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*)

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ABSTRACT

Birds display a tremendous variety of carotenoid-based colors in their plumage, but the mechanisms underlying interspecific variability in carotenoid pigmentation remain poorly understood. Because vertebrates cannot synthesize carotenoids de novo, access to pigments in the diet is one proximate factor that may shape species differences in carotenoid-based plumage coloration. However, some birds metabolize ingested carotenoids and deposit pigments that differ in color from their dietary precursors, indicating that metabolic capabilities may also contribute to the diversity of plumage colors we see in nature. In this study, we investigated how the acquisition and utilization of carotenoids influence the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). We supplemented the diet of captive goldfinches with red carotenoids to determine whether males, which are typically yellow in color, were capable of growing red plumage. We also deprived cardinals of red dietary pigments to determine whether they could manufacture red carotenoids from yellow precursors to grow species-typical red plumage. We found that American goldfinches were able to deposit novel pigments in their plumage and develop a striking orange appearance. Thus, dietary access to pigments plays a role in determining the degree to which goldfinches express carotenoid-based plumage coloration. We also found that northern cardinals grew pale red feathers in the absence of red dietary pigments, indicating that their ability to metabolize yellow carotenoids in the diet contributes to the bright red plumage that they display.

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Introduction

The evolution of colorful plumage in birds has been of interest to biologists for more than a century (Darwin 1871; Wallace 1889). Many species use carotenoid pigments to color their feathers red, orange, and yellow (Fox 1976), and carotenoid-based pigmentation has been the subject of many recent studies investigating the proximate and ultimate bases for variability in plumage color displays (Olson and Owens 1998; Hill 1999). Most emphasis has been placed on how carotenoid ornaments function as sexually selected traits within species, such that females prefer to mate with the most brightly colored males who are in the best condition (Andersson 1994).

Comparatively less attention has been paid to the factors that underlie the tremendous diversity of carotenoid-based plumage colors we see among avian species in nature. Essential to elucidating the mechanisms by which birds differ in the color of carotenoid displays is an understanding of the steps involved in producing carotenoid-based feathers. Vertebrates cannot synthesize carotenoid pigments de novo, so they must acquire them from the diet at the time they are growing their colorful plumage so that they can be deposited in feathers (Völker 1938; Brush 1981). As a result, access to dietary pigments may differ among species and contribute to the variation in avian plumage colors that exist. However, some species metabolize ingested carotenoids and deposit plumage pigments that differ in color from their dietary precursors (Brush 1990*a*). Thus, both carotenoid acquisition and utilization may shape interspecific differences in carotenoid-based plumage pigmentation.

Early work on the proximate determinants of carotenoid pigmentation focused on the physiological bases for color dif-

ferences among species. Using captive feeding experiments in which dietary carotenoid content was manipulated, and by comparing the plumage pigments present in closely related species, many concluded that the differences in carotenoid-based plumage color among species were attributable to biochemical and physiological mechanisms under genetic control (Brush and Johnson 1976; Hudon et al. 1989; Hudon 1991). For example, Test (1969) separately fed captive yellow- and red-shafted flickers (*Colaptes auratus*) the same diet and found that they still grew differently colored feathers. Also, reducing the amount of red pigments in the diets of captive American flamingos (*Phoenicopterus ruber*; Fox and McBeth 1970) and black rosy finches (*Carpodacus roseus*; French 1959) induced birds to grow pale feathers but did not prevent them from developing species-typical red plumage coloration. Collectively, these results suggest that species-specific carotenoid-based plumage pigmentation in birds is affected little by diet and is instead maintained primarily as a result of specific metabolic pathways.

The idea that dietary access to pigments influences interspecific variation in carotenoid coloration has gained support recently from studies in which novel carotenoids have been added to the diets of birds. The introduction of Morrow's honeysuckle (*Lonicera morrowii*) into the eastern United States and into the diet of cedar waxwings (*Bombycilla cedrorum*) over the last 40 yr has been responsible for the appearance of waxwings with orange-tipped tail bands, rather than species-typical yellow pigmentation (Hudon and Brush 1989; Brush 1990*b*; Mulvihill et al. 1992; Witmer 1996). Yellow-breasted chats (*Icteria virens*) and Kentucky warblers (*Oporornis formosus*) also develop a wash of orange color in their feathers from consuming honeysuckle berries (Mulvihill et al. 1992). An orange variant of the bananaquit (*Coereba flaveola*), a species that typically displays yellow carotenoid-based plumage pigmentation, has been identified in the French West Indies and is also attributed to a new source of dietary pigment (Hudon et al. 1996).

