whitney U tests were used to compare differences between males and females (Z-statistic). Mean±SE are given for each group.

room. Each curve was generated relative to a white standard (WS-1, Ocean Optics). Using SpectraSuite software (Ocean Optics), we averaged 20 sequential spectra for each of five arbitrarily selected locations on each pouch sample. To obtain an average spectral curve for displaying males and for incubating males, the median percent reflectance for each 10 nm increment was averaged for the 10 individuals from each category, across the wavelengths relevant to the avian visual spectrum (300–700 nm).

2.1. Statistical analysis

We used SYSTAT 11 (Systat Software, Inc.) for all statistical analyses. We used Mann–Whitney U tests to compare differences in the carotenoid concentrations and percent composition of each carotenoid present in the plasma of male and female great frigatebirds. We used a two-way ANOVA to compare differences between sex and three time periods. We conducted post-hoc analyses of differences between individual groups using Fisher's least significant difference test.

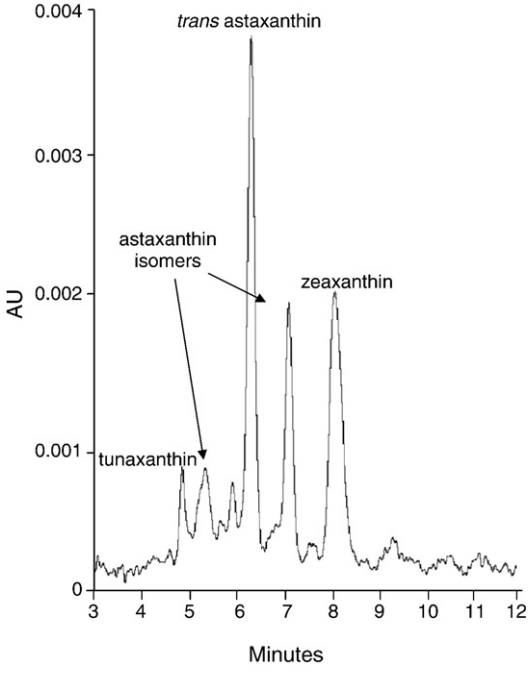

Fig. 3. Great frigatebird plasma carotenoid concentrations by sex and breeding status. Sample sizes were courting males (61), courting female 10), incubating males (10), incubating females (10), non-breeding males (11), non-breeding females (10). Error bars represent standard errors of the means.

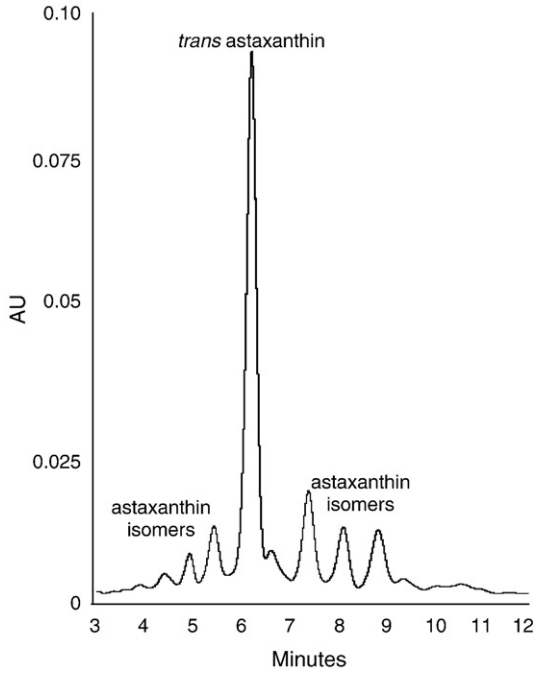

Fig. 4. Representative two-dimensional HPLC chromatogram depicting carotenoids detected in the pouch tissue of frigatebirds.

3. Results

Great frigatebird blood plasma contained three carotenoid pigments: astaxanthin, tunaxanthin, and zeaxanthin (total = 1.06±0.06 μg/mL; mean±SE). Astaxanthin accounted for 84.5±0.010% of the total plasma carotenoids (0.87±0.04 μg/mL).

