Differential Accumulation and Pigmenting Ability of Dietary Carotenoids in Colorful Finches

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ABSTRACT

Many animals develop bright red, orange, or yellow carotenoid pigmentation that they use to attract mates. Colorful carotenoid pigments are acquired from the diet and are either directly incorporated as integumentary colorants or metabolized into other forms before deposition. Because animals often obtain several different carotenoids from plant and animal food sources, it is possible that these pigments are accumulated at different levels in the body and may play unique roles in shaping the ultimate color expression of individuals. We studied patterns of carotenoid accumulation and integumentary pigmentation in two colorful finch species—the American goldfinch (*Carduelis tristis*) and the zebra finch (*Taeniopygia guttata*). Both species acquire two main hydroxycarotenoids, lutein and zeaxanthin, from their seed diet but transform these into a series of metabolites that are used as colorful pigments in the plumage (goldfinches only) and beak (both species). We conducted a series of carotenoid-supplementation experiments to investigate the relative extent to which lutein and zeaxanthin are accumulated in blood and increase carotenoid coloration in feathers and bare parts. First, we supplemented the diets of both species with either lutein or zeaxanthin and measured plasma pigment status, feather carotenoid concentration (goldfinches only), and integumentary color. Zeaxanthin-supplemented males grew more colorful feathers and beaks than lutein-supplemented males, and in goldfinches incorporated a different ratio of carotenoids in feathers (favoring the accumulation of canary xanthophyll B). We also fed goldfinches different concentrations of a standard lutein-zeaxanthin mix and found that at physiologically normal and high concentrations, birds circulated proportionally more zeaxanthin over lutein than occurred in the diet. Collectively, these results demonstrate that zeaxanthin is preferentially accumulated in the body and serves as a more potent substrate for pigmentation than lutein in these finches.

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Introduction

Among the suite of sexually selected traits that animals exhibit, including long tails, weaponry, and elaborate courtship dances, the physiological and biochemical bases for carotenoid coloration are perhaps best described (reviewed in McGraw and Hill 2001; McGraw et al. 2001; Hill 2002). Animals develop their striking red, orange, and yellow integumentary colors by acquiring carotenoid pigments from a diet that contains either plant matter or herbivorous prey. These diet-derived molecules are then circulated through the bloodstream and can be metabolized into more oxidized forms before being incorporated into skin, scales, feathers, and other bare parts. Animals that acquire more carotenoids from the diet (Grether et al. 1999; Hill et al. 2002; Mahler et al. 2003) or accumulate more in the body (Hill et al. 1994; Bortolotti et al. 1996; McGraw et al. 2003) develop the brightest carotenoid-based colors.

Despite the wealth of information on the basic processes that underlie carotenoid pigmentation (Stradi 1998), little is known about how specific dietary carotenoids are processed or are used to develop sexy colors. Animals often acquire a host of carotenoids in the diet that differ in molecular characteristics, such as bond structure, hydrophobicity, and light absorbance, which may allow them to be differentially accumulated in the body and play very different roles in shaping the ultimate color expression of individuals. One interesting and viable hypothesis is that the utilization systems of colorful animals are attuned to handling particular pigments over others, perhaps selectively accumulating those carotenoids that impart the brightest colors or serve as the best metabolic substrates for the synthesis of integumetary colorants.

To test these ideas, we studied patterns of carotenoid accumulation from the diet and into colorful tissues in two colorful songbird species—the American goldfinch (*Carduelis tris-*

tis, family Fringillidae) and the zebra finch (*Taeniopygia guttata*, family Estrildidae). Males from both species incorporate carotenoid pigments into the beak to develop sexually dichromatic and sexually attractive coloration (Burley and Coopersmith 1987; Johnson et al. 1993; McGraw et al. 2002*a*, 2002*b*). Male goldfinches also color their sexually selected body plumage with high concentrations of carotenoids (McGraw et al. 2002*a*). In previous work, we found that lutein and zeaxanthin are the two main carotenoids obtained by these finches through their seed diet (McGraw et al. 2001, 2002*b*; Gregory 2002). Goldfinches transform these yellow hydroxycarotenoids into a suite of canary xanthophylls that are used to color the feathers and beak (McGraw et al. 2001); zebra finches, in contrast, use lutein and zeaxanthin to manufacture red ketocarotenoids that appear in the bill (McGraw et al. 2002*b*).

