ELSEVIER

Comparative Biochemistry and Physiology, Part B 139 (2004) 45–51

CBP

www.elsevier.com/locate/cbpb

The evolution of carotenoid coloration in estrildid finches: a biochemical analysis

K.J. McGraw[a,*], J.G. Schuetz[b]

[a]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, United States
[b]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, United States

Received 4 March 2004; received in revised form 9 June 2004; accepted 10 June 2004

Abstract

The estrildid finches (Aves: Passeriformes: Estrildidae) of Africa, Asia, and Australia have been the focus of several recent tests of sexual selection theory. Many estrildids display bright red, orange, or yellow colors in the beak or plumage, which typically are generated by the presence of carotenoid pigments. In this study, we used high-performance liquid chromatography to investigate the carotenoid content of feathers and other colorful tissues in seven species of estrildids. Star finches (*Neochmia ruficauda*) and diamond firetails (*Stagonopleura guttata*) circulated two main dietary carotenoids (lutein and zeaxanthin) through the blood and liver and used both to acquire a yellow plumage color. However, five other estrildids (common waxbill, *Estrilda astrild*; black-rumped waxbill, *Estrilda troglodytes*; zebra waxbill, *Amandava subflava*; red avadavat, *Amandava amandava*; and zebra finch, *Taeniopygia guttata*) circulated these same dietary carotenoids along with two metabolites (dehydrolutein and anhydrolutein) through the blood and/or liver and used all four as yellow plumage colorants. We subsequently tracked the distribution of these pigments using a published phylogeny of estrildid finches to determine the evolutionary pattern of carotenoid metabolism in these birds. We found that finches from the most ancient tribe of estrildids (Estrildini) possessed the ability to metabolize dietary carotenoids. Although carotenoids from the most ancestral extant estrildid species have yet to be analyzed, we hypothesize (based on their relationships with other songbirds known to have such metabolic capabilities) that these finches inherited from their ancestors the capability to metabolize carotenoids. Interestingly, later in estrildid evolution, certain taxa lost the ability to metabolize dietary carotenoids (e.g., in the Poephilini), suggesting that the occurrence of carotenoid metabolism can be labile and is likely shaped by the relative costs and benefits of color signaling across different species.

Keywords: Estrildidae; Carotenoids; Plumage coloration; Sexual selection

1. Introduction

The estrildid finches (Aves: Passeriformes: Estrildidae) are a diverse group of nearly 140 species found in Africa, Asia, and Australia (Goodwin, 1982). Based on several lines of morphological, natural history, and biochemical evidence, they have been divided into three main tribes within the family: (1) Estrildini—waxbills restricted largely to Africa; (2) Poephilini—grassfinches centered in Australasia; and (3) Lonchurini—mannikins found throughout the paleotropics (Delacour, 1943; Christidis, 1987a,b). Each group contains species that display a dazzling array of colors in their feathers and bare parts (e.g., beak, legs), ranging from the deep blues in parrot finches (*Erythrura* spp.) to the spectacular reds in the avadavats (*Amandava* spp.). There has been a recent surge of interest in the evolutionary significance of these bright colors in estrildids, and the role that they play in facilitating sexual mate choice (Burley and Coopersmith, 1987; Burley and Symanski, 1998; Witte and Curio, 1999; Collins and Luddem, 2002) and imprinting (Plenge et al., 2000).

One of the most common color patterns seen in estrildids is rich red, orange, and yellow pigmentation, which is

* Corresponding author. Current address: Department of Animal Science, University of California—Davis, One Shields Avenue, Davis, CA 95616, USA. Tel.: +1 530 752 2401; fax: +1 530 752 0175.
E-mail address: kjmcgraw@ucdavis.edu (K.J. McGraw).

1096-4959/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2004.06.006

typically conferred on songbirds by carotenoid pigments (Brush, 1978; Stradi, 1998). Zebra finches (*Taeniopygia guttata*), for example, use carotenoids to acquire their red beak and leg coloration (McGraw et al., 2002a). Carotenoid-based colors also have captured the recent attention of behavioral ecologists due to their ability to serve as honest signals of both foraging successes (because carotenoids are limited in nature; Endler, 1983; Hill, 1992; Grether et al., 1999; Hill et al., 2002) and general health (because carotenoids are active antioxidants and immunostimulants; Lozano, 1994; Møller et al., 2000; McGraw and Ardia, 2003) of individuals. While there is now an enormous literature on the proximate control mechanisms and signaling function of carotenoid colors in animals like fishes and birds (reviewed in Hill, 1999, 2002), the historical paths by which birds evolve their conspicuous, carotenoid-containing colors are less well characterized.