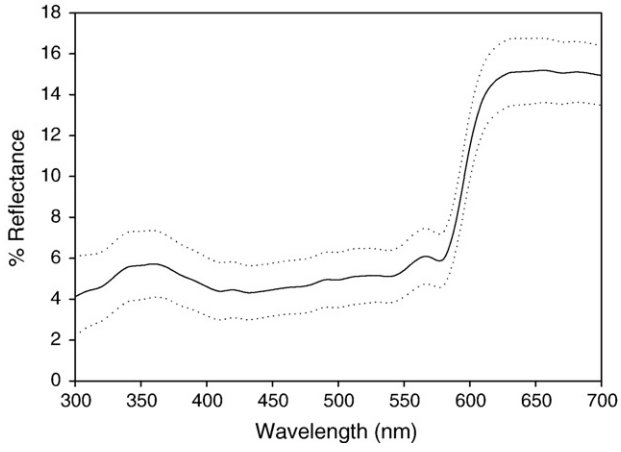

Fig. 5. Mean spectral reflectance curve of the throat pouch of displaying male great frigatebirds (n=10). Measurements were averaged from 5 readings taken from the non-inflated portion of skin under the bill. Solid line represents the mean reflectance curve. Dotted lines represent the upper and lower 95% confidence limits.

Zeaxanthin and tunaxanthin accounted for 2.8±0.003% and 12.7±0.010% of the total plasma carotenoids, respectively (0.04±0.004 μg/mL; 0.16±0.02 μg/mL). A representative 2-dimentional HPLC chromatogram of plasma is presented in Fig. 2. Males had significantly higher astaxanthin concentrations in their plasma than did females, when individuals from all breeding stages were combined (Table 1). The relative proportion of astaxanthin was significantly higher in males compared to females, while the relative proportions of tunaxanthin and zeaxanthin did not differ between males and females (Table 1). Total carotenoid concentrations in plasma differed significantly between the sexes (two-way ANOVA, sex: $F_{1,106}=36.08$, $P<0.001$; Fig.3, but not in relation to breeding category (two-way ANOVA, $F_{2,106}=0.77$, $P=0.47$; Fig. 3). However, there was a significant interaction between sex and breeding category (two-way ANOVA, sex * category interaction: $F_{2,106}=18.976$, $P<0.001$; Fig. 3). Post-hoc comparisons showed that non-breeding males circulated significantly higher carotenoids compared with courting males (Fisher's least-significant difference, $P<0.001$), courting females ($P=0.002$), incubating females ($P<0.001$), and non-breeding females ($P<0.001$). In addition, incubating males circulated significantly higher levels compared with courting males ($P=0.004$), incubating females ($P<0.001$), and non-breeding females ($P<0.001$). Courting males circulated higher levels than incubating females ($P=0.007$) and non-breeding females ($P=0.006$). Finally, courting females circulated higher levels than incubating females ($P=0.003$) and non-breeding females ($P=0.003$). Astaxanthin was the only carotenoid present in gular pouch tissue. Pouch tissue concentrations (1268.8±282.5 μg/g; mean±SE) were extremely high compared to total plasma concentrations. One gular pouch had an astaxanthin concentration exceeding 3000 μg/g (3668.0). A representative 2-dimentional HPLC chromatogram for pouch tissue is presented in Fig. 4.

The average reflectance curve for gular pouches of displaying males ($n=10$; 5 spectra averaged for each) showed a distinctive pattern containing an initial peak in the UV range at approximately 360 nm. In addition, small absorption valleys occurred at approximately 542 nm and 577 nm, followed by a plateau at approximately 630 nm (Fig. 5). The average reflectance curve for gular pouches of incubating males ($n=10$, 5 spectra averaged for each) was similar to that of displaying males. There was an initial peak in the UV range at approximately 360 nm, and the final plateau again appears at approximately 630 nm. However, the absorption valleys present in the displaying male pouch spectra at 542 nm and 577 nm are not present in the incubating male pouch (Fig. 6).