From all of this work, it is clear that more experimental studies in which carotenoid access is manipulated are needed to better understand the specificity of pigmentation systems among species and thus the degree to which carotenoid access and utilization determine species-specific plumage color patterns in birds. In this study, we performed captive feeding experiments in two passerines exhibiting carotenoid-based pigmentation to ask two questions about the physiological capabilities of pigmentation among species: (1) Can yellow-pigmented species grow red plumage when fed abundant red pigments? (2) Can red-pigmented species maintain red plumage when deprived of red dietary pigments and fed only yellow carotenoids in the diet? Specifically, we investigated whether male American goldfinches (*Carduelis tristis*), which display yellow carotenoid-based plumage coloration (Middleton 1993), could grow red plumage when fed the red pigment canthaxanthin in their diet during molt. We also tested whether male northern cardinals (*Cardinalis cardinalis*), which are red in color (Halkin and Linville 1999), could maintain red plumage pigmentation in the presence of only yellow dietary pigments. After birds had completed molt in captivity, we quantified the color of plumage in both species with a reflectance spectrophotometer and identified carotenoids in the food and feathers using high-performance liquid chromatography (HPLC) to determine the metabolic fate of dietary pigments.

Methods

American Goldfinches

Between January 15 and 24, 1999, we captured 58 male goldfinches in basket traps at thistle feeders in Lee County, Alabama. We randomly divided the males into four groups of 13–15 males and housed the flocks in separate outdoor cages (3.7 m long × 1.5 m wide × 2.4 m high). All groups were fed a basal, ad lib. diet of sunflower hearts and water throughout the course of the study. Two dishes of food and water were placed on the floor of each cage. Water was treated with 6.6 drops L^{-1} of Premium Multi-Drops high-potency multivitamins (Eight in One Pet Products, Hauppauge, N.Y.) and 0.001 g mL^{-1} of sulfadimethoxine, a drug that suppresses coccidial infections in these birds (McGraw and Hill 2000). No finches showed signs of ectoparasitism (e.g., avian pox, mycoplasmal conjunctivitis) or died during their time spent in captivity.

We started our carotenoid supplementation experiment on February 15, 1 mo before birds began their prealternate molt in mid-March. Two control groups ($n = 30$ birds) were fed the normal seed and water diet for the course of the study. For two experimental groups ($n = 28$ males), we supplemented the basal diet with carotenoids by dissolving a red pigment, canthaxanthin (0.001 g mL^{-1} of Roxanthin Red 10 WS canthaxanthin beadlets, Roche Vitamins, Parsippany, N.J.), in the water. We used this carotenoid treatment because canthaxanthin is readily absorbed and deposited into plumage by closely related red cardueline finches (Stradi 1998; Inouye et al. 2001) and because this pigment concentration is sufficient to turn captive males from these species red (Hill 1992). All groups of goldfinches completed their prenuptial molt by June 1.

Northern Cardinals

Ten male cardinals were captured using mistnets in Lee County from August 14 to 21, 1997. We housed males in an outdoor cage identical to those described above. Cardinals were fed an ad lib. diet of red millet, white millet, sunflower seeds, and vitamin-supplemented and medically treated water. We provided no carotenoid supplements to these birds. All cardinals completed their single annual (prebasic) molt in the fall by October 1, 1997.

Plumage Scoring

After molt, we scored the carotenoid-based plumage coloration (hue) of male goldfinches and cardinals using a handheld Colortron spectrophotometer (sensu Hill 1998). We took three measurements of carotenoid-based pigmentation from both the ventral and dorsal sides of the animals and averaged these six scores to compute mean plumage hue for each individual. For comparison with captive birds, we captured and scored the freshly molted plumage of 29 wild male goldfinches in Ithaca, New York, from May 1 to 15, 2000, and 16 wild male cardinals from Lee County, Alabama, from August 14 to 21, 1997. The Colortron assigns hue scores based on a 360° color wheel, so values decrease from yellow to orange to red.

Seed and Feather Carotenoid Analyses

Because we were interested in determining the degree to which these birds physiologically incorporated either novel or reduced levels of dietary pigment into their plumage, we used HPLC techniques to identify the carotenoids present in the diet and feathers of both cardinals and goldfinches. To analyze seed carotenoids, we separately pulverized 0.5 g of sunflower hearts, red millet, and white millet to a fine powder with a mortar and pestle. The seed lipids were extracted three times with 3 mL tetrahydrofuran. We centrifuged the suspension at 3,000 rpm for 3 min and evaporated the supernatant to dryness under a stream of nitrogen. We redissolved the residue from both types of millet in 100 μL of HPLC phase A (methanol-acetonitrile, 50 : 50, v/v, +0.05% triethylamine) plus 50 μL of HPLC phase B (methanol-methylene chloride, 50 : 50, v/v, +0.05% triethylamine) and centrifuged off a white precipitate before HPLC analysis. Because of the high triglyceride content in sunflower seeds, xanthophylls and carotenes were partitioned between 1 mL dimethylformamide (DMF) and 4 mL hexane, respectively. We shook and centrifuged this suspension for 2 min, removed the hexane supernatant, and re-extracted the DMF phase with another 4 mL hexane. After shaking and centrifuging, the hexane phases were combined, and the hexane and DMF were evaporated to dryness under nitrogen. We resuspended the carotenes and xanthophylls in 1 mL of hexane and ethanol, respectively, for quantification of total carotenoids by spectrophotometry (Bausch and Lomb Spectronic 1001). We applied the following formula:

$$\frac{A \times \text{volume of extract (mL)}}{E \times \text{seed mass (g)}},$$

where A is the absorbance of the sample at λ_{max} (448 for xanthophylls and 453 for carotenes) and E is the extinction coefficient at 1%/1 cm of the relevant carotenoids at λ_{max} (2,550 for xanthophylls in ethanol and 2,592 for carotenes in hexane; Bauernfiend 1981).