To begin to understand carotenoid-based color evolution from a phylogenetic perspective, it is first important to classify variation in the types of color displays we see. Carotenoid colors come in two basic biochemical forms in animals. Some species use carotenoids directly acquired from food to color their skin, scales, or feathers; others transform these dietary pigments into different forms that appear in the integument and often are more colorful or more oxidized (Stradi, 1998). It has been argued that metabolically derived forms of carotenoids are more complex and energetically demanding to produce (Hill, 2000) and thus may be expected to have evolved from a more simple form of coloration that directly involves dietary carotenoids (Hill, 1996). Although the types of carotenoids have now been identified from nearly 100 species of birds spanning 17 families (Brambilla et al., 1999), we have never before had the opportunity to combine phylogenetic and biochemical data to test this hypothesis regarding the evolutionary trajectory of carotenoid metabolism and coloration in birds.

In this study, we sampled carotenoids from seven different estrildid finch species that represented each of the three main tribes in the family. Carotenoid data were also available for two additional species—red-headed parrot finch (*Erythrura psittacea*) and gouldian finch (*Chloebia gouldiae*)—in Brambilla et al. (1999). The diet of estrildids is primarily—if not exclusively in some species—made up of seeds (Goodwin, 1982), which typically contain a suite of carotenoids, including both xanthophylls (lutein, zeaxanthin) and carotenes (β-cryptoxanthin, β-carotene) (Goodwin, 1980; McGraw et al., 2001). We drew blood and plucked feathers from living birds and acquired liver tissue (a purported site of carotenoid metabolism in birds; Brush, 1990) from euthanized birds to determine whether dietary or metabolically derived carotenoids were present. Because decent phylogenetic information is available for estrildids (Christidis, 1987a,b), we were able to trace the evolutionary history of carotenoid pigmentation in this clade.

2. Materials and methods

2.1. Study species

We studied seven estrildid finch species that display either red, orange, or yellow coloration in their feathers or bare parts—star finch (*Neochmia ruficauda*), diamond firetail (*Stagonopleura guttata*), zebra finch (*T. guttata*), common waxbill (*Estrilda astrild*), black-rumped waxbill (*Estrilda troglodytes*), zebra waxbill (*Amandava subflava*), and red avadavat (*Amandava amandava*). The diamond firetail, zebra finch, and star finch are native Australian estrildids; the waxbills are found in Africa; and the avadavat inhabits parts of India and southeast Asia (Goodwin, 1982). Both sexes in all species acquire a red or reddish-orange beak as adults, but they vary in the extent of red, orange, and yellow feather pigmentation. Zebra finches have no carotenoid-pigmented feathers (McGraw and Wakamatsu, 2004), whereas waxbills express a red postorbital stripe (all three species) and either splashes of red in the breast (*E. astrild* and *E. troglodytes*) or deep yellow and orange breast coloration (*A. subflava*). Avadavats exhibit strongly sexually dichromatic plumage, as males are bright red and females are yellow. Male and female star finches both display yellow bellies; males also have red facial feathers. Both male and female diamond firetails restrict red plumage to their rump.

2.2. Animal sources and housing

All common and zebra waxbills were captured in the wild by J.G.S. in KwaZulu-Natal, South Africa between January and April 2000 and imported into the USA in May 2000. Star finches, black-rumped waxbills, and red avadavats were purchased from local pet stores (presumably all of which were captive-bred); diamond firetails were obtained from a local aviculturalist. All birds were housed in captivity by the authors for at least 11 months prior to sampling. They were kept in hardware cloth cages (either 46×46×76 cm or 92×92×183 cm in size) in groups of 2–20 in an animal-approved indoor room on the campus of Cornell University. The primary diet of all birds at this time was Kaytee® Forti-Diet™ (Kaytee Products, Chilton, WI) (see McGraw et al., 2002a for details of the composition of this finch seed mix) and water, and was supplemented every 2–3 days with greens (lettuce, sprouts, spinach, broccoli, or cucumber) and weekly with boiled egg and mealworms.