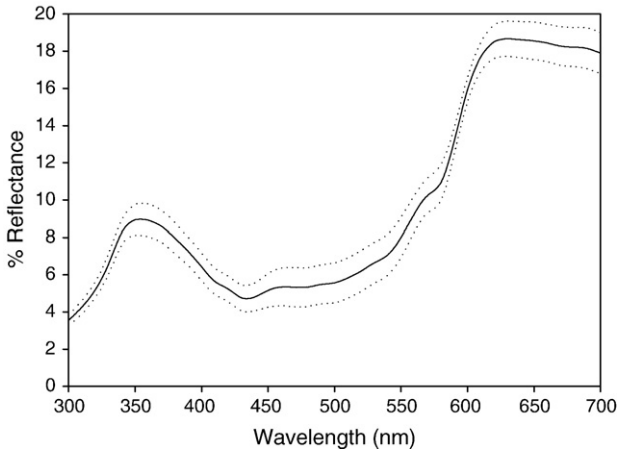

Fig. 6. Mean spectral reflectance curve of the throat pouch of incubating male great frigatebirds ($n=10$). Measurements were averaged from 5 readings from the skin patch under the bill. Solid line represents the mean reflectance curve. Dotted lines represent the upper and lower 95% confidence limits.

4. Discussion

Carotenoids are a known source of ornamental coloration in a variety of bird tissues, including feathers (Stradi, 1999; McGraw et al., 2001; McGraw and Hardy, 2006), bills (Bennett et al., 1996; McGraw and Ardia, 2005), and legs (Bortolotti et al., 1996; Casagrande et al., 2006). Far less attention has been given to colorful fleshy parts in birds (e.g. wattles, combs, eyerings, pouches) and the potential role that carotenoids play in the expression of these colors (but see Negro et al., 2006; Mougeot et al., 2007). Because the concentration of astaxanthin, a red carotenoid, in male great frigatebird throat pouch tissue was so high, it is likely that astaxanthin contributes substantially to the red color of these ornaments. This has implications for the information content of this display signal, as astaxanthin is a known stimulant of the immune system and a powerful antioxidant (Hussein et al., 2006; McGraw, 2006). Previous work on this population demonstrated variation in displaying male pouch color (Dearborn and Ryan, 2002). Males capable of displaying astaxanthin-rich pouch tissue may be advertising superior fitness if only excess carotenoids beyond what are needed for basic physiological functions is available for diversion into throat pouch tissue. If females select mates based on coloration of their throat pouches, selection of males with the most astaxanthin-rich pouches could potentially result in direct benefits to females. Perhaps the most intensely colored males are in better condition and are more efficient foragers, able to provide superior parental care. In addition, females could gain indirect benefits if the ability of males to acquire or assimilate carotenoids is a genetic trait that could be inherited by their offspring.

High concentrations of carotenoids have been reported in other avian tissues, such as in the feathers of American goldfinches (McGraw and Gregory, 2004). One throat pouch tissue analyzed in our study exceeded 3000 μg/g, and two additional pouch tissues exceeded 2000 μg/g; these to our knowledge are the highest reported concentrations of carotenoids in any bird tissue. Astaxanthin concentration in throat pouch tissue was remarkably high compared to the total carotenoid concentrations found in blood plasma. In addition, of the three carotenoids circulated in great frigatebird blood plasma, only astaxanthin was deposited in throat pouch tissue. It is rare that pure astaxanthin is deposited in avian tissues, as it is almost always accompanied by other 4-oxo-carotenoids (Stradi, 1999). This study, to our knowledge, is the third demonstration of pure astaxanthin deposition in bird tissue and the first in bare skin. Interestingly, the two previous

examples may both be the result of human introduction of astaxanthin into the environment (Negro and Garrido-Fernandez, 2000; McGraw and Hardy, 2006). Astaxanthin present in frigatebird blood plasma suggests that it is acquired directly from a food source, rather than being manufactured from another precursor. The dietary source of astaxanthin in great frigatebirds on Tern Island is not well understood. Great frigatebird diet has been reported to consist of primarily flying fish and squid (Metz and Schreiber, 2002). Initial analyses of diet samples revealed moderate levels of astaxanthin in some fish (F. Juola and K.J. McGraw pers. obs.). Astaxanthin is commonly acquired through aquatic food sources such as fish and crustaceans (Goodwin, 1984), and some squid species are highly enriched in astaxanthin (K. McGraw, M. Hipfner, and J. Dale, pers. obs.), making this a likely source for astaxanthin accumulation in great frigatebirds.