We injected 20 μL of each sample into a Hitachi L-6200 HPLC (Hitachi, Tokyo) fitted with a Develosil RPAqueous RP-30 HPLC column (250 × 4.6 mm i.d.). The hexane phase of sunflower hearts was not analyzed by HPLC because the triglycerides partitioned with the carotenes during the extraction and attempts to saponify and remove these lipids proved unsatisfactory. A gradient system was used for analysis at a constant flow rate of 1.2 mL min^{-1}: isocratic elution with 92 : 8 (v/v) A : B for 10 min, followed by a linear gradient up to 30 : 70 A : B through 26 min, and finishing with isocratic elution at 30 : 70 A : B for 12 min. Carotenoids were detected at 450 nm using a Hitachi L-4250 UV/VIS detector, and peak areas were integrated with an HP 3390A integrator. We identified pigments present in the seeds by comparing their retention times to those of authentic reference carotenoids provided by Roche Vitamins (lutein, 13.9 min; zeaxanthin, 16.0 min; β-cryptoxanthin, 28.8 min; β-carotene, 36.8 min). The concentration of each carotenoid type in red and white millet was determined by comparison to an internal standard (echinenone, Roche Vitamins, 1 mg mL^{-1}, retention time of 29.8 min); specific xanthophyll concentrations in sunflower seed were calculated by comparing the relative proportions of individual pigments, as reflected by their HPLC peak areas, to the total carotenoid content determined by spectrophotometry.

To identify feather carotenoids, we plucked newly molted plumage patches of seven captive cardinals, five wild male goldfinches, three captive goldfinches fed plain seed, and three captive goldfinches fed canthaxanthin. Hudon (1991) previously published the carotenoid pigments present in the plumage of wild male cardinals. We followed the extraction and identification protocols of Stradi et al. (1995*a*) to analyze feather pigments. The carotenoid-pigmented barbules of each bird were washed with hexane, placed separately in 3 mL methanol, ground in a micronizer, filtered from inorganic salts and keratin, and evaporated at room temperature. The residue was dissolved in 200 μL acetone, after which time the precipitate was filtered off and the filtrate containing the pigment was evaporated under dry nitrogen. The remaining pigment residue was dissolved in mobile phase (acetonitrile/methanol, 70 : 30) and injected into a Gymcotec A 110 instrument for HPLC analysis using two sequential Lichrocart Purosphere RP-18 columns (250 × 4 mm i.d.) at a flow rate of 0.5 mL min^{-1}. Data were acquired between 230 and 600 nm with a diode-array detector (HP 1050 series), and peak area was integrated at 450 nm. Three-dimensional chromatograms were recorded using HP Chem software. Mass spectrometry was carried out with an HP 5988 A particle beam instrument for pigment identification.

Statistical Analyses

Plumage hue data were not normally distributed for any group (Shapiro-Wilks W-test, $P < 0.05$), so we used nonparametric

Mann-Whitney *U*-tests (Z reported) to compare mean hue scores among the groups within each species. Cages of goldfinches within a diet treatment did not differ significantly in plumage hue (both $P > 0.2$), so we pooled them for analyses.

Results

American Goldfinches

From the basal sunflower seed diet fed to captive goldfinches, we isolated two primary yellow carotenoid pigments, lutein and zeaxanthin (Fig. 1; Table 1), with minor amounts of unidentified carotenes. Captive males that were fed only this seed diet during molt deposited two different primary carotenoids into their yellow plumage: canary xanthophylls A and B (Fig. 2). These are the same two pigments found in the carotenoid-based plumage of wild male goldfinches (Fig. 2).

Captive males fed sunflower seed during molt grew drab yellow plumage compared to wild males ($Z = 6.29$, $P < 0.0001$; Fig. 3). Males whose diets were supplemented with canthaxanthin molted into a striking orange appearance (refer to Fig. A in the online edition of *Physiological and Biochemical Zoology* for a color image of these birds). Canthaxanthin was identified as the main pigment (>75%) present in the feathers of these birds (Fig. 4). The plumage of canthaxanthin-supplemented males differed significantly in hue from captive males that did not receive supplements ($Z = 6.62$, $P < 0.0001$) and from wild males ($Z = 6.58$, $P < 0.0001$; Fig. 3).