We performed two types of carotenoid-supplementation experiments with captive birds to investigate the degree to which lutein and zeaxanthin differentially accumulate in the body and contribute to integumentary color. In experiment 1, we supplemented goldfinches and zebra finches with high concentrations of either lutein or zeaxanthin to determine (when presented alone) the extent to which each dietary xanthophyll was extracted from the diet and elevated beak or feather coloration. We drew blood to obtain a record of diet-derived carotenoids, scored integumentary color with a reflectance spectrophotometer, and plucked feathers in goldfinches to determine the types and amounts of carotenoids in colorful plumage using high-performance liquid chromatography (HPLC). In experiment 2, we administered low, medium, and high concentrations of a standard lutein-to-zeaxanthin mix (matching that found in the seeds they eat; McGraw et al. 2001) to different groups of goldfinches and measured the carotenoid content of blood and feathers to understand potential interactions between these carotenoids and how this may translate into plumage pigmentation.

Material and Methods

Experiment 1: American Goldfinches

In February 2002, 10 male American goldfinches were captured from the wild using basket traps at bird feeders in Lee County, Alabama. Males were randomly split into groups of five and housed indoors in two small wire cages (0.6 m long × 0.4 m wide × 0.4 m tall). Windows maintained natural day length cycles. We fed the finches an ad lib. diet of sunflower hearts and tap water. Water was treated with a coccidiostat throughout molt to eliminate coccidial infections (sensu McGraw and Hill 2000) that can inhibit dietary carotenoid uptake (Allen 1987).

Our dietary carotenoid supplementation experiment spanned the duration of the pre-alternate molt (March 15–May 15, 2002). We were interested in using water-soluble carotenoids to elevate carotenoid levels to the upper range of the physiological limit. By determining the concentration of carotenoids in the captive seed diet, the amount of food and water consumed daily, and levels of blood carotenoids in wild, molting male goldfinches, we could estimate the amount needed to add to water to achieve this. Sunflower seeds contain a very low concentration of carotenoids (1 μg/g total xanthophylls, 70 : 30 lutein : zeaxanthin ratio) relative to other bird foods (McGraw et al. 2001), and as a result, male goldfinches circulate a low concentration of plasma carotenoids (ca. 8 μg/mL) when fed this captive diet during molt (Gregory 2002). Molting males in the wild, however, circulate as much as 60 μg/mL carotenoids through blood (McGraw and Gregory 2004). Thus, we were interested in increasing daily carotenoid intake sevenfold, which, even if we assumed 100% extraction efficiency from the diet, would not exceed physiological limits in plasma. Molting goldfinches consume an average of 2.3 g of sunflower seed (and thus 2.3 μg carotenoids) per day during molt (Gregory 2002), and in pilot tests we found that molting goldfinches drink approximately 2 mL of water per day. So, to achieve a daily intake of 16.3 μg carotenoids, we could add 7 μg/mL carotenoids to water. Ultimately, one group of five finches was administered a 7 μg/mL dose of lutein beadlets (Roche Vitamins, Parsippany, N.J.) dissolved in the drinking water, whereas the other group was fed an equal dose of zeaxanthin beadlets.

We drew blood from males before the experiment (March 13) and on 1 d during molt (April 12) to determine plasma-carotenoid content using HPLC. Plasma-carotenoid extraction and HPLC methods follow those outlined in McGraw et al. (2002*b*). At the end of molt, we scored plumage and beak color using the Colortron II reflectance spectrophotometer and determined feather-carotenoid concentration using HPLC (see McGraw et al. 2002*a* for methods on both procedures). We use all three tristimulus color scores (hue, saturation, and brightness) generated by the Colortron to investigate the relative effects of these two carotenoids on different components of color. We were unable to document the carotenoid profiles of beaks in goldfinches and zebra finches (described subsequently) because bill pigments are esterified and proved difficult to analyze chromatographically.