Carotenoid concentrations in great frigatebird blood plasma were similar to those reported for other large, non-passerine birds, and lower than those reported for small passerine birds (Tella et al., 2004). Carotenoid concentrations in blood plasma differ widely across avian species (Parker, 1996; Tella et al., 1998; Slifka et al., 1999; McGraw, 2005), with phylogeny being a significant predictor of circulating carotenoid levels (Tella et al., 2004). Additional factors such as diet, (Tella et al., 2004), occurrence of ornamental coloration (Tella et al., 2004), and efficiency in assimilating consumed carotenoids into the blood system (McGraw, 2005) have been used to explain higher levels of circulating carotenoids in passerine birds. In addition, body size may play a role. In vertebrates, food consumption is proportional to body mass raised to the 3/4 power (Peters, 1983). The increase in blood volume is directly proportional to the increase in species body mass. Thus, larger animals eat less food, and presumably fewer carotenoids, per body mass unit than smaller species, and incorporate relatively fewer carotenoids into their proportionally equal blood volume (Tella et al., 2004; McGraw, 2005).

As has been found in other dichromatic species (Hill, 1995; Bortolotti et al., 1996; Negro et al., 1998; McGraw and Ardia, 2005), male great frigatebirds circulate higher concentrations of carotenoids then do females. Dietary differences or differences in the absorption capacities of males and females could mechanistically account for this difference in carotenoid levels. Interestingly, incubating and non-breeding males exhibited higher levels of circulating carotenoids compared with displaying males. A prediction of higher circulating carotenoid levels in more colorful males (while displaying) seems intuitive if the assumption is that males flood their system with more carotenoids during times of peak color production. However, circulating carotenoids diverted into tissues such as skin, beaks, or feathers may deplete carotenoid levels circulating in the blood. For this reason, predictions of either higher or lower circulating carotenoid levels in more colorful males seem plausible. Lower carotenoid levels in displaying males lend support to the idea that carotenoids are depleted by diversion into throat pouch tissue. Courting females had higher circulating carotenoid levels than incubating and non-breeding females, a pattern similar to that reported in American kestrels (Negro et al., 1998). It's possible that females circulated higher carotenoid levels during courtship in preparation for egg-laying, as carotenoids are a known component of egg yolk (Fletcher 1992; Royle et al., 1999).

The average spectral curve presented here is the first reported for any frigatebird throat pouch. A spectral curve derived solely from astaxanthin-based red tissue would predict a single absorption valley at approximately 470 nm, followed by a steeper upslope and plateau at around 550 nm. However, gular pouch tissue is highly vascular. Blood filled capillaries are visible in inflated pouches (F. Juola pers. obs.) The presence of oxyhemoglobin produces a characteristic spectral pattern of absorption bands at 542 nm and 577 nm (Zonios et al., 2001). Great Frigatebird throat pouches exhibited absorption bands at these locations. This suggests that increased blood flow and the presence of hemoglobin may contribute substantially to the overall coloration and spectral properties of these throat pouches during the display period. This notion is supported by the rapid fading of pouch color from red to orange once a male has secured a mate, something that could be accomplished through diversion of blood flow. Furthermore, one key difference between the spectral curves of displaying males and those of incubating males was the lack of absorption bands expected with the presence of hemoglobin in incubating males. This supports the notion that gular pouch coloration is produced through a combination of carotenoid deposition and increased blood flow.

A secondary peak appeared in the UV-range of the pouch reflectance spectrum. UV-signaling in birds has been the focus of a number studies in recent years, since the advent of more sophisticated analytical methods. It has been demonstrated that females of some species use UV-reflecting feathers to assess male quality and prefer those males that exhibit greater UV reflection (Bennett et al., 1996; Hunt et al., 1999). UV-reflectance in avian skin parts is not well documented. Some examples have been reported in passerine species (Hunt et al., 2003; Jourdie et al., 2004) and in the orbital combs of the red grouse (*Lagopus lagopus scoticus*; Mougeot and Redpath, 2004). Interestingly, UV brightness in the red orbital combs of the red grouse was negatively correlated with parasite infection rates (Mougeot et al., 2005), suggesting that UV reflectance could serve as a reliable signal of parasite resistance. It has been demonstrated that feathers with carotenoid-based colors create such UV spectral patterns by absorbing light from a structural white tissue that in which the carotenoids are embedded (Shawkey and Hill, 2005). Frigatebird pouch tissue was comprised of two tissue layers, an inner tissue layer that was pink or white, and an outer dermal layer that contained the astaxanthin pigments (K. McGraw pers. obs.). It is possible that, as in grouse (Mougeot et al., 2007), the pouch tissue gets its UV reflectance from the white background layer.