2.3. Sampling protocol

In April 2001, we sampled blood from seven live adult birds representing three of the species (two male and one female *E. astrild*; one male and one female *A. subflava*; and two male *S. guttata*) to determine plasma carotenoid signatures. Several estrildids had been euthanized (and stored at −80 °C indefinitely) as part of a separate study in

April 2001 and April 2003, and on April 23, 2003, we dissected the liver from eight thawed adults representing six of the species (one male and one female *A. amandava*; one *E. troglodytes* of unknown sex; one male *E. astrild*; one male *S. guttata*; one male and one female *A. subflava*; and one male *N. ruficauda*) to determine liver carotenoid content. Finally, from the five euthanized birds (two *A. amandava*; two *A. subflava*; and *N. ruficauda*), we plucked yellow feathers for plumage carotenoid analysis. We focused on only yellow pigments and colors in this study because red pigments in the feathers and beaks proved difficult to identify chromatographically, as they generated broad peaks that are characteristic of esterified carotenoids. Data for zebra finches in this study are taken from McGraw et al. (2002a).

2.4. Carotenoid analyses

Biochemical methods for identifying carotenoids in finch plasma, liver, and feathers follow previously published procedures (McGraw et al., 2002a for plasma and liver; McGraw et al., 2002b for plumage). At the time of collection, blood was centrifuged in heparinized microcapillary tubes for 10 min at 1500×*g* and plasma was removed and stored at −80 °C in 1.5-ml screw-cap microcentrifuge tubes. Carotenoids were extracted from 10 μl of thawed plasma or 10 mg of liver by adding 100 μl of ethanol. We vortexed the mixture and added 100 μl of *tert*-butyl methyl ether. After vortexing again, we centrifuged the solution for 3 min in a microcentrifuge (Eppendorf model 5414). To remove carotenoids from feathers, we first washed them in ethanol and hexane (sequentially) for 15 min each to remove surface lipids. We then blotted the feathers dry on filter paper, trimmed off the yellow-pigmented barbules, and added these to 1 ml of acidified pyridine in a 9-ml glass tube (sensu Hudon and Brush, 1992; McGraw et al., 2002b). We filled the headspace of the tube with argon and held the solution at 95 °C for 3 h. After cooling to room temperature, we added 1 ml of water, vortexed the mix, and then added 1 ml of *tert*-butyl methyl ether. We shook the tube for 2 min and centrifuged at 1500×*g* for 5 min. At this point in both procedures, the supernatant (containing the carotenoids) was transferred to a new high-performance liquid chromatography (HPLC) tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 200 μl of HPLC mobile phase (acetonitrile:methanol:chloroform, 46:46:8, vol/vol/vol) and vortexed prior to analysis.

We injected 50 μl of each sample into a Waters™ 717 plus Autosampler HPLC (Millipore, Bedford, MA, USA) fitted with a Develosil RPAqueous RP-30 column (250×4.6 mm ID; Nomura Chemical, Japan). We used an isocratic system (HP 1050 Series Isocratic Pump) at a constant flow rate of 1.2 ml/min for 45 min, which is sufficient time to allow both carotenes and xanthophylls to elute. Data were collected from 250 to 600 nm using a Waters™ 996 photodiode array detector (Waters Chromatography, Milford, MA, USA). Feather pigments were identified by comparing their retention times (t_R) and absorption properties (λ_{max} values) to authentic reference carotenoids donated by Roche Vitamins (Parsippany, NJ, USA), Dr. Frederick Khachik (University of Maryland, USA), and Dr. Riccardo Stradi (University of Milan, Italy).

3. Results

3.1. Carotenoids in plasma, liver and tissue

Among the four carotenoids identified in the diet of these birds (McGraw et al., 2002a), lutein (retention time, t_R=6.2 min; wavelength of maximum absorbance, λ_{max}=448 and 477 nm), zeaxanthin (t_R=6.6 min, λ_{max}=453 and 482 nm), and β-cryptoxanthin (t_R=19.7 min, λ_{max}=453 and 482 nm) were present in the blood and livers of estrildids; lutein and zeaxanthin (but not β-cryptoxanthin) were also found in feathers (Fig. 1). Although present in food, we found no evidence of β-carotene in any sample. As in seeds, lutein was the predominant dietary carotenoid.