Northern Cardinals

We isolated four major pigments from the seeds provided to captive cardinals: lutein, zeaxanthin, β-cryptoxanthin, and β-carotene (Fig. 1; Table 1). Captive males fed this seed diet during molt deposited four different primary pigments into their ornamental plumage—α-doradexanthin, astaxanthin, canthaxanthin, and adonirubin (Fig. 5)—the same as those found in the plumage of wild males (Hudon 1991). In contrast to the typically yellow carotenes and hydroxy-xanthophylls present in the

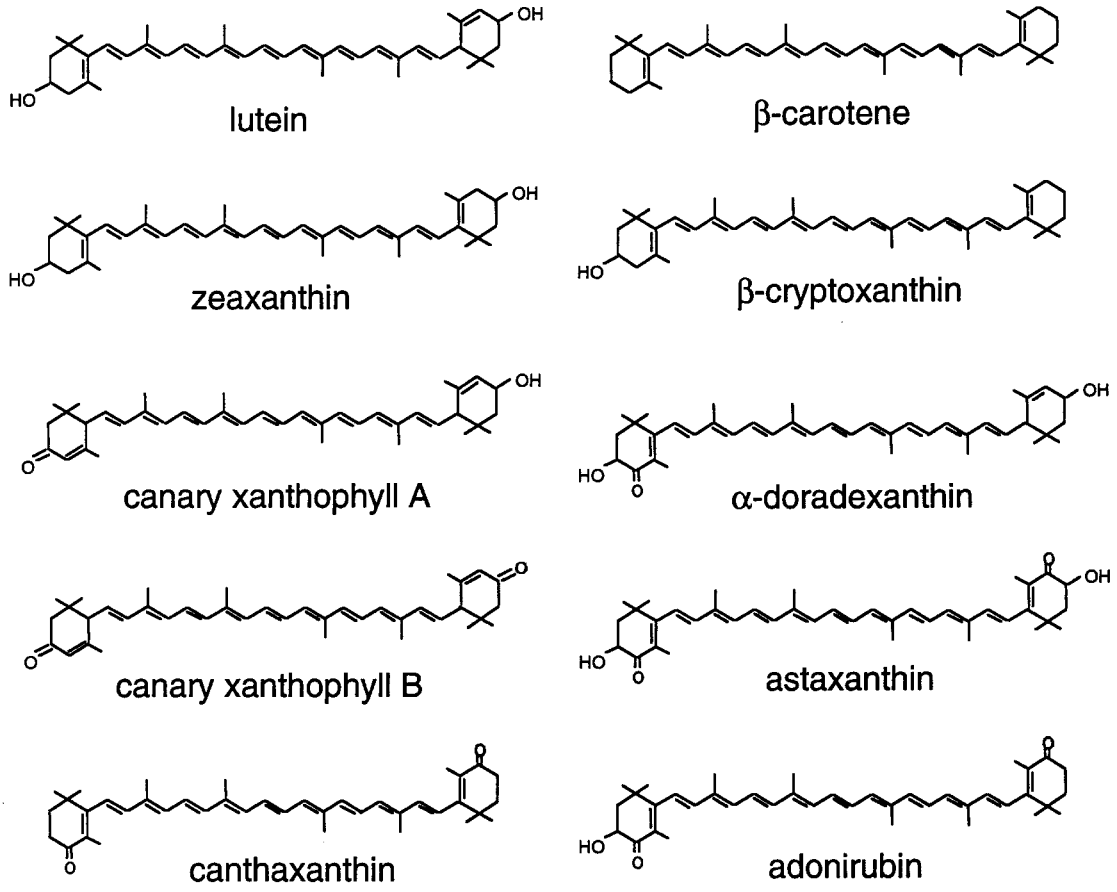

Figure 1. Carotenoid pigments identified in the seeds and feathers of American goldfinches and northern cardinals

Table 1: Major carotenoid pigments identified in the seeds fed to captive American goldfinches and northern cardinals

Seed Type and Pigment	Concentration[a]	Percentage of Total
White millet:		
Total	7.26 ± .08	…
Lutein	5.01 ± .06	69.0 ± .76
Zeaxanthin	2.10 ± .05	28.9 ± .60
β-cryptoxanthin	.06 ± .007	.8 ± .14
β-carotene	.09 ± .016	1.3 ± .21
Red millet:		
Total	.74 ± .27	…
Lutein	.28 ± .12	36.0 ± 6.56
Zeaxanthin	.46 ± .21	58.3 ± 6.03
β-cryptoxanthin	.02 ± .004	1.9 ± .87
β-carotene	.03 ± .01	4.3 ± 1.52
Sunflower seed:		
Total	1.06 ± .11	…
Lutein	.68 ± .05	65.2 ± 2.52
Zeaxanthin	.41 ± .06	33.7 ± 2.52
Carotene	.02 ± .01	1.7 ± .43

Note. Means ± 1 SD are reported for each pigment type based on triplicate extractions performed on separate 0.5-g seed portions.

[a] Micrograms of pigment per gram of seed.

seeds, the keto-carotenoids that we found in the plumage typically exhibit red hues. Although captive cardinals fed this seed diet grew plumage that differed significantly in hue from wild males (Fig. 6), they were still able to grow red feathers on a diet that contained yellow pigments (refer to Fig. B in the online edition for a color image).

Discussion

American Goldfinches

Using HPLC, we identified two primary yellow carotenoid pigments—canary xanthophylls A and B—in the yellow nuptial plumage of wild male *Carduelis tristis*. These xanthophylls are also the main carotenoids found in the yellow feathers of other *Carduelis* finches (Stradi et al. 1995*b*) and are present in lower concentrations in the red plumage of various *Carpodacus* relatives (Stradi et al. 1997; Inouye et al. 2001). When provided with a sunflower seed diet during molt that contained lutein and zeaxanthin as the primary dietary carotenoids, captive male goldfinches grew yellow feathers and, like birds in the wild, deposited canary xanthophylls A and B as plumage pigments. This suggests that wild males metabolize two yellow dietary pigments and deposit more oxidized yellow carotenoids into their ornamental plumage (Fig. 7; sensu Stradi 1998).