Acknowledgements

We thank Angela Anders, Beth Flint, Dominique Horvath and the U.S. Fish and Wildlife Service for logistical support and access to Tern Island, Morgan Gilmour for assistance in the

field, and William A. Searcy for comments on the original manuscript. Funding sources included the University of Miami, Department of Biology's Kushlan/Frohring Graduate Research Support Fund, and the Maytag endowment.

References

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B., Sorci, G., 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. Am. Nat. 164, 651–659.

Andersson, S., Prager, M., 2006. Quantifying colors. In: Hill, G.E., McGraw, K.J. (Eds.), Bird Coloration. Mechanisms and Measurements, vol. 1. Harvard University Press, Cambridge, MA.

Bendich, A., 1989. Carotenoids and the immune response. J. Nutr. 119, 112–115.

Bennett, A.T.D., Cuthill, I.C., Partridge, J.C., Maeir, E.S., 1996. Ultraviolet vision and mate choice in zebra finches. Nature 380, 433–435.

Blas, J., Pérez-Rodríguez, L., Bortolotti, G.R., Viñuela, J., Marchant, T.A., 2006. Testosterone increases bioavailability of carotenoids: insights into the honesty of sexual signaling. PNAS 103, 18633–18637.

Blount, J.D., Houston, D.C., Moller, A.P., 2000. Why egg yolk is yellow. Trends Ecol. Evol. 15, 47–49.

Blount, J.D., Metcalfe, N.B., Birkhead, T.R., Surai, P.F., 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 300, 125–127.

Bortolotti, G., Negro, J.J., Tella, J.L., Marchant, T.A., Bird, D.M., 1996. Sexual dichromatism in birds independent of diet, parasites and androgens. Proc. R. Soc. Lond., Ser. B 263, 1171–1176.

Bortolotti, G., Blas, J., Negro, J.J., Tella, J.L., 2006. A complex plumage pattern as an honest social signal. Anim. Behav. 72, 423–430.

Casagrande, S., Csermely, D., Pini, E., Bertacche, V., Tagliavini, J., 2006. Skin carotenoid concentration correlates with male hunting skill and territory quality in the kestrel *Falco tinnunculus*. J. Avian Biol. 37, 190–196.

Dearborn, D.C., Ryan, M.J., 2002. A test of the Darwin-Fisher theory for the evolution of male secondary sexual traits in monogamous birds. J. Evol. Biol. 15, 307–313.

Dearborn, D.C., Anders, A.D., 2006. Demography and reproductive ecology of great frigatebirds. Atoll Res. Bull. 543, 159–171.

Endler, J.A., 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34, 76–91.

Figuerola, J., Gutierrez, R., 1998. Sexual differences in levels of blood carotenoids in Cirl buntings *Emberiza cirlus*. Ardea 86, 245–248.

Fletcher, D.L., 1992. Methodology for achieving pigment specifications. Poult. Sci. 71, 733–743.

Fox, D.L., 1979. Biochromy: Natural Coloration of Living Things. University of California Press, Berkeley.

Goden, J.G., McDonough, H.E., 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. Behav. Ecol. 14, 194–200.

Goodwin, T.W., 1984. The biochemistry of the carotenoids. Animals, vol 2. Chapman and Hall, New York, NY.

Grether, G.F., Hudon, J., Millie, D.F., 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proc. R. Soc. Lond., Ser. B 266, 1317–1322.

Hill, G.E., 1995. Seasonal variation in circulating carotenoid pigments in the house finch. Auk 112, 1057–1061.

Hill, G.E., 1999a. Is there an immunological cost to carotenoid-based ornamental coloration? Am. Nat. 154, 589–595.

Hill, G.E., 1999b. Mate choice, male quality, and carotenoid-based plumage coloration. Proc. Int. Ornithol. Congr. 22, 1654–1668.

Hill, G.E., McGraw, K.J., 2006. Bird Coloration. Function and Evolution, vol. II. Harvard University Press, Cambridge, MA.