Two metabolically derived carotenoids, presumably manufactured primarily from lutein and/or zeaxanthin (sensu Stradi, 1998; McGraw et al., 2002a), were also present in the blood, liver, and feathers of certain species: (1) dehydrolutein (t_R=6.9 min, λ_{max}=448 and 477 nm) and (2) anhydrolutein (t_R=13.2 min, λ_{max}=448 and 477 nm) (Fig. 1). Anhydrolutein was the more common of the two (Fig. 1).

3.2. Species-specific carotenoid profiles

Estrildid species varied widely in the extent to which they circulated or stored dietary and/or metabolically derived carotenoids, although for all individuals within a species, all fluids and tissues consistently contained the same suite of pigments (Fig. 2). We were able to divide the nine species into two categories based on their carotenoid signatures: (1) 'dietary carotenoid users' (two species) and (2) 'carotenoid metabolizers' (seven species). *S. guttata* and *N. ruficauda* were the species that had only dietary carotenoids (lutein and zeaxanthin) in plasma, liver, and feathers (Fig. 2). Of the seven remaining species, six of them also had lutein and zeaxanthin in the blood, liver, and feathers, but these were accompanied by both dehydrolutein and anhydrolutein in all tissues and fluids (as well as β-cryptoxanthin in blood and liver). The seventh, *C. gouldiae*, deposits lutein, zeaxanthin, and dehydrolutein in yellow feathers (no evidence of anhydrolutein; Brambilla et al., 1999).

3.3. Phylogenetic pattern

We were then interested in determining the phylogenetic relatedness of these two groups of estrildids that differed in

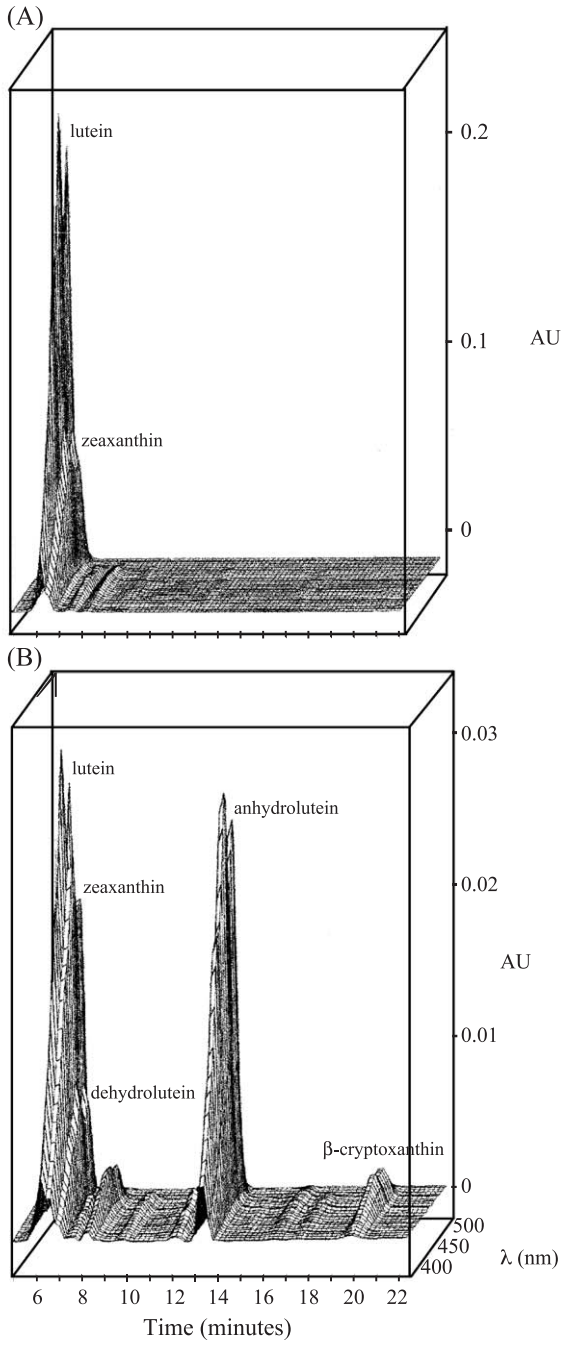

Fig. 1. Representative three-dimensional HPLC chromatograms of the two types of carotenoid signatures we encountered in estrildid fluids and tissues. (A) Dietary carotenoids only (lutein+zeaxanthin), and (B) dietary carotenoids plus metabolically derived forms (including dehydrolutein and anhydrolutein). We are unable to determine in our study whether β-cryptoxanthin appearing in panel B occurs because it is synthesized from lutein (e.g., McGraw et al., 2002a) or is simply better assimilated from the diet by the particular species that show this carotenoid profile.