Experiment 1: Zebra Finches

Twenty male zebra finches were housed individually in small wire cages in an indoor room on the campus of Cornell University (see McGraw et al. 2002*b* for housing details). Birds were fed Kaytee Forti-Diet finch blend (see McGraw et al. 2002*b* for composition) as a baseline diet. As previously described with goldfinches, one randomly selected group of 10 was fed a dose of lutein beadlets, whereas the other group was fed an equal dose of zeaxanthin. Elevating carotenoids to the upper limit of the physiological range was difficult here because nothing is known of carotenoid circulation in wild zebra finches. In captivity, adult males in breeding condition can accumulate

levels like goldfinches as high as 60–65 μg/mL, but on average circulate ca. 25–30 μg/mL (Blount et al. 2003; McGraw and Ardia 2003). Thus, we were interested in doubling the daily dose that zebra finches obtained from seeds. The millet-based diet of zebra finches contributes more carotenoids to the baseline diet (7 μg/g; McGraw et al. 2001) than do sunflower seeds. Since zebra finches consume similar amounts of food (2.3 g/d; McGraw et al. 2003) and water (2.5 mL/d; Zann 1996) to goldfinches, we boosted carotenoid levels by again adding 7 μg of either lutein or zeaxanthin per milliliter drinking water, raising daily consumption levels from ca. 16 μg to 32 μg.

Birds were supplemented with carotenoids for 1 mo, which was sufficient time for blood-carotenoid levels and beak color to change in response to dietary supplementation in previous studies of zebra finches (Blount et al. 2003; McGraw and Ardia 2003). After this time, we drew blood to determine plasma-carotenoid content (sensu McGraw et al. 2002*b*) and measured beak color with the Colortron. We also scored beak color at the start of the experiment.

Experiment 2

Fifty additional male American goldfinches were captured as described previously and housed in six large outdoor aviaries in groups of 10 (see McGraw and Hill 2000 for housing conditions). They were fed the same sunflower-seed diet as in experiment 1 for goldfinches, but here all birds received both lutein and zeaxanthin beadlets in the ratio at which they are found in sunflower seeds (70 : 30, McGraw et al. 2001; lutein and zeaxanthin occur at a ratio of 70 : 30 in the blood of wild, molting male goldfinches as well; McGraw and Gregory 2004) and varying only in total concentration among the groups. For the duration of molt, two cages ($n = 19$ males) received a physiologically low dose (0.1 μg/mL) of this lutein-zeaxanthin mix, two cages ($n = 16$ males) received a physiologically intermediate dose (1 μg/mL), and two cages ($n = 15$ males) received a physiologically high dose (10 μg/mL). These doses were chosen because (1) in this experiment conducted after experiment 1, we previously found that a 7-μg/mL dose still did not push males to the upper limit of physiological values (discussed subsequently), allowing for an increase to 10 μg/mL, and (2) order of magnitude changes would generate a wide range of supplemental values. We sampled blood once during molt to determine plasma carotenoid concentration. At the end of molt, we again scored plumage coloration and determined feather-carotenoid content.

Results

Experiment 1: American Goldfinches

Before carotenoid supplementation, birds in the two treatment groups did not differ significantly in plumage color (hue: $F = 0.37$, $P = 0.56$; saturation: $F = 2.6$, $P = 0.15$; brightness: $F = 0.07$, $P = 0.79$) or any measure of plasma-carotenoid content (total pigment concentration: $F = 0.06$, $P = 0.81$; lutein concentration: $F = 0.002$, $P = 0.97$; zeaxanthin concentration: $F = 0.94$, $P = 0.36$; lutein/zeaxanthin ratio: $F = 5.0$, $P = 0.06$). Pre-experimental goldfinches fed the low-carotenoid, sunflower-seed diet in captivity for 3–4 weeks before the study circulated an average (±SE) of 5.5 ± 0.45 μg/mL total carotenoids through blood (3.7 ± 0.33 μg/mL lutein, 1.8 ± 0.14 μg/mL zeaxanthin).

Our dietary carotenoid treatments were successful in elevating zeaxanthin in zeaxanthin-supplemented (ZS) males and lutein in lutein-supplemented (LS) males. Total plasma-carotenoid concentration increased by nearly an order of magnitude in both groups after supplementation (Fig. 1). This occurred because of a 10-fold increase in plasma-lutein concentration in LS males (paired *t*-test of lutein levels before and during molt, $t_4 = 8.2$, $P = 0.0012$) and a 22-fold increase in plasma zeaxanthin concentration in ZS males ($t_4 = 7.7$, $P = 0.0015$; Fig. 1). The raw increase in zeaxanthin levels in ZS males did not differ significantly from the comparable increase in lutein levels in LS birds (unpaired *t*-test, $t_8 = 0.41$, $P = 0.69$), although the percent increase did ($t_8 = 4.17$; $P = 0.003$), which is likely due to the lower baseline concentration of zeaxanthin compared with lutein in the seed diet (McGraw et al. 2001). Total plasma-carotenoid concentration also failed to differ between the groups after supplementation ($t_8 = 0.51$, $P = 0.62$).