Hill, G.E., Inouye, C.Y., Montgomerie, R., 2002. Dietary carotenoids predict plumage coloration in wild house finches. Proc. R. Soc. Lond., Ser. B 269, 1119–1124.

Hunt, S., Cuthill, I.C., Bennett, A.T.D., Griffiths, R., 1999. Preferences for ultraviolet partners in the blue tit. Anim. Behav. 58, 809–815.

Hunt, S., Kilner, R.M., Langmore, N.E., Bennett, A.T.D., 2003. Conspicuous, ultraviolet-rich mouth colours in begging chicks. Proc. R. Soc. Lond., Ser. B 270, S25–S28 (suppl.).

Hussein, G., Sankawa, U., Goto, H., Matsumoto, K., Watanabe, H., 2006. Astaxanthin, a carotenoid with potential in human health and nutrition. J. Nat. Prod. 69, 443–449.

Jourdie, V., Moureau, B., Bennett, A.T.D., Heeb, P., 2004. Ultraviolet reflectance by the skin of nestlings. Nature 431, 262.

Kodric-Brown, A., 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociob. 25, 393–401.

Lozano, A., 1994. Carotenoids, parasites, and sexual selection. Oikos 70, 309–311.

Madsen, V., Dabelsteen, T., Osorio, D., Osorno, J.L., 2007. Morphology and ornamentation in male magnificent frigatebirds: variation with age class and mating status. Am. Nat. 169, S93–S111.

Massaro, M., Davis, L.S., Darby, J.T., 2003. Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antipodes*). Behav. Ecol. Sociobiol. 55, 169–175.

McGraw, K.J., 2005. Interspecific variation in dietary carotenoid assimilation in birds: links to phylogeny and color ornamentation. Comp. Biochem. Physiol. B 142, 245–250.

McGraw, K.J., 2006. Mechanics of carotenoid-based coloration. In: Hill, G.E., McGraw, K.J. (Eds.), Bird Coloration. Mechanisms and Measurements, vol 1. Harvard University Press, Cambridge, MA.

McGraw, K.J., Ardia, D.R., 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. Am. Nat. 162, 704–712.

McGraw, K.J., Gregory, A.J., 2004. Carotenoid pigments in male American goldfinches: what is the optimal biochemical strategy for becoming colourful? Biol. J. Linn. Soc. 83, 273–280.

McGraw, K.J., Ardia, D.R., 2005. Sex differences in carotenoid status and immune performance in zebra finches. Evol. Ecol. Res. 7, 251–262.

McGraw, K.J., Hardy, L.S., 2006. Astaxanthin is responsible for the pink plumage flush in Franklin's and ring-billed gulls. J. Field Ornithol. 77, 29–33.

McGraw, K.J., Hill, G.E., Stradi, R., Parker, R.S., 2001. The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and Northern Cardinals (*Cardinalis cardinalis*). Physiol. Biochem. Zool. 74, 843–852.

McGraw, K.J., Adkins-Regan, E., Parker, R.S., 2002. Anhydrolutein in the zebra finch: a new, metabolically derived carotenoid in birds. Comp. Biochem. Physiol. B 132, 811–818.

McGraw, K.J., Gregory, A.J., Parker, R.S., Adkins-Regan, E., 2003. Diet, plasma carotenoids, and sexual coloration in the zebra finch (*Taeniopygia guttata*). Auk 120, 400–410.

McGraw, K.J., Hill, G.E., Navara, K.J., Parker, R.S., 2004. Differential accumulation and pigmenting ability of dietary carotenoids in colorful finches. Physiol. Biochem. Zool. 77, 484–491.

McGraw, K.J., Nolan, P.M., Crino, O.L., 2006. Carotenoid accumulation strategies for becoming a colourful House Finch: analyses of plasma and liver pigments in wild moulting birds. Funct. Ecol. 20, 678–688.

McGraw, K.J., Toomey, M.B., Nolan, P.M., Morehouse, N.I., Massaro, M., Jouventin, P., 2007. A description of unique fluorescent yellow pigments in penguin feathers. Pigm. Cell Res. 20, 301–304.

Metz, V.G., Schreiber, E.A., 2002. Great Frigatebird (*Fregata minor*). In: Poole, A., Gill, F. (Eds.), The Birds of North America, No. 681. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, DC.