When provided with the red carotenoid pigment canthaxanthin in the diet, however, captive male goldfinches grew orange plumage by depositing canthaxanthin directly into their feathers. Canthaxanthin is commonly found in the plumage of many birds, including various finches (Stradi et al. 1997) and woodpeckers (Stradi et al. 1998). Thus, like the species mentioned previously that exhibit yellow carotenoid-based plumage pigmentation (Mulvihill et al. 1992; Hudon et al. 1996), male American goldfinches have the capacity to grow orange plumage when provided with novel pigments in the diet during molt. Because orange plumage has never been recorded in a wild

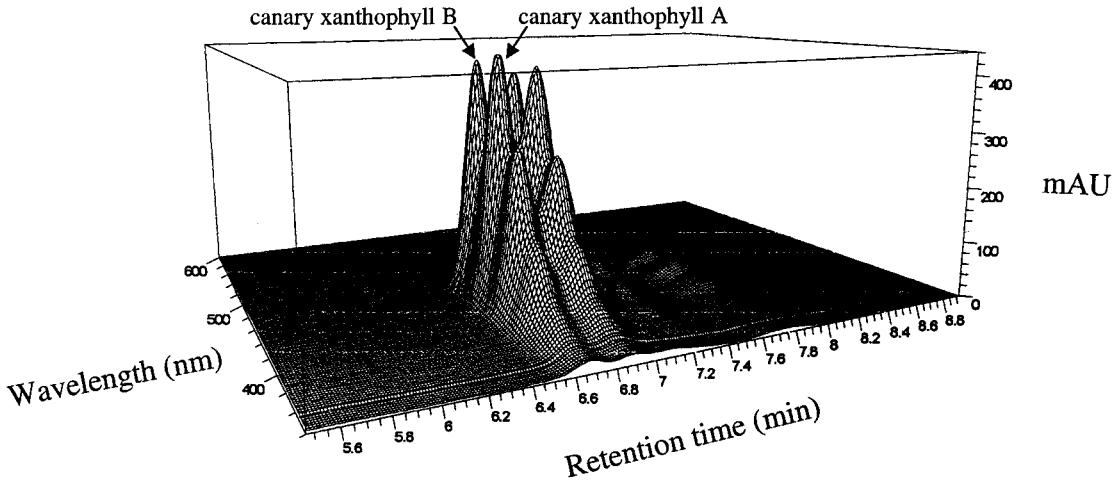

Figure 2. Representative three-dimensional HPLC chromatogram for carotenoid pigments found in the plumage of male American goldfinches. Primary pigments include canary xanthophylls A and B.

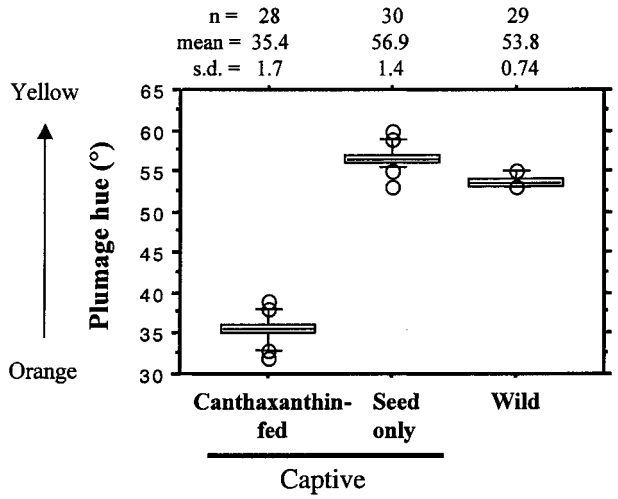

Figure 3. Quantitative comparison of the differences in plumage hue between wild male goldfinches and captive males fed canthaxanthin and plain seed. Feather patches were scored with a Colortron reflectance spectrophotometer after all birds had completed molt. Horizontal bars in the plot indicate the tenth, twenty-fifth, fiftieth, seventy-fifth, and ninetieth percentiles, and points give data for individuals outside of these ranges.

male goldfinch, this result suggests that goldfinches do not consume sufficient quantities of orange or red pigments in nature to color their plumage orange or red. In fact, although the specific dietary items of molting wild goldfinches are not completely described, there is no evidence that, during the spring, goldfinches forage on fruits or berries (Middleton 1993) that contain large quantities of orange and red carotenoids (Brush 1978). Instead, goldfinches are primarily granivorous and consume grass, tree, and composite seeds in addition to leaf buds and dandelion flowers (Middleton 1993), which all are likely to contain the yellow pigments lutein and zeaxanthin primarily and few orange or red pigments (Völker 1934; Goodwin 1980; Klaui and Bauernfiend 1981). Thus, the type of dietary carotenoids ingested during the prealternate molt is a factor that limits the expression of carotenoid-based plumage coloration in male goldfinches.