Treatment differences in plasma-pigment composition had important consequences for the types and amounts of carotenoids found in feathers and the color of the carotenoid-based plumage and beak. ZS males deposited a significantly higher concentration of one of the feather carotenoids—canary xanthophyll B ($t_8 = 6.0$, $P = 0.0003$; Fig. 2). In contrast, the yellow feathers of LS males contained a higher concentration of canary

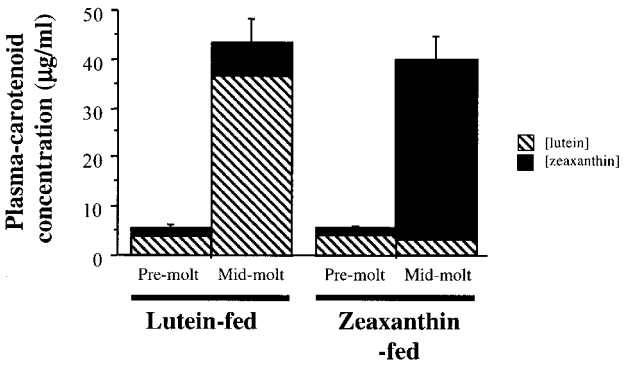

Figure 1. Effect of dietary carotenoid supplementation on the plasma-carotenoid status of captive male American goldfinches. Mean + SE are shown here and in all other figures. One group of five birds was provisioned with water-soluble lutein beadlets for the duration of the prenuptial molt, whereas another group of five was administered zeaxanthin. Before molt, birds were housed in captivity on a sunflower seed diet for 3–4 wk.

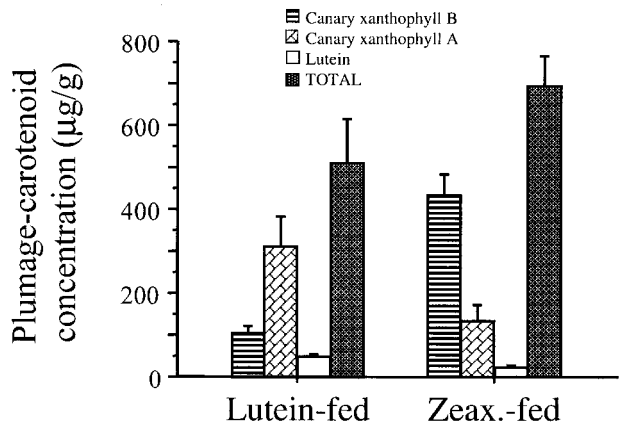

Figure 2. Effect of dietary carotenoid supplementation (see Fig. 1 for methods) on the concentration and composition of plumage carotenoids in male goldfinches.

xanthophyll A ($t_8 = 2.3$, $P = 0.05$) and tended to have more lutein ($t_8 = 1.9$, $P = 0.09$) than ZS males (Fig. 2). Yellow feathers did not differ in total carotenoid concentration between the groups ($t_8 = 1.41$, $P = 0.20$; Fig. 2).

Plumage saturation and beak hue were two measures of color that differed between ZS and LS males, with ZS males growing more saturated plumage ($t_8 = 2.4$, $P = 0.05$) and having a more orange bill ($t_8 = 2.6$, $P = 0.03$) than LS males (Fig. 3). These two measures capture most of the variation in carotenoid-based color expression in this species and are known to reflect other aspects of individual quality, such as incidence of parasitism (McGraw and Hill 2000). We found no other significant differences in beak or plumage color between the groups (all $P > 0.1$).

Experiment 1: Zebra Finches

Treatment groups did not differ in any measure of beak color before the start of the experiment ($P > 0.4$ for tristimulus scores). After supplementation, ZS and LS zebra finches, like goldfinches, also differed significantly in both plasma-carotenoid status and beak coloration. Zebra finches circulate four main carotenoids through blood—lutein, zeaxanthin, 2′,3′,-anhydrolutein, and β-cryptoxanthin—three of which are obtained from the diet and one of which (anhydrolutein) is a metabolite (McGraw et al. 2002*b*). ZS males circulated a significantly higher concentration of zeaxanthin ($t_{19} = 2.3$, $P = 0.03$) but lower concentration of β-cryptoxanthin ($t_{19} = 2.1$, $P = 0.05$) in blood than LS males (Fig. 4). There were no treatment differences in the plasma concentration of lutein ($t_{19} = 0.2$, $P = 0.83$), anhydrolutein ($t_{19} = 1.4$, $P = 0.18$), or total plasma-carotenoid concentration ($t_{19} = 0.4$, $P = 0.72$; Fig. 4).