carotenoid metabolism. As we are still awaiting a comprehensive species-by-species phylogenetic hypothesis for this family, we used the basic three-tribe classification system previously outlined and supported by Christidis (1987a,b), in which Estrildini is said to be the most ancestral tribe, followed by Lonchurini, and then most recently derived Poephilini. Four of our study species (*E. astrild*, *E. troglodytes*, *A. amandava*, and *A. subflava*) are members of the ancestral Estrildini, and all show carotenoid-metabolizing capabilities. The two Lonchurini species (gouldian finch and red-headed parrot finch) also incorporate carotenoid metabolites into feathers (although a bit differently than other metabolizers; see above). The remaining three species are from Poephilini and, interestingly, it is in this group that we saw evidence for dietary carotenoid use only (e.g., *N. ruficauda* and *E. guttata*). The zebra finch, however (also from Poephilini), does show the ability to metabolize carotenoids (McGraw et al., 2002a).

4. Discussion

We obtained and used information on the carotenoid pigments present in nine species of estrildid finches to develop the first phylogenetic test of the evolution of carotenoid coloration in animals. It is argued that sexually selected traits like bright colors serve as honest signals of mate quality because they are demanding to produce (Zahavi, 1975; Grafen, 1990; Johnstone, 1995). Traits are generally thought to evolve historically from less elaborate to more elaborate forms, but this has rarely been tested and may not be as common as was once thought (Wiens, 2001). In the case of carotenoid colors, it has been suggested that simple use of yellow diet-derived carotenoids as colorants predated the appearance of more costly, red metabolically derived forms (Hill, 1996). Thus, we reconstructed carotenoid metabolism in a clade of colorful songbirds to trace the evolutionary history of these pigments.

We found two main pigmentation strategies in the estrildid species under study, which spanned each of the three major tribes in the family. Seven of the nine species were able to metabolize dietary carotenoids. The blood, liver, and feathers of these finches contained carotenoid metabolites (anhydrolutein and/or dehydrolutein) in addition to dietary compounds (e.g., lutein, zeaxanthin). Our evidence for metabolism in these birds is identical to that in a previous study of zebra finches, in that our analyses of diet carotenoids yielded no anhydrolutein or dehydrolutein (McGraw et al., 2002a; also see Stradi, 1998 for predicted precursor–product relationships for bird carotenoids). The fluids and tissues of the remaining two finches (including feathers in *N. ruficauda*) contained only dietary carotenoids. When mapped phylogenetically, members of the two more ancestral estrildid tribes (Estrildini, then Lonchurini) were in fact capable of carotenoid metabolism. It was only in the most derived clade (Poephilini) that we saw species that were not capable of transforming dietary carotenoids, and, even in this tribe, all species did not show the same profile (e.g., see carotenoid metabolites in *T. guttata*; McGraw et al., 2002a).

From these results, we offer two main conclusions about the evolution of carotenoid metabolism and coloration in this