A series of biochemical factors may allow goldfinches and other yellow-pigmented species to deposit red pigments into their plumage. Canthaxanthin was deposited unmodified into feathers by goldfinches and thus did not require a specific enzyme-conversion system to incorporate this pigment into plumage displays. Canthaxanthin also has a very similar chemical structure to the two canary xanthophylls found in goldfinch plumage (Fig. 1). All three carotenoids contain oxo-groups, and oxo-carotenoids are absorbed preferentially by birds and fish (Schiedt 1989). In fact, canthaxanthin is a positional isomer of canary xanthophyll B, differing only in the degree of conjugation of the hydrocarbon chain and in the location of the carbonyl groups. Rhodoxanthin, the pigment that cedar waxwings obtain from honeysuckle berries to grow orange plumage (Brush 1990*b*), is also a positional isomer of the canary xanthophylls typically found in yellow waxwing tail bands (Stradi 1998). Thus, birds may be predisposed to depositing certain carotenoids into feathers based on their structural similarity to natural plumage pigments and unlikely to deposit novel pigments that are less similar in structure to those typically used to color feathers.

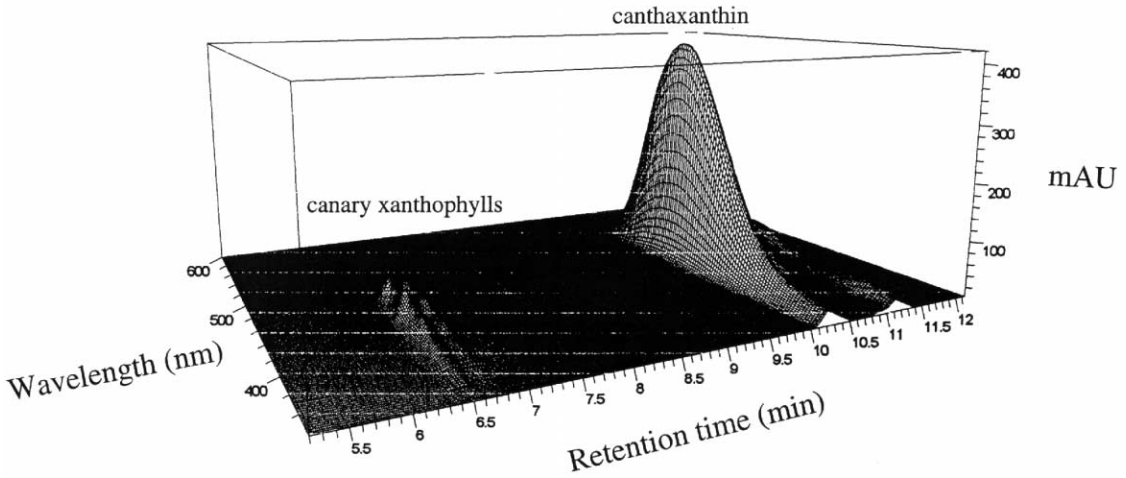

Figure 4. Representative three-dimensional HPLC chromatogram for carotenoid pigments found in the plumage of captive male American goldfinches fed a diet supplemented with canthaxanthin. The primary pigment is canthaxanthin.

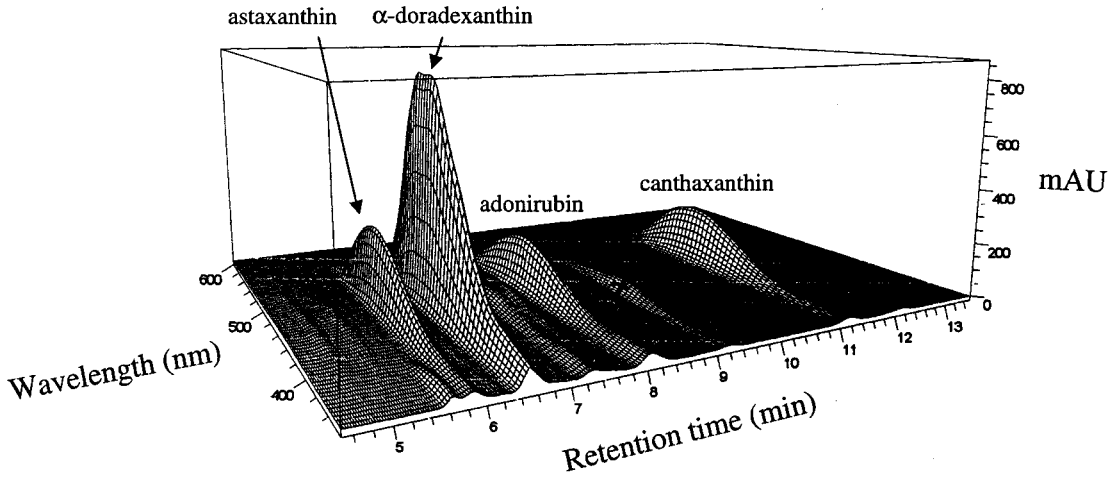

Figure 5. Representative three-dimensional HPLC chromatogram for carotenoid pigments found in the plumage of captive male northern cardinals. Major pigments include α-doradexanthin, astaxanthin, canthaxanthin, and adonirubin.