We detected no group differences in absolute values for beak hue, saturation, or brightness after the experiment (all $P > 0.2$). However, we did find that the beaks of ZS males shifted significantly more toward a redder hue than those of LS males over the course of the carotenoid treatment ($t_{19} = 2.2$, $P = 0.04$; Fig. 5; $P > 0.5$ for changes in saturation and brightness).

Experiment 2

Goldfinches from the three treatment groups did not differ in plumage coloration before carotenoid supplementation during molt (Navara and Hill 2003). After 2 mo of treatment, however, the groups circulated significantly different carotenoid levels through blood (ANOVA: $F_{2,47} = 77.5$, $P < 0.0001$ for total concentration; $P < 0.05$ for all pairwise post hoc Fisher's protected least significant difference [PLSD] tests). Plasma from high-supplement (HS) males contained more than three times more carotenoids than the medium-supplement (MS) birds and nearly six times that of the low-supplement (LS) group (Fig. 6*A*). When broken down by specific dietary carotenoid, however, the group patterns were quite different for lutein versus zeaxanthin accumulation. There were still overall differences among groups in the levels of both carotenoids in plasma (lutein: $F_{2,47} = 3.3$, $P = 0.05$; zeaxanthin: $F_{2,47} = 97$, $P < 0.0001$), but HS males did not have significantly higher levels of lutein

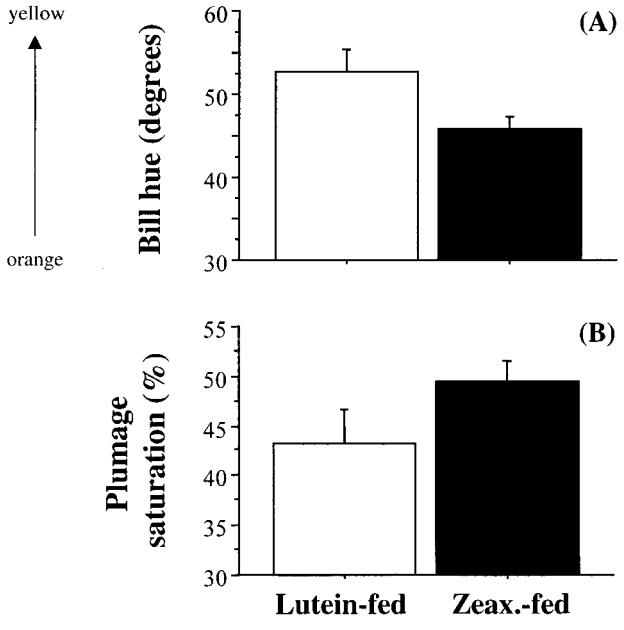

Figure 3. Effect of dietary carotenoid supplementation (see Fig. 1 for methods) on two measures of sexually selected coloration in male goldfinches: (*A*) beak hue and (*B*) plumage saturation. Beak hue is measured in degrees around a 360° color wheel, with lower values corresponding to more orange hues. Plumage saturation is measured as a percentage relative to absolute reflectance (*white*) and absorbance (*black*) standards.

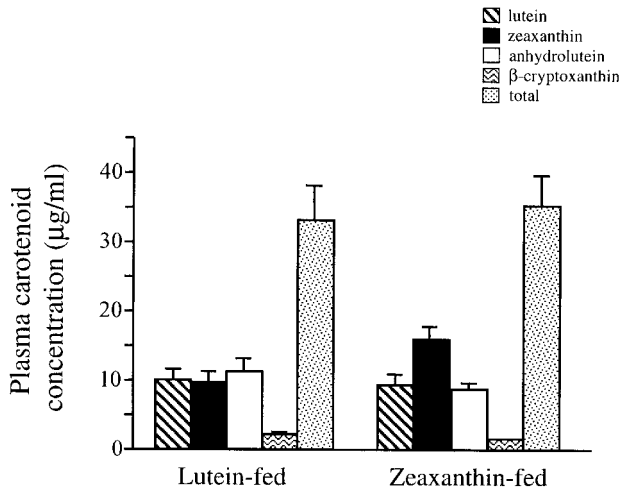

Figure 4. Effect of dietary carotenoid supplementation on the plasma-carotenoid status of captive male zebra finches. Groups of 10 birds were provisioned with a dose either of lutein or of zeaxanthin for 4 wk.