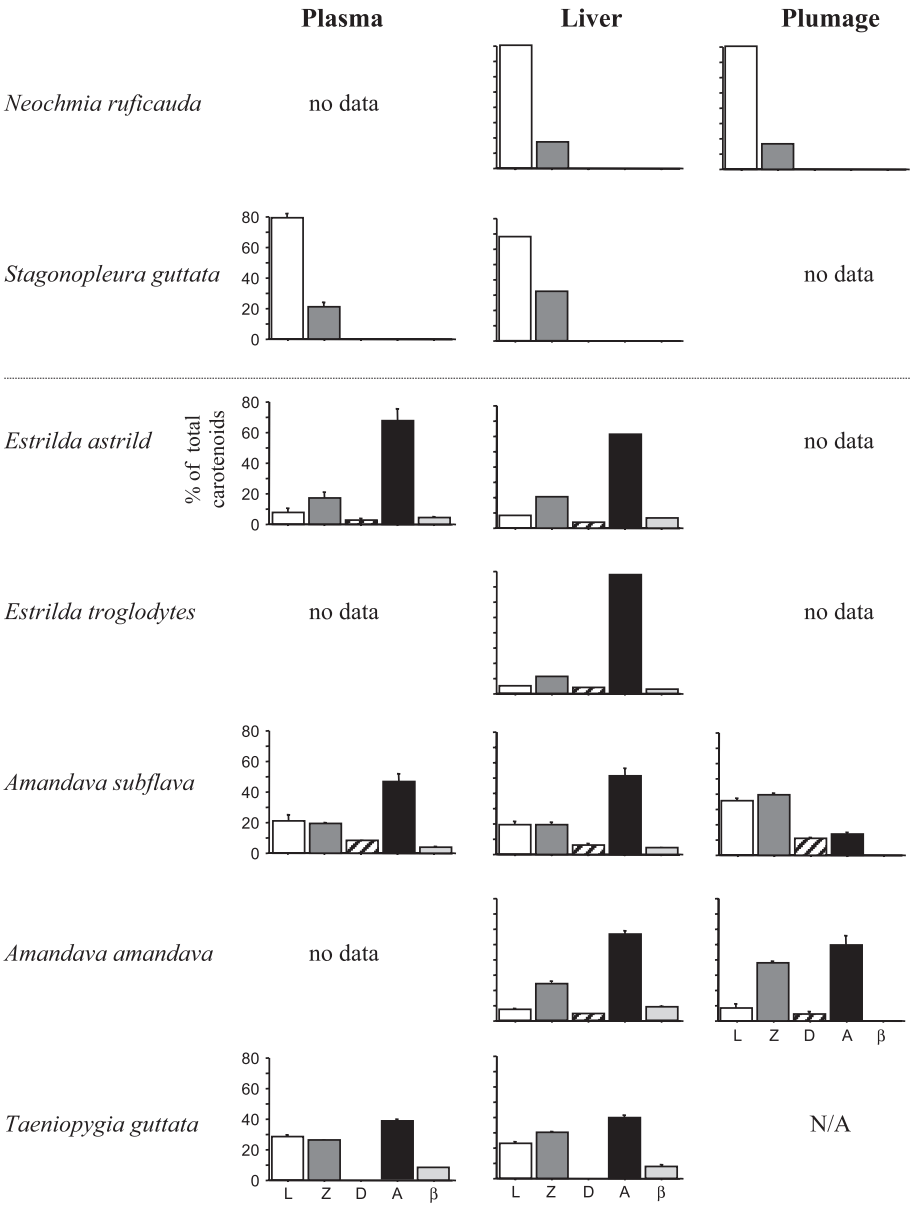

Fig. 2. Occurrence of different carotenoids (percent of total) in the plasma, liver, and plumage of seven estrildid finch species. Mean±S.E.M. is shown for cases where multiple individuals were sampled per species. Abbreviations for the different pigment types on the *X*-axis: L=lutein; Z=zeaxanthin; D=dehydrolutein; A=anhydrolutein; β=β-cryptoxanthin. Note empty cells ('no data') where we were not able to sample fluids or tissues from certain species. The dashed horizontal line separates 'dietary carotenoid users' (above) from 'carotenoid metabolizers' (below).

lineage. First, it does not appear that the use of dietary carotenoids is ancestral in this clade. Although we admittedly did not sample carotenoids from every species in this family, and would have to draw on the pigment systems of the most basal taxa to truly suggest an ancestral state, we can look at: (a) a cursory analysis of red coloration in this group and (b) the outgroups to further support carotenoid transitions among estrildids. Recall that red colors were not studied here for logistical reasons. Red carotenoids are rare, if not absent, from the diets of granivorous passerines (Hill, 1996) and instead have been repeatedly shown to be synthesized by red-colored songbirds from basic yellow dietary xanthophylls (e.g., Stradi, 1998; McGraw et al., 2001). If we work under this assumption for estrildids (*T. guttata*, McGraw et al., 2002a; *E. psittacea*, Brambilla et al., 1999) and particularly the Estrildini (in which 14 of 16 genera show some red color in the integument; Goodwin, 1982), then these reds should contain metabolic derivatives, which would corroborate the notion that carotenoid metabolism is phylogenetically ancient among estrildids. Also, estrildids are nested within the parvorder Passerida and superfamily Passeroidea (Sibley and Ahlquist, 1990; Ericson et al., 2000). Across the series of phylogenies that have been built for this group, families of songbirds like the waxwings (family Bombycillidae) and long-tailed tits (family Aegithalidae) consistently fall out as more ancestral than the estrildids and also possess the