Pigment processing systems may also differ between yellow- and red-pigmented songbirds. Yellow-plumaged species like goldfinches may not utilize dietary pigments as effectively as those species that are naturally redder in color. Whereas male goldfinches grew orange plumage on a canthaxanthin-supplemented captive diet, male house finches, which express red plumage in the wild, develop bright red pigmentation when fed this same concentration of canthaxanthin in captivity (Hill 1992). Because they are smaller birds, goldfinches may have consumed less water and fewer pigments than house finches. However, males in our study shed considerable amounts of canthaxanthin in their feces (as indicated by orange-colored droppings; K. McGraw, personal observation), which suggests that there may be some difference in the way these two species absorb or process ingested pigments that allows house finches to deposit more canthaxanthin into plumage than goldfinches. In addition, although goldfinches convert dietary pigments into xanthophylls, they may not have the capability of metabolizing certain carotenoids that are oxidized by other species to grow orange or red plumage. In support of this, goldfinches fed a concentration of β-carotene (a purported dietary precursor to canthaxanthin in certain carduelines; Stradi 1998) identical to that of canthaxanthin in this study failed to grow orange plumage in captivity (K. McGraw, unpublished data).

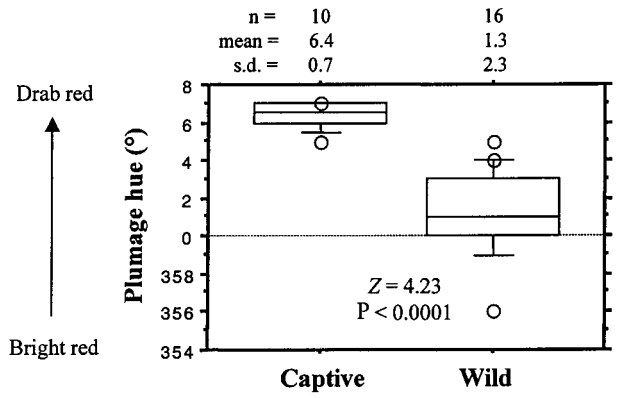

Figure 6. Quantitative comparison of the differences in plumage coloration between wild northern cardinals and captive birds fed a seed-only diet. See Figure 4 for description of boxplot.

Northern Cardinals

Hudon (1991) identified four major red pigments in the carotenoid-based plumage of wild male northern cardinals: canthaxanthin, astaxanthin, adonirubin (formerly phoenicoxanthin), and α-doradexanthin. In our study, when fed a seed diet composed of four yellow carotenoids—lutein, zeaxanthin, β-carotene, and β-cryptoxanthin—birds did not grow yellow plumage; instead, molting captive male cardinals deposited the same four red pigments that are found in the plumage of wild birds. There is little evidence that colorful songbirds store carotenoids and use previous stores to pigment feathers (Brockmann and Völker 1934; Hill 1992; Inouye 1999), which supports the idea that birds in our study derived these red plumage carotenoids from dietary pigments available to them during molt and not those previously accumulated from the wild. Based on likely 4-oxidation reactions that appear to be common in birds (Stradi et al. 2001), we propose that cardinals convert each of the dietary pigments into different red carotenoids that are deposited in plumage (Fig. 7; sensu Stradi 1998).