than did MS males (Fisher's PLSD, $P = 0.12$; Fig. 6*A*). In fact, lutein concentration was on average nearly 3 μg/mL lower in HS males than in MS males. Instead, it was the extraordinary increase in zeaxanthin concentration that gave HS males such high blood-carotenoid levels. HS birds circulated 45 μg/mL more zeaxanthin than MS males and nearly 50 μg/mL more than LS males. The percentage of zeaxanthin in plasma for HS males was on average 83%, whereas MS and LS males contained 37% and 27%, respectively. Again, note that the plasma-carotenoid levels in the high group, like those for goldfinches in experiment 1, were within the range of values found in wild, molting birds.

Among-group differences in plasma-carotenoid concentration and composition had significant consequences for plumage color (namely, saturation; Navara and Hill 2003). Here, we investigated the carotenoid content of these feathers and found that, like that in experiment 1 for goldfinches, HS males (circulating predominantly zeaxanthin in plasma) used a significantly higher concentration of canary xanthophyll B to color their feathers than the other treatment groups (Fig. 6*B*; $F_{2,47} = 7.7$, $P = 0.0012$; $P < 0.01$ for post hoc pairwise tests using HS; $P = 0.34$ for MS vs. LS). Conversely, HS males did not deposit more canary xanthophyll A into feathers; instead, levels were comparable to those in MS males ($F_{2,47} = 3.2$, $P = 0.05$ for groupwise test; $P = 0.82$ for post hoc test comparing HS and MS). Total feather-carotenoid content differed only between HS and LS males ($F = 5.7$, $P = 0.006$ for groupwise test; $P < 0.0015$ for pairwise comparison).

Discussion

We experimentally tested whether dietary xanthophylls are differentially accumulated in the body and serve different pigmenting roles in two songbird species that use carotenoids as sexual colorants. Birds and other vertebrates such as reptiles and fishes are known to preferentially accumulate xanthophylls over carotenes (e.g., Schiedt 1998), but little is known about any differences in the physiological fate of xanthophylls such as lutein and zeaxanthin that commonly appear in bird diets (McGraw et al. 2001). Domestic chickens (*Gallus domesticus;* Surai and Sparks 2001; Surai 2002) and red-legged partridges (*Alectoris rufa*; Bortolotti et al. 2003), for example, deposit concentrations of these xanthophylls in egg yolks in the same ratios that are found in the diet. Some prior studies have identified unusually high accumulation of zeaxanthin over lutein (e.g., in quail, Toyoda et al. 2002; in humans, Handelman et al. 1999), but it is not clear whether this occurred because of higher dietary levels or physiological factors.

Physiological Accumulation

In experiment 1, male American goldfinches that were fed the baseline seed diet only, which contained a very low concentration of carotenoids (which poorly pigments the feathers of these birds; McGraw et al. 2002*a*), accumulated more lutein than zeaxanthin in blood, which directly reflected the relative concentration of the two pigments in sunflower seed. The same was true of goldfinches in experiment 2 that were supplemented with a very low concentration of lutein and zeaxanthin (appearing in the same ratio as in seeds) in water. Goldfinches fed seeds plus concentrated supplements of either lutein alone or zeaxanthin alone accumulated the respective pigments at high levels in the blood, with no difference between lutein and zeaxanthin uptake. These results indicate that, when presented in low dietary concentrations or in isolation of one another, goldfinches do not selectively accumulate one xanthophyll over another. This would rarely occur in nature, however, because

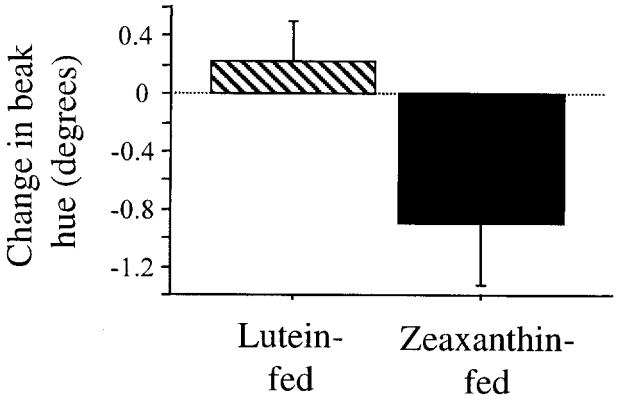

Figure 5. Effect of dietary carotenoid supplementation (see Fig. 4 for methods) on the change in beak color in male zebra finches. Negative changes in hue indicate shifts toward redder colors.