capability of metabolizing dietary carotenoids (Stradi, 1998; Brambilla et al., 1999). Some of the closest relatives of estrildids, the weaver finches (family Ploceidae) (Christidis, 1987a,b; Sorenson and Payne, 2001), also metabolize dietary carotenoids (Brambilla et al., 1999; Dale, 2000). Thus, it is most parsimonious to conclude that estrildids inherited from their ancestors their tendency to metabolize carotenoids.

Our second primary conclusion is that patterns of carotenoid use appear to be labile within certain groups of estrildids (e.g., the Poephilini). Whereas most of the basal finch lineages may metabolize carotenoids, in more derived taxa, we found species that circulated and deposited only dietary colorants. This is consistent with previous studies showing widespread reversals in sexual trait evolution in animals (reviewed in Wiens, 2001), from more to less elaborate forms.

Implicated in such reversals are a suite of environmental (e.g., food availability, lighting conditions, predation), social (e.g., mating preferences, competitive interactions), and genetic (e.g., drift) mechanisms. Across the estrildids, we generally know little about the costs of acquiring rich carotenoid coloration or the fitness benefits of these colors; this really is true for the repertoire of sexual advertisements within any given lineage of animals (Hill and McGraw, 2004). However, there is evidence for certain estrildids that there are physiological demands to being colorful (e.g., in *T. guttata*; McGraw et al., 2003) and that, in this same species, the most colorful individuals are preferred as mates (Burley and Coopersmith, 1987; Blount et al., 2003). Thus, it may be that sexual selection pressures covary with the costs of color display, and that in certain species where mating pressures are relaxed, there is not as great a need to manufacture carotenoids and bear the high costs of coloration. However, there is also evidence that males of certain species in this family (e.g., long-tailed finch, *Poephila acuticauda*) become less conspicuously colored to mimic females and reduce aggression with rivals (Langmore and Bennett, 1999). From these observations, it is clear that several factors may interact to shape the coloration systems of these birds. In future work, we encourage coupling behavioral and physiological studies of this sort with phylogenetic data so that we may better understand the array and balance of selective forces that generate historical patterns of sexual trait expression in these and other animals.

Acknowledgements

We thank T. Bruce, M. Swett, and L. Yang for their assistance in the field; N. Chan, S. Malone, A. Rakow, D. Theodorson, and M. Yi for help with animal care; and the Environmental Protection Agency (STAR fellowship to K.J.M.), Cornell University Mellon Foundation, American Museum of Natural History, and National Science Foundation (doctoral dissertation improvement grant IBN no. 0104481 to J.G.S.) for funding. The Institutional Animal Care and Use Committee at Cornell University (protocol no. 99-106) approved all procedures reported here.

References

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.

Brambilla, L., Canali, G., Mannucci, E., Massa, R., Saino, N., Stradi, R., Zerbi, G., 1999. Colori in volo: il piumaggio degli uccelli ricerca scientifica e cultura umanistica. Universita degli Studi di Milano, Milan, Italy.

Brush, A.H., 1978. Avian pigmentation. In: Brush, A.H. (Eds.), Chemical Zoology, vol. X. Academic Press, New York, pp. 141–161.

Brush, A.H., 1990. Metabolism of carotenoid pigments in birds. FASEB J. 4, 2969–2977.

Burley, N., Coopersmith, C.B., 1987. Bill color preferences of zebra finches. Ethology 76, 133–151.

Burley, N.T., Symanski, R., 1998. "A taste for the beautiful": latent aesthetic mate preferences for white crests in two species of Australian grassfinches. Am. Nat. 152, 792–802.

Christidis, L., 1987a. Biochemical systematics within palaeotropic finches (Aves: Estrildidae). Auk 104, 380–392.

Christidis, L., 1987b. Phylogeny and systematics of estrildine finches and their relationships to other seed-eating passerines. Emu 87, 119–123.