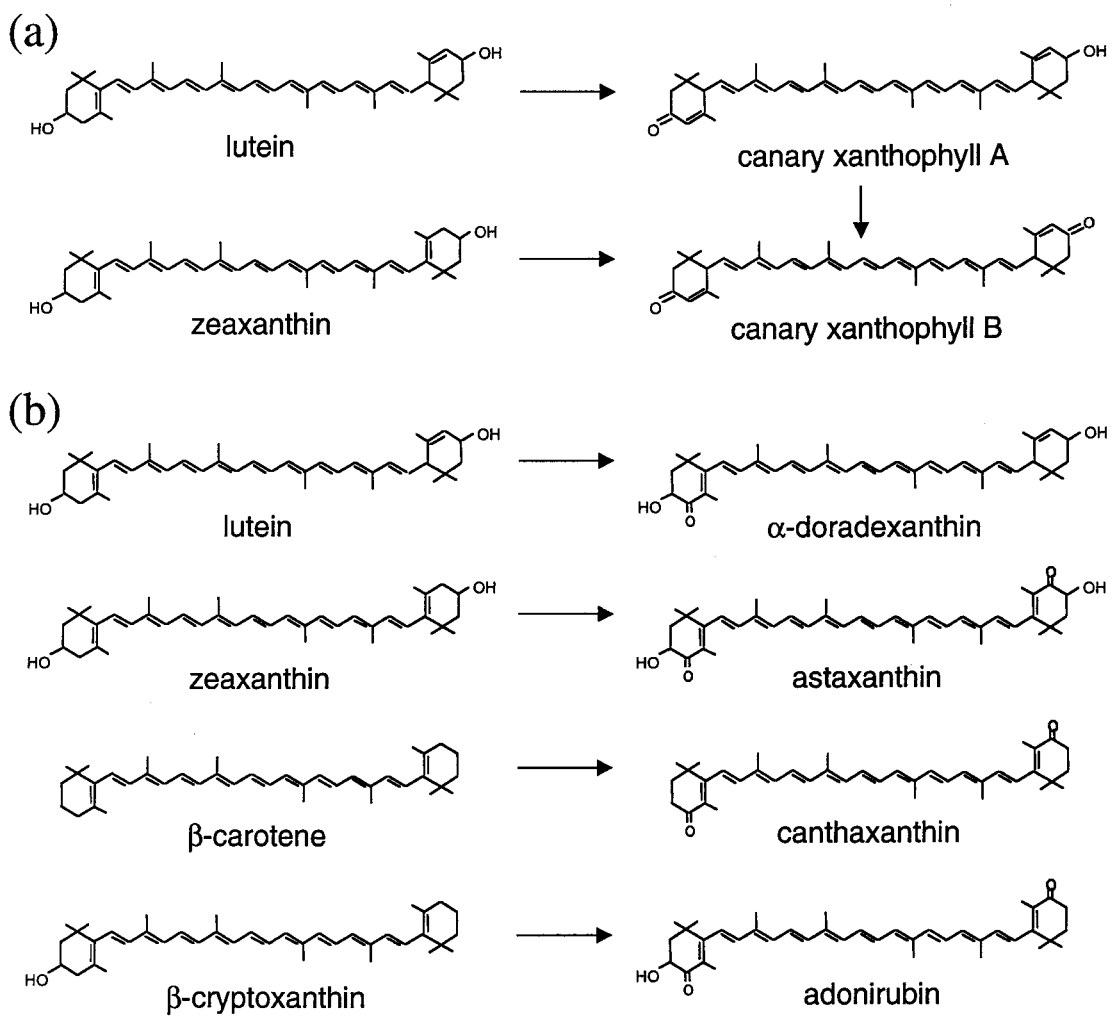

Figure 7. Proposed conversion pathways of dietary carotenoids in (*a*) American goldfinches and (*b*) northern cardinals (sensu Stradi 1998)

Our results suggest that northern cardinals possess the metabolic capabilities of growing red plumage by oxidizing yellow dietary precursor pigments. Thus, carotenoid utilization facilitates the maintenance of species-typical coloration in this species. Captive birds in our study likely molted into a less intense red coloration not because of access to different pigments but because they had access to a lower concentration of pigments than they would in the wild. Wild male cardinals consume a diet composed of 60% fruit during molt (Linville and Breitwisch 1997), which likely provides them with a higher concentration of pigments than the seeds in our captive studies (Brush 1978). In captivity, American goldfinches fed sunflower seeds, which presumably contain less of the same two pigments that males ingest from their natural seed diet, also molted into a less colorful yellow plumage than wild finches. Although some have speculated that the health of captive birds can also affect plumage displays (Hudon 1994), goldfinches and cardinals still incorporated red pigments into their feathers on our experimental diets.

Is there any particular metabolic conversion pathway that allows cardinals to maintain their red coloration? Stradi et al. (2001) investigated components of carotenoid assimilation in red-pigmented cardueline finches by providing a captive diet rich in lutein (proposed precursor for α-doradexanthin) and deficient in β-cryptoxanthin (hypothetical precursor for adonirubin). α-doradexanthin is found in the wild plumage of only a few red carduelines (e.g., *Carpodacus roseus*, *Pyrrhula pyrrhula*), and only these species were able to retain their red color in captivity, presumably because of their ability to oxidize lutein as opposed to depositing it directly into feathers to grow yellow plumage. Lutein is present in nearly all plant matter (Goodwin 1980), so it will be important in future studies of red-colored

species to remove lutein from the diet and determine the degree to which individuals can maintain species-typical plumage pigmentation.

Conclusions

Because of their direct link to dietary sources, carotenoid-based plumage colors offer a unique opportunity to investigate the importance of environmental factors in shaping variable expression of sexually dimorphic traits among species. Ecological parameters such as altitude (Badyaev 1997), light environment (Endler and Thery 1996; Andersson 2000), predation pressure, and parasite burden (Johnson 1991) have been considered in a comparative framework to assess plumage color variability among avian taxa. Here, we examined the direct means by which birds used pigments available to them in the diet to understand better the differences in the pigmentation systems of two passerines displaying different carotenoid-based ornamental colors. Both dietary access to pigments and the ability to utilize different pigments contributed to the expression of species-typical coloration in American goldfinches and northern cardinals. Although this marks a beginning to understanding the proximate factors controlling plumage pigmentation in these two species, both carotenoid acquisition and utilization remain unstudied in most other wild birds. Not only do we rarely know the types and amount of pigments that species consume while growing their colorful feathers, but most aspects of carotenoid physiology are as of yet undescribed. For example, pigment conversion pathways are only hypothetical and based on feeding experiments with unlabeled pigments. Moreover, we do not know in any species with carotenoid-based plumage coloration the anatomical site where these metabolic conversions occur (e.g., liver, feather follicle) and, accordingly, have not yet attempted to identify the enzymes that may catalyze these oxidation reactions (Brush 1990*a*). These fundamental components of pigment-based plumage displays must become a priority in future research before we can fully understand carotenoid-signaling systems at any level of analysis.

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