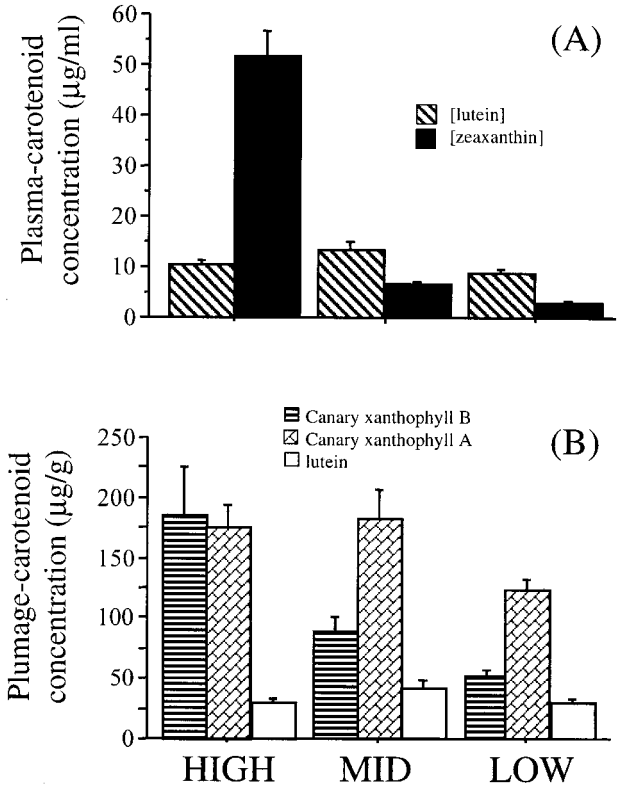

Figure 6. Effect of a different dietary carotenoid supplementation protocol on the concentration and composition of carotenoids in (*A*) plasma and (*B*) feathers in male American goldfinches. We used three groups in this experiment and administered the following treatments for the duration of the pre-alternate molt: (1) a low dose of lutein and zeaxanthin occurring in the same ratio as that found in seeds, (2) an intermediate dose of the lutein-zeaxanthin mix, and (3) a high dose of the lutein-zeaxanthin mix.

lutein and zeaxanthin nearly always coexist in plant matter and at higher concentrations than is found in sunflower seeds (Goodwin 1980).

When supplemented with both lutein and zeaxanthin at physiologically higher concentrations, however, male goldfinches accumulated proportionally more zeaxanthin and less lutein than appeared in the diet. For example, although zeaxanthin made up only 30% of dietary carotenoids in experiment 2, it comprised 83% of blood carotenoids in birds that received the highest dose. This suggests that zeaxanthin is selectively accumulated due to the competitive interactions of these two carotenoids during processing (van den Berg 1999). Carotenoids are absorbed from food by the mucosa of the small intestine via incorporation into mixed micelles, where they are transferred to lipoproteins that transport carotenoids through blood (Erdman et al. 1993; Furr and Clark 1997). The polarity of molecules and the saturation of micelles and lipoprotein particles are two primary factors that affect carotenoid absorption and transport (Parker 1996). The subtle differences in molecular structure and polarity between these two carotenoids imply a very specialized regulatory system involving a binding protein (Zagalsky 1995). At lower dietary carotenoid concentrations, micelles or lipoproteins are likely not fully saturated, and there are sufficient binding sites for both carotenoids to be maximally taken up. When both xanthophylls appear at higher dietary concentrations, however, zeaxanthin seems to outcompete lutein for binding substrates in these birds.