Mougeot, F., Redpath, S.M., 2004. Sexual ornamentation relates to immune function in male red grouse *Lagopus lagopus scoticus*. J. Avian Biol. 35, 425–433.

Mougeot, F., Redpath, S.M., Leckie, F., 2005. Ultra-violet reflectance of male and female red grouse, Lagopus lagopus scoticus: sexual ornaments reflect nematode parasite intensity. J. Avian Biol. 36, 203–209.

Mougeot, F., Martínez-Padilla, J., Pérez-Rodríguez, L., Bortolotti, G.R., 2007. Carotenoid-based colouration and ultraviolet reflectance of the sexual ornaments of grouse. Behav. Ecol. Sociobiol. 61, 741–751.

Negro, J.J., Garrido-Fernandez, J., 2000. Astaxanthin is the major carotenoid in tissues of White Storks (*Ciconia ciconia*) feeding on introduced crayfish (*Procambarus clarkii*). Comp. Biochem. Physiol. B 126, 347–352.

Negro, J.J., Botolotti, G.R., Tella, J.L., Fernie, K.J., Bird, D.M., 1998. Regulation of integumentary colour and plasma carotenoids in American kestrels consistent with sexual selection theory. Funct. Ecol. 12, 307–312.

Negro, J.J., Grande, J.M., Tella, J.L., Garrido, J., Hornero, D., Donázar, J.A., Sanchez-Zapata, J.A., Benítez, J.R., Barcell, M., 2002. Coprophagy: an unusual source of essential carotenoids. Nature 416, 807–808.

Negro, J.J., Sarasola, J.H., Fariñas, F., Zorrilla, I., 2006. Function and occurrence of facial flushing in birds. Comp. Biochem. Physiol. A 143, 78–84.

Nelson, J.B., 1975. The breeding biology of frigatebirds: a comparative review. Living Bird 14, 113–155.

Olson, V.A., Owens, I.P.F., 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol. Evol. 13, 510–514.

Parker, R.S., 1996. Absorption, metabolism, and transport of carotenoids. FASEB J. 10, 542–551.

Peters, R.H., 1983. The Ecological Implications of Body Size. Cambridge University Press, Cambridge, U.K.

Royle, N.J., Surai, P.F., McCartney, R.J., Speake, B.J., 1999. Parental investment and egg yolk lipid composition in gulls. Funct. Ecol. 13, 298–306.

Saks, L., McGraw, K.J., Horak, P., 2003. How feather colour reflects its carotenoid content. Funct. Ecol. 17, 555–561.

Shawkey, M.D., Hill, G.E., 2005. Carotenoids need structural colours to shine. Biol. Lett. 1, 121–124.

Slifka, K.A., Bowen, P.E., Stacewicz-Sapuntzakis, M., Crissey, S.D., 1999. A survey of serum and dietary carotenoids in captive wild animals. J. Nutr. 129, 380–390.

Stradi, R., 1999. Pigmenti e sistematica degli uccelli. In: Brambilla, L., Canali, G., Mannucci, E., Massa, R., Saino, N., Stradi, R., Zerbi, G. (Eds.), Colori in volo: il piumaggio degli uccelli. Universita degli Studi di Milano, Milan, Italy.

Tella, J.L., Negro, J.J., Rodriguez-Estrella, R., Blanco, G., Forero, M.G., Blazquez, M.C., Hiraldo, F., 1998. A comparison of spectrophotometry and color charts for evaluating total plasma carotenoids in wild birds. Physiol. Zool. 71, 708–711.

Tella, J.L., Figuerola, J., Negro, J.J., Blanco, G., Rodriguez-Estrella, R., Forero, M.G., Blazquez, M.C., Green, A.J., Hiraldo, F., 2004. Ecological, morphological, and phylogenetic correlates of interspecific variation in plasma carotenoikd concentrations in birds. J. Evol. Biol. 17, 156–164.

Velando, A., Beamonte, R., Torres, R., 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. Oecologia 149, 543–552.

Zonios, G., Bykowski, J., Kollias, N., 2001. Skin melanin, hemoglobin, and light scattering properties can be quantitatively assessed in vivo using diffuse reflectance spectroscopy. J. Invest. Dermatol. 117, 1452–1457.