Collins, S.A., Luddem, S.T., 2002. Degree of male ornamentation affects female preference for conspecific versus heterospecific males. Proc. R. Soc. Lond. B269, 111–117.

Dale, J., 2000. Ornamental plumage does not signal male quality in red-billed queleas. Proc. R. Soc. Lond. B267, 2143–2149.

Delacour, J., 1943. A revision of the subfamily Estrildinae of the family Ploceidae. Zoologica 28, 69–86.

Endler, J.A., 1983. Natural and sexual selection on colour patterns in poeciliid fishes. Environ. Biol. Fisches 9, 173–190.

Ericson, P.G.P., Johansson, U.S., Parsons, T.J., 2000. Major divisions in oscines revealed by insertions in the nuclear gene c-*myc*: a novel gene in avian phylogenetics. Auk 117, 1069–1078.

Goodwin, T.W., 1980. The Biochemistry of the Carotenoids. Plants, vol. I. Chapman & Hall, London.

Goodwin, D., 1982. Estrildid Finches of the World. Cornell Univ. Press, Ithaca, NY.

Grafen, A., 1990. Biological signals as handicaps. J. Theor. Biol. 144, 517–546.

Grether, G.F., Hudon, J., Millie, D.F., 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proc. R. Soc. Lond., B266, 1317–1322.

Hill, G.E., 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. Auk 109, 1–12.

Hill, G.E., 1996. Redness as a measure of the production cost of ornamental coloration. Ethol. Ecol. Evol. 8, 157–175.

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

Hill, G.E., 2000. Energetic constraints on expression of carotenoid-based plumage coloration. J. Avian Biol. 31, 559–566.

Hill, G.E., 2002. A Red Bird in a Brown Bag: The Function and Evolution of Ornamental Plumage Coloration in the House Finch. Oxford Univ. Press, Oxford.

Hill, G.E., McGraw, K.J., 2004. Correlated changes in male plumage coloration and female mate choice in cardueline finches. Anim. Behav. 67, 27–35.

Hill, G.E., Inouye, C.Y., Montgomerie, R., 2002. Dietary carotenoids predict plumage coloration in wild house finches. Proc. R. Soc. Lond. B269, 1119–1124.

Hudon, J., Brush, A.H., 1992. Identification of carotenoid pigments in birds. Methods Enzymol. 213, 312–321.

Johnstone, R.A., 1995. Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. Biol. Rev. 70, 1–65.

Langmore, N.E., Bennett, A.T.D., 1999. Strategic concealment of sexual identity in an estrildid finch. Proc. R. Soc. Lond. B266, 543–550.

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

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., Wakamatsu, K., 2004. Melanin basis of ornamental feather colors in male zebra finches. Condor 106, 686–690.

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., 2002a. Anhydrolutein in the zebra finch: a new, metabolically derived carotenoid in birds. Comp. Biochem. Physiol. B132, 813–820.

McGraw, K.J., Hill, G.E., Stradi, R., Parker, R.S., 2002b. The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American goldfinch. Comp. Biochem. Physiol. B131, 261–269.

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.

Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N., Surai, P.F., 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence, or detoxification ability? Avian Poult. Biol. Rev. 11, 137–159.

Plenge, M., Curio, E., Witte, K., 2000. Sexual imprinting supports the evolution of novel male traits by transference of a preference for the colour red. Behaviour 137, 741–758.

Sibley, C.G., Ahlquist, J.E., 1990. Phylogeny and Classification of Birds: A Study in Molecular Evolution. Yale Univ. Press, New Haven, CT.

Sorenson, M.D., Payne, R.B., 2001. A single ancient origin of brood parasitism in African finches: implications for host–parasite coevolution. Evolution 55, 2550–2567.

Stradi, R., 1998. The colour of flight. Carotenoids in Bird Plumage. Solei Gruppo Editoriale Informatico, Milan, Italy.

Wiens, J.J., 2001. Widespread loss of sexually selected traits: how the peacock lost its spots. Trends Ecol. Evol. 16, 517–523.

Witte, K., Curio, E., 1999. Sexes of a monomorphic species differ in preferences for mates with a novel trait. Behav. Ecol. 10, 15–21.

Zahavi, A., 1975. Mate selection—a selection for a handicap. J. Theor. Biol. 53, 205–214.