We are not aware of any comparable demonstration of hydroxycarotenoid discrimination in animals. However, some birds are known to preferentially transfer dietary ketocarotenoids such as canthaxanthin to tissues (e.g., in lesser black-backed gulls [*Larus fuscus*], Blount et al. 2002; in chickens, Hencken 1992). In fact, when given canthaxanthin supplements in the diet, male American goldfinches will readily deposit it at a high concentration in feathers, at the expense of the usual yellow carotenoids, to acquire orange plumage (McGraw et al. 2001, 2002*a*). It is likely that the aforementioned physiological mechanism of carotenoid discrimination is at work here as well, with canthaxanthin outcompeting both lutein and zeaxanthin for binding sites due to its molecular structure (specifically its highly oxidized and more conjugated state).

Zebra finches also showed the ability to circulate more zeaxanthin than lutein in the blood, in spite of the fact that lutein was more concentrated in the diet (given the higher carotenoid concentration in millet than sunflower seeds and the higher proportion of lutein than zeaxanthin in millet). However, because zebra finches (unlike goldfinches) circulate a carotenoid metabolite through blood, we cannot be sure whether zeaxanthin was truly selectively accumulated over lutein or whether lutein was selectively metabolized into anhydrolutein before being circulated in blood (McGraw et al. 2002*b*). Under the second scenario, however, we would have anticipated significantly higher levels of anhydrolutein (or lutein + anhydrolutein levels) in LS males than in ZS males, which we did not see ($P > 0.4$ for unpaired t-test for group differences in lutein + anhydrolutein).

Pigmenting Efficacy

Although the molecular mechanism of selective zeaxanthin accumulation in the blood of finches is unclear, the benefits to zeaxanthin retention are. Elevated zeaxanthin status imparted brighter integumentary coloration on both American goldfinches and zebra finches. We provide three lines of evidence in support of this: (1) in experiment 1, ZS goldfinches acquired more orange beaks and more saturated yellow feathers than LS males, despite having equal total plasma-carotenoid concentrations, (2) ZS zebra finches acquired redder beaks than LS males, also despite having equal total plasma-pigment levels, and (3) goldfinches from experiment 2 that circulated pro-

portionally more zeaxanthin in blood acquired the brightest beak and plumage colors (although this result is obviously confounded by total pigment concentration).

Zeaxanthin itself is known as a "more colorful" carotenoid than lutein because of its more conjugated chromophore. However, this cannot explain why zeaxanthin-enriched finches in this study developed brighter beak and feather colors because both species metabolize these xanthophylls that circulate through blood into different forms that are used as actual display pigments (McGraw et al. 2001, 2002*a*, 2002*b*). We quantified the types and amounts of feather carotenoids in goldfinches to investigate the biochemical basis for zeaxanthin enhancement of plumage coloration. It is conceivable that higher zeaxanthin levels could result in simply a greater concentration of metabolically derived pigments, or in a specific mix of carotenoid metabolites, that conferred a more saturated plumage color. We found that the feathers of zeaxanthin-rich males had a different carotenoid signature from those of lutein-fed males. Zeaxanthin-replete males had higher concentrations of canary xanthophyll B, whereas lutein-enriched males deposited more canary xanthophyll A; yet the groups in experiment 1 did not differ in total feather-carotenoid concentration. These findings confirm our earlier hypotheses regarding product-precursor relationships for carotenoid metabolism in goldfinches (Stradi 1998; McGraw et al. 2001) as well as the importance of canary xanthophyll B as a plumage colorant in this species (McGraw et al. 2002*a*). Canary xanthophyll B appears to bestow brighter colors on goldfinch feathers than other plumage pigments at a given concentration, and the selective accumulation of zeaxanthin directly allows finches to manufacture more of these valuable feather carotenoids. Although the extinction coefficient (which provides objective information about the light-absorbing and light-scattering capabilities of biochemicals) is unknown for both of these canary xanthophylls, we presume that it is higher for canary xanthophyll B than A because of the presence of a conjugated, end-ring keto group (instead of a hydroxl).

Conclusions

The fact that lutein is predominant in the plasma of many birds (e.g., Saino et al. 1999; Slifka et al. 1999; Negro et al. 2001) is a reflection of how common lutein is in the diet of birds relative to zeaxanthin (Goodwin 1980). With zeaxanthin comparatively rarer in nature, it is conceivable that birds may actively seek out zeaxanthin-rich foods as a strategy to maximize carotenoid accumulation and the expression of sexual coloration (via synthesis of the most colorful metabolites). Studies of carotenoid intake are difficult in wild birds for many reasons (e.g., Hill et al. 2002), but they should serve as nice complements to physiological studies such as these to deepen our understanding of the carotenoid-pigmentation system of colorful animals